SCIENCE-BASED FACTS & KNOWLEDGE ABOUT WILD ANIMALS, ZOOS AND SARS-COV-2 VIRUS

This Q&A was produced by the EAZWV Infectious Diseases Working Group

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Preliminary note: the scientific content of this factsheet was collected from reliable sources such as OIE, European National references laboratories, WHO, and scientific literature.

A massive amount of new science is available daily online, but please pay close attention to the source [e.g. pre-print server vs. peer-reviewed]. Several preprints on animal topics roaming the internet since the spring of 2020 ended up getting rejected or changed their conclusion more than 6 months later, so caution is important.

Here you can find a good resource for daily publications: Lit Cov (see online references)

Contents

Context

The COVID-19 is a viral infectious disease (last "d" =disease) transmitted between humans, first described in Wuhan China on the 31st December 2019[1]. As of December 2021, the virus spread globally to more than 192 countries, causing the death of more than five million people worldwide, with infections rising again due to the emergence of highly transmissible variants.

The virus name is SARS-COV-2 and it belongs to Coronavirus family. This name was given because of real genetic proximity of this virus with the SARS virus of 2002-2003 outbreak. On the 11th of March 2020, the WHO officially declared it pandemic.[2] One month later, it was also declared as a notifiable disease in animals by O.I.E. as SARS-Cov-2 was found able to infect some domestic and wild species.

Questions / Answers

These are selected questions that visitors, authorities, zoo professionals or other stakeholders may ask regarding COVID-19 risk assessment related to zoo animals.

Coronaviruses in general

- Coronaviruses are very common in Mammals and Birds [3]. They are not always associated with disease and there are a lot of non-symptomatic carriers, often occurring in many domestic and wild species.
- This RNA virus family is comprised between 4 main groups [4]
  - Alphaconoravirus: mainly found in bats, but this group also contains:
    - The Feline Coronavirus FeCov with its two forms (FeCV and FIP) [5]
    - The canine coronavirus type I and II [6]
    - Human viruses like HCov 229-E, often a component of the common cold
  - Betacoronavirus: most represented in mammals, from carnivores[7] to hoofstock[8910 11 12] from hedgehogs[13] to bats. It also contains the 3 more recent emerging coronaviral diseases:
    - subgenera Merbecovirus : MERS CoV[14]
    - subgenera Sarbecovirus : SARS CoV and SARS Cov-2
    - subgenera Embecovirus: HCoV-OC43 and HCov-HKU1, two of the more prevalent infectious agents of the common cold in humans
  - Gammacoronavirus: viruses from cetaceans (beluga, dolphins), and a dozen of purely avian viruses[15]
  - Deltacoronavirus: mostly avian species specific coronaviruses[15] , and some porcine one, recently recovered from leopard cats [16]
- Chiropterans are well known to be host of many viruses, including various coronaviruses at the same time [17,18]. These include also some very specific coronaviruses that are specific to one species or only one genus of bats.
- After their first year of life, more than 80% of domestic species including dogs, cats, cattle, and pigs, are seropositive for at least one coronavirus, without expressing clinical signs.

SARS-CoV-2

Which animal species is the SARS-CoV-2 associated with?

- SARS-CoV2 shows 96.3% genomic identity with Bat-CoV-RaTG13 that had been previously detected in the intermediate horseshoe bat (*Rhinolophus affinis*) from southwest China's Yunnan Province [19]. This RaTG13 virus was involved into pneumonia cases in miners in 2012[20], with clinical signs very similar to COVID19. 2020 and 2021 studies revealed other very close sarbecoviruses, showing the evolution history of SARS-CoV-2 is more complex and that RATG13 is not the direct ancestor of SARS-CoV-2.
  - In Japan[21] : 1 sarbecovirus (Rc-o319) was retrieved from 2013-samples of *Rhinolophus cornutus*, with 81.5% sequence identity to SARS Cov2.
  - In Cambodia[22]: 2 sarbecoviruses were found from 2010-samples of *Rhinolphus shameli*, with 92.6% nucleotide identity across the genome, representing a new sublineage of SARS-CoV-2 related viruses.
  - In Thailand[23], a single isolate was discovered recently in 5 different bats of *Rhinolophus acuminatus* species, and named RaCS203. This virus shares 95.86% sequence identity with SARS-Cov-2 and serological survey in the same colony of bats and in a pangolin revealed SARS -Cov-2 neutralizing antibodies.
  - In north Laos , a more recent study[24] discovered 3 very close viruses in 3 different *Rhinolphus* species (*R.malayanus, R.marshalli* and R.*pusillus*) the closest known to date.

- There is still a difference within the **R**eceptor **B**inding **D**omain RBD of the spike (S) protein between SARS-Cov-2 and all these Rhinolphus viruses as they're still lacking the furin cleavage site into the RBD region (between S1 and S2 suunits of (S)pike protein), which is an important feature of this virus, allowing successful fusion to human cells.
- Pangolin coronaviruses have been described from Malayan pangolins (*Manis javanica*) confiscated in 2017 and 2018. Regarding the short RBD region, the Pangolin-CoV is more similar to SARS-CoV-2 region than the Bat-CoV-RaTG13. The Pangolin-CoV shares all five key amino acids in invading human cells with SARS-CoV-2 whereas Bat-CoV-RaTG13 genome only shares one out of five [26]. However, it is important to note that pangolins or any other species have not been confirmed to be intermediary or amplification host in this SARS-CoV-2 outbreak. On a whole genome basis, the CoVs from pangolins are very dissimilar to the SARS-CoV-2 [27,28].

- A reviewed hypothesis [29] states that the simplistic scenario Bats>Pangolin>Human is not applicable. The authors propose that a multitude of SARS-CoV-2 similar coronaviruses circulate widely in wildlife and humans and that spread in humans is driven by post-exposure host-driven selection, rather than the suggested preadaptation to the human host.
- Great caution should be paid to all new papers and information released about animal species susceptibility to the virus[30,31]
    - While the objective is sometimes to assess a potential role of animal species in transmission, it **mostly is to identify potential animal models for treatment and vaccination testing**. Several studies have been performed on previously non receptive species , transformed in models like transgenic mice [32] that were modified to have the human ACE2 gene.
    - The methods employed vary significantly as seen by the following examples:
        - in vivo assays (where immune system effects of hosts are mostly not considered).
        - computer models (prediction of molecular binding abilities).
        - experimental infection using **high infective** doses of SARS-CoV-2 **injected directly in nose, trachea or blood stream.**
    - Hence, while these types of studies provide valuable information, findings may not be directly applicable to real life situations (e.g., where animals are not exposed to this extreme range of viral loads).
    - Over the past two years several animal species have proven susceptible to the virus through natural infection. So far only few of these examples have found their way to peer-reviewed literature.

COVID and Carnivores

Table A1: Extant knowledge about Caniform species sensitivity to SARS-CoV-2. **(N/A : no assessment source found)**

		Species	Calculated prediction of Viral "S" to Host' ACE2 receptor binding	In vivo experimental infection success *(blank: no data yet)*	Natural transmission (Human > Animal)
CANIFORM	CANIDAE	Domestic Dog *Canis familaris*	Medium	YES	YES
		Red Fox Vulpes			Only seroconversion noticed in one study[33]
		Racoon Dog *Nyctereutes prycionides*	ACE2 matching also proven in [34]	YES[35]	
	URSIDAE	Polar bear *Ursus maritimus*	Low[36,37]		
		Brown bear *Ursus arctos*	Low[36]		
	PROCYONIDAE	Racoon *Procyon lotor*	Medium[26]	YES[38]	
		Coati *Nasua nasua*	N/A		YES (UDSA, 2021)
	AILURIDAE, MUSTELIDAE & MEPHITIDAE	American mink *Neovison vison*	Very low[37]		YES[39–41] *+ Animal to Human transmission*[42]
		European mink *Mustela lutreola*	Very low (17/25)		*Reported but likely mismatched with European farmed American mink*
		Sea otter *Enhydra lutris*	Low (17/25) [43,44] Very high [36]		

		Asian small-clawed otters *Aonyx cinereus*	N/A		YES (USDA, 2021)
		Striped skunk *Mephitis*	N/A	YES[38]	
		Red panda *Ailurus fulgens*	Very low[37]		
	PINNIPEDIA	California Sealion *Zalophus californianus*	Low[36]		
		Harbour Seal *Phoca vitulina*	High[36]		
		Walrus *Odobenus rosmarus*	Very High[36]		

Table A2: Extant knowledge about Feliform species sensitivity to SARS-CoV-2. **(N/A : no assessment source found)**

		Species	**Calculated prediction of Viral "S" to Host' ACE2 receptor binding**	**In vivo experimental infection success** ***(blank: no data yet)***	**Natural transmission** **(Human > Animal)**
FELIFORM	FELIDAE	Tiger, Lion *Panthera Leo* *Panthera tigris*	Medium[37] 94% Binding residue of ACE2 from[45]		YES [46–48]
		Puma *Puma concolor*	Medium[37]		YES (OIE, 12.08.2020), USDA 2021
		Snow Leopard *Panthera uncia*	Medium[37]		YES (OIE, 18.12.2020)
		Fishing Cat *Prionailurus viverrinus*	N/A		YES (USDA, 2021)
		Leopard cat *Prionailurus bengalensis*	N/A		
		Canadian Lynx *Lynx canadensis*	Medium[37]		YES
		Clouded Leopard *Neofelis nebulosa*	Medium[37]		YES
	HYENIDAE	Spotted Hyaena *Crocuta crocuta*	Reported as high as Golden Hamster or most NHP in [49]		YES (USDA 2021)
	HERPESTIDAE	Meerkat *Suricatta suricata*	Very low[37]		
	VIVERRIDAE	Civet cat *Paradoxyrus hermaphoditus*	Likely		

		Masked palm civet *Paguma larvata*	Very low[37]		
		Binturong *Arctictis binturong*	N/A		YES (USDA, 2021)
	EDUPLERIDAEAE	Fossa *Cryptoprocta ferox*	Very low		

- Carnivores are the mammal order showing the highest number of non-experimental proven infection. Among them, mainly 3 species stand for most of the reports: dog, cat and American mink. In zoos, lions, tigers and snow leopards have represented the most cases. There is a current concern about hyper sensibility of snow leopards, as some positive cases eventually died in US zoos. Causes of death are still being investigated, in order to sort out comorbidities factors that may have played a role in the lethal issue of theses specific cases,

Details regarding Mustelids :

Notes on Mink

- On the 26th of April, two mink fur farms in the Netherlands were reported to have American mink (*Neovison vison*) infected with SARS-Cov2. The two farms are situated in close proximity and within a region of the Netherlands with a high incidence of Covid19 in humans. Animals exhibited respiratory and GI-tract signs and the population (around 20.000 animals) experienced an increased mortality rate. The Dutch government decided not to move animals or their manure anymore and cordoned the area with a 400 m perimeter to human circulation (walk, cycle path) as preventative measure. A Dutch statement emphasizes the contamination is of human origin and that mink are of negligible risk to humans. Air circulation devices and filters are currently being analysed to check for virus particle presence.
- Regarding numbers of infected individuals, mink are currently the most abundant species in terms of non-experientially infected animals. SARS-Cov2 has been detected in mink farms in many countries. The following two examples illustrate the initial panic, which now appears to have been replaced by a more pragmatic approach:
  - **The Netherlands** : on the 18th of June 2020,15 farms were contaminated. One month later, the number increased to 25 with only 8 reporting clinical signs. At the time of writing, there were 33 infected farms. All animals from infected farms are culled (=1.5 million animals, so 30% of total Dutch farming minks). Transmission from mink to mink seems very efficient and involves fomites but also aerogenic routes. Reverse viral transmission from animals to 2 workers is highly suspected. Pelting ended at the end of 2020, then the whole mink industry is now terminated in this country.
  - **Denmark:** Initially 4 farms located in the same area (North Jutland) showed a 50% prevalence and minimal clinical signs, and a stamping out policy was employed. However, despite efforts to contain the outbreak, in June–November 2020, SARS-CoV-2-infected mink were detected in 290 of 1,147 Danish mink farms. In North Denmark Region, 30% (324/1,092) of people found connected to mink farms tested SARS-CoV-2-PCR-positive and approximately 27% (95% confidence interval (CI): 25–30) of SARS-CoV-2-strains from humans in the community were mink-associated. Measures proved insufficient to mitigate spread, and in November 2020, the government ordered culling of all Danish mink[42].
- In the second half of 2020, a lot of other European countries reports positive mink farms : Spain, Poland, France, , Canada, Greece, Lithuania, Italy, Sweden. In North America, Canada reported on case in British Columbia, while in the USA, at least 4 states saw outbreak in farms : Utah (with 6 different farms between September and November), Michigan, Oregon, Wisconsin (3 farms).
- All countries are describing that SARS-Cov-2 infection in mink is not readily identifiable due to its mild clinical signs and relatively short course in animals. Morbidity and mortality reports are variable, from zero (detection only by survey) to a mortality rate that can reach sometimes 12%. Therefore, countries with mink farming mainly implement an Early Warning System strategy, with a strict monitoring of direct and indirect health indicators among farmed animals (e.g. any animals dying of "natural causes" Is screened for SARS-Cov-2). If positive, then strategies may differ between countries, from immediate and global culling to selective screening and culling.

- At the end of 2021, there are 3 strategies on fur animals depending on countries or even regions of some countries
    - Accelerating the phasing out of fur farm industry (France, Netherlands.)
    - Temporarily ban breeding (Denmark)
    - Vaccination is being considered in several countries but, until 31st of December 2021, only applied in Finland.

Details regarding Pinniped and Cetaceans:

- While the real risk in these species still needs to be assessed, caution is advised, particularly in visitor contact programs with captive marine mammals (sealions, dolphins, etc.). Moreover, efficiency of life support system sterilization units (either chlorine, UV or ozone) to effectively remove coronavirus risk from water remains uncertain based on several studies on human water and wastewater treatment[50] , even free chlorine concentration of 0.5mg/L may not be enough to remove SARS-Cov-2 from water.

COVID and non-human primates

Table B : Extant knowledge about Non-Human Primates species sensitivity to SARS-CoV-2 from [2643,44,51] N/A= not assessed yet

	Species	**Calculated prediction of Viral "S" to Host' ACE2 receptor binding**	**In vivo experimental infection success** *(blank: no data yet)*	**Natural transmission** **(Human > Animal)**
PROSIMIANS	Coquerel sifaka *Propithecus coquereli*	High[37,52]		
	Blue eyed black lemur *Eulemur flavifrons*	High[37,52]		
	Aye *Daubentonia madagascarensis*	High[37,52]		
	Cheirogalus *Cheirogalus medius*	Low[37,52]		
" NEW WORLD"	Owl Monkey *Aotus nancymaae*	Low		Screening in wild NWM so far led to all negative animals[53]
	Squirrel Monkey *Saimiri boliviensis*		**YES**[54]	
	Common Marmoset *Callithrix jacchus*		**YES**[55]	
" OLD WORLD"	Hamadryas baboon *Papio hamadryas*	Very High	**YES**[55]	
	Cynomolgus monkey *Macaca fascicularis*	Likely	**YES**[56]	
	Rhesus macaque *Macaca mulatta*	Very High	**YES**[57–59] and reinfection could not occur at T0+28 days new challenge	
	African Green Monkey *Chlorocebus aethiops*		**YES**[59–61] Cytokine storm is seen in this species, like human and unlike Rhesus Macaques	**Yes**[62] Pre-screening procedure in experimental animals revealed one monkey already exposed to European SARS Cov2 strain
APES	Orangutan *Pongo pygmaeus*	Very High[30,37,52]		
	Chimpanzee	Very High		**YES** (seroconversion reported)

	Pan troglodytes			
	Gorilla *Gorilla gorilla*	Very High		**Yes** Animals signs : from transient lethargy to pneumonia. Monoclonal antibodies used in a male.

While non-human primates, and especially apes, are likely susceptible to SARS-Cov-2, it should be noted that outside of experimental infections, the number of reports on natural infections in NHP is rather low, (one African green monkey prior to experimental setting [62] and several gorilla troops), while a lot of primates have been tested in a few European zoos, mostly by faecal PCR (chimpanzees, gorillas, gibbons), but also by nasal/tracheal PCR + serology (lemurs, baboons etc.) with negative results.

Prosimians

- There is no information on the susceptibility of prosimians, other than that they show great diversity in their ACE2 configuration across the various prosimians (e.g. Sifakas with ACE2 very similar to human, while mouse lemur is more distant). In Madagascar, CoVid19 is a major concern in the human population far from any health care facilities. A paper still in preprint [63] is describes the theoretical sensibility of prosimians according to the binding energy needed in ACE2 /S junction, and placed Sifaka, Avahi and Aya-Aye at the upper risk level, followed by varecia, Elumrus and Prolemurs. Transmission to lemurs within National Parks / Reserves or in captive settings (hotels, zoos,..) has been therefore identified has a threat to lemur conservation, but so far, no contamination was confirmed. Four Ring tailed lemurs *Lemur catta*, found dead in Madagascar in April 2020 were assessed for SARS-Cov-2 by Pasteur Institute and were negative. Prosimian like Microcebus or Galago are predicted with a lesser risk[52,63].

Old World Monkeys

- According to the genetic and physiological (immunology) proximity between human and non-human primates, SARS-CoV-2 is likely to be able to enter NHP cells, to replicate, to provoke clinical signs, and maybe to be transmitted between animals. So far, all these milestones have only been confirmed in rhesus macaques and gorillas (see table D.)
- Feral NHP species, living close to human activities, like macaques in South Asian cities, or monkeys fed by humans in temples etc. are likely to be at risk for infection from humans. Other interfaces between wild NHP and humans (poaching, hunting, tourism) are also thought to be driver of contamination[64].

Apes

- Coronavirus transmission was previously proven from Humans to apes with HCoV OC43, one of the human coronavirus involved in the common cold [65], when wild chimpanzees became infected by humans visiting their habitat in Taï National Park in Cote d'Ivoire. Therefore, high level of hygiene, distance and/or PPE use paired with staff health monitoring are more than ever mandatory in the care of great apes.
- On the 11th of January 2021, two coughing gorillas in San Diego Zoo's troop tested positive for SARS-Cov-2 in faecal samples [66]. Eight animals were exposed and the source of contamination was reported as being an asymptomatic member of the staff, later found positive by PCR. Sequence of gorilla isolates matched the sequence of the positive symptomatic carrier keeper. Social distancing and protective measures were already implemented at the time of contamination. Gorillas did not receive specific treatment (no antibiotics, no antivirals) other than fluids and vitamins, except for the male silverback that received monoclonal antibodies.
- On the 25th of February 2021, Prague Zoo reported a male gorilla with tiredness and loss of appetite that was PCR positive with SARS-Cov-2. There was improvement after a few days of only under supportive care , including NSAIDs, Vitamin, Antibiotic and Ivermectin (see lower in Treatment Section)
- Atlanta Zoo managed an SAS-Cov-2 infection in their gorillas when 18 individuals out of 20 showed clinical signs during the month of September 2021. All animals recovered within weeks after support.

New World Monkeys

- Based on New World primate sensibility to SARS-CoV-1 [67], one can envisage that they could be less susceptible to SARS-CoV-2 than Old World Monkeys, as they were known to be inadequate animal models for SARS infection. However, as seen in Table D below, prediction from ACE2 receptor modelling highlight some species (common marmoset, Night or Howler monkeys) as within "medium" range receptors, with same amount of changed amino acids as lions or tigers (4 out of 25) on the RBD. One study sampled wild neotropical primates in two regions of Brazil in 2021 and found neither PCR nor seropositive animals in early 2021[53]

Sensitivity of other mammals

Table C: Extant knowledge about Ungulates species sensitivity to SARS-CoV-2. N/A= not assessed yet

Species	**In Vitro** **Viral Particle entry** **Computer & molecular prediction of ACE2 receptor binding** **From** [26,37,68]	**In vivo experimental infection success** *(blank: no data yet)*	**Natural transmission** **(Human > Animal)**
Swine *Sus scrofa domesticus*	Viral particle entry in vitro Likely Low	On study failed to get positive PCR and seroconversion in Infected animals and sentinel [69], while another succeed to infect animals[70]	
Cattle *Bos taurus*	No particle entry in vitro Likely	**YES**[71,72] , but hardly got infected and no transmission ton contact animals	
Sheep *Ovis aries*	Medium	While one experimental infection failed[73], another recent one (in preprint) succeed to infect 8 sheep with historical and alpha Variant, but with very mild infection and no transmission to contact sheep.	
African elephant *Loxodonta Africana*	Unlikely Low		
Camel *Camelus bactrianus*	Medium		
Giraffe *Giraffa sp.*	Medium		
Hippopotamus *Hippopotamus hippopotamus*	Medium		**YES** (Antwerp zoo press release)
Alpaca *Vicugna pacos*	Medium	Experimental infection failed[73] but n=2	
Horse *Equus caballus*	Low	Experimental infection failed[73] but n=1	
Reindeer *Rangifer tarandus*	High		
White Tail Deer *Odocoileus virginainus*	High	**YES**[74] However, experiment was performed on 6 weeks old hand reared fawns infected intranasally with very high viral load. Infected animals exhibited subclinical infection, shed virus in nasal secretions and were able to infect nearby naïve fawns	**YES** (see below in "Wildlife" Section)

Table D: Extant knowledge about miscellaneous (non NHP, non-carnivore, non Ungulates) species sensitivity to SARS-CoV-2 from N/A= not assessed yet

Species	**In Vitro** **Viral Particle entry** **Computer & molecular prediction of ACE2 receptor binding** **From** [26,37,68]	**In vivo experimental infection success** *(blank: no data yet)*	**Natural transmission** **(Human > Animal)**
Manatee *Trichechus manatus*	Low[36]		
Giant anteater *Myrmecophaga*	High		
Mouse *Mus musculus*	No *in vitro* particle entry Unlikely Very low	Although it was not possible with historical strain, a recent study[75] proved mice can be infected with recent V.O.C. such as B.1.1.7 (Alpha)	
Rat *Rattus rattus*	Unlikely Very low		
Chinese hamster *Cricetulus griseus*	Likely High	**YES** [76] Older hamsters exhibit more weight loss. Young animals launch earlier and stronger immune response.	
Syrian hamster *Mesocricetus auratus*			
Horseshoe bat *Rhinolophus sp.*	*In vivo* Particle entry Likely Or Very low		
Daubenton's bat *Myotis daubentoni*	No *in vitro* particle entry		
Vampire bat *Desmodus rotundus*	Likely or Very low		
Fruit Bat *Rousettus aegyptiacus*		**YES**[77] and one out of 3 contact animal was infected	Not detected in situ in Egypt [78]
Deer Mouse *Peromyscus maniculatus*	N/A	Yes[79] Respiratory and intestinal viral invasion, but also presence of virus within the brain	
Guinea pig *Cavia porcellus*	N/A Unlikely		Not detected in case of pet owned by positive owner[80]
Chinese Tree Shrews *Tupaia bellangeri chinensis*	N/A	**YES** [81] Enter upper resp. tract, lungs, intestines, and brain. No fatalities	
Chicken *Gallus*	Unlikely (3/5)	No. Failed to get positive PCR and seroconversion	
Mallard duck *Anas platyrynchos*	N/A	No. Failed to get positive PCR and seroconversion	

- Usual pest species found in zoos such as rodents (mice, rats) or birds (crows, pigeons) are very unlikely to be vectors for the SARS-CoV-2, although gulls were suspected to have acted as vectors / fomites during the Danish outbreak in mink. Even if rodents can harbour multiple other coronaviruses[82], mice and rats seem to be poor hosts for the SARS-CoV-2, as they lack the ACE2 receptor matching amino acids [26].
- However, the susceptibility of Cricetidae family could be relevant: there are not only common experimental models, but also some of the species like deer mice, are very widespread in some continents. Therefore, monitoring of these

pests could be valuable as they may be seen as a potential spill over, source of perpetuation and risk of reverse zoonosis[79,83]

Concerns about strains, mutations, and variants in animals?

- SARS-Cov-2 virus encodes an exonucelase that is increasing genome correction during transcription phase and then decreasing mutation rate compared to other virus like influenza[84]. However, SARS-Cov-2 is a 30.000 bases long genome[85], making it the longest of the known RNA viruses.
- The classification of sequences, clades, variants and strains is somehow puzzling, as their nomenclature is more or less related to the repository database where they've been deposited, namely GISAID, NextStrain, NCBI or PANGOLIN. While there are currently (in December 2021) more than 6 million sequences from humans in GISAID database, only 1500 sequences are recorded in the same database out of animals. In NCBI, this ratio is 2.5 million human sequences versus 250 animals. Therefore, it remains very important that any positive PCR animal sample must be sequenced and added to theses shared bases.
- At the time of writing this update, there are 44 specific strains or variants recovered from animals: 10 from big cats (lion and tigers), 25 from mink (American mink, but there are also some wrong attributions to *Mustela lutreola* because of the blurry denomination "European farmed mink"), 7 from cats and 2 from dogs.

Table E. Strains or variants identified in non-domestic species

Species	Strain and/or Clade and or Variant	Comment
Gorilla	B.1.429 in the USA reported cases	Variant very widespread in human in California
	B.1.1.7 (Alpha)	From the Czech Republic outbreak
Tigers	B1.177.21. in Sweden	
	NY-CDC-2929 in the Bronx cases, Clade G	The strains from tigers and tiger keepers clustered with clade G), while the lion sequences clustered with clade V[48]
Lions	Clade V in Bronx cases	
	Clade 21J (Delta)	In Singapore case (Nextstrain link)
Tiger, Cougar Snow Leopard, Leopard, Lion	B.1.617.2 (Delta)	Delta variant, mostly seen since September 2021 in big cats infection, seems to produce heavier clinical signs than previous V.O.C. Found recently (2022) in a dead wild leopard cub, cause of death was predator attack, but SARS-Cov-2 antigens were found into several organs (see below in "Free Ranging Wildlife")[86]
Leopard Cat *Prionailurus bengalensis*	B.1.1.7 (Alpha)	Found in Croatia (Nexstrain reference)
Mink	"Cluster 5" : name of the mink variant, found in 12 human, and carrying several mutations (5 to 7) on the spike protein	Danish Statens Serum Institute announced existence of this Cluster 5 on the 3rd of November 2020. On the 19th of November, SSI reported they didn't find any new Cluster 5 cases, and so classified this cluster as extinct.
White Tail Deer	Mainly B.1.2 and more rare B1.596 and B1.582 in Ohio study[87]	

- Except from mink, all other variants / strains retrieved from wild species are very close to human strain from staff that contaminated them and are not worrying any experts. Regarding mink variants (found in humans), the ECDC produced a rapid risk assessment on the 12th of November 2020, with the conclusion that risk is low for general population in areas with high density of mink farms and moderate to high for medically vulnerable people in the same area. The risk increases to moderate for general population with occupational exposure to mink farms, and very high for medically vulnerable with the same occupational exposure.
- Among the recent Variants of Concern (V.O.C.) in humans, Delta (B.1.617.2) has been retrieved from both Gorillas and big cats and is suspected to produce enhanced clinical signs compared to historical Wuhan strain or Alpha & Beta variants. The recent V.O.C. "Omicron" (B.1.1.529) has not been reported in any zoo animals at the time of writing this document. WHO supported a series of studies on animals in December 2021, but there is barely any feedback yet, except a very early preprint[88] suggesting very reduced respiratory signs, lesions and viral load in hamsters compared to other variants.

- A recent Statement was made by OIE (see link), to address the hypothesis that B.1.1.529 variant ("Omicron") would have emerged from an animal reservoir. This hypothesis was emitted by some specialist based on the apparition of some mutations on Omicron spike that are associated to adaptation to mice ACE2[89]. While it can't be totally dismissed, there is no current evidence about such an animal origin.

Diagnostics in Animals

Antigen Detection

- **RT-PCR :** In human beings, large variety of tests are used, but the core tool remains RT-PCR detection of viral RNA mainly from nasopharyngeal and oropharyngeal swabs, and more recently from rectal swabs or faeces. These tests are qualitative (positive/negative and they can be used to find presence of precise sequences such as in current variants. They are also quantitative to assess viral load: the higher the CT value, the less viral load detected. Moreover, more labs have now set of probes to evaluate the presence of mutation that are specific to the current variant of concern (V.O.C).

  ***Note** : Several national and private commercial laboratories are now offering RT-PCR in animals. In some countries, there is still a requirement to allow the test only through the validation of an official state veterinarian. The detection of COVID-19 virus in animals now meets the criteria for reporting to the OIE through WAHIS, in accordance with the OIE Terrestrial Animal Health Code as a disease. Therefore, any detection of the COVID-19 virus in an animal (including information about the species, diagnostic tests, and relevant epidemiological information) should be reported to the OIE. Please see the OIE guidelines for testing.*

- **RAPID ANTIGENIC TEST:** Aside from these RT-PCR tests, more than 70 antigenic rapid test are also available on the market. They are built to detect the presence of protein from the Nucelocapsid (NP) through the use of lateral flow cassette system. In humans, the specificity of these test is often more than 98%, but their sensitivity is one of their major drawbacks[90], as it can be low and then really decrease the negative predictive value. In humans, asymptomatic carriers are often shedding low viral load, that can still be detected by RT-PCR but not by rapid antigen test. In animals, these tests have been used with nasal and rectal swabs on big cats with success and with good correlation with RT-PCR.
- **Samples to be used:**
  - **Oral/Nasal**: In big cats, is seems that there is a very good correlation between oral and nasal viral loads, so direct testing on swabs of both origins could be equivalent. Some studies even report a better sensitivity and constancy over infection course than nasopharyngeal samples. In animals, saliva excretion was found in several species such as macaques[91,92] and ferrets[93,94].
  - **Faeces**: If the animal are not trained for nasal swabs or anaesthetised, faeces remains a convenient option as faecal shedding lasts for several days to week[47]. However, faecal shedding usually occurs later than nasal or oral shedding (likely due to progressive gastric absorption of oropharyngeal fluids), so that false negative could occur in the first period of infection if based only on faecal monitoring alone. On the other hand, faecal shedding lasts longer than oronasal shedding. Rapid Antigenic test can be used to have point of care results, but it should be known that using them on faecal samples is likely to jeopardize the results and predictive values, as wild animals faeces contains a lot of interfering molecules compared to a oronasal fluids. There could be a matrix effect affecting the results, so that one should always double check with RT-PCR anyway.

Serological Antibodies test: There are different techniques included in this category : Rapid flow tests, different kind of ELISAs, different targeted antigens, etc. Some human tests may have further potential in other animal species such as non-human primates or carnivores:

- **Double antigen sandwich ELISA** based on recombinant viral protein that could detect both IgM and IgG antibodies [95]. Different viral antigens constitute the test target: Whole "S" Spike protein, sub unit S1, Sub Unit S2, Whole RBD region antigen or N195 (nucleoplasmid antigen). Multiplexes of these different antigens could be used as well to test all at once. The double sandwich technique may circumvent species specificity problems, so that this technique could theoretically be efficient in all mammal species.
- **The rapid detection tests** are becoming more and more available, not only in hospitals or labs, but also from pharmacies or even over the counter. In humans, specificity for IgG and IgM detection is around 90%, while sensitivity for IgG detection at 2 to 3 weeks after onset of symptoms is between 92 and 100%[90]. The tests are based on lateral flow immunochromatography, **and some of them are** using *Staphylococcus aureus* proteins A and/or G conjugate to reveal Ig G and Ig M. Those conjugates may function for numerous animal species Immunoglobulin detection, **but not all**. Hence, the literature must be consulted before trying to apply any kind of non-validated test to animals, and results would be of course without any established predictive values. [96]

As many animals already harbour other species-specific coronaviruses, the SARS-CoV-2 specificity of the test must be precisely monitored [95].

- RT-PCR test does not cross-react with other coronavirus (e.g. feline coronavirus), granting a good specificity.
- Note: available commercial tests for feline or canine coronavirus (ELISA) do not cross-react with SARS-COV-2

Treatment of infected animals

It is not recommended to treat seropositive but asymptomatic / PCR negative animals. In case of positive PCR without any clinical signs, we also recommend to hold off treatment. Asymptomatic animals (big cats) have been opportunistically screened as PCR positive for few days and never showed any symptoms.

- **Treatment** should be considered:
    - to alleviate clinical signs, mainly respiratory and digestive
        - Anti-emetic like Maropritant or Odanstron
        - NSAIDs like Meloxicam
        - Albuterol and O_2 supply for severe respiratory distress.
    - To avoid secondary infections, mainly bacterial and fungal (e.g. Mycoplasma)
        - recommended antibiotics for animals are of the class of macrolide, especially Azithromycin. This antibiotic has a good lung distribution, a proven ***in vitro*** activity against SARS-Cov-2[97], and there are current hypotheses about its antiviral propriety on RNA viruses, as well as immunomodulatory abilities that may reduce apparition of immune system overreaction such as cytokine storms[98].
        - Other antibiotics used as a synergy were: Amoxicillin + clavulanic acid, Ceftiofur and Enrofloxacin
- **Corticosteroid use**: in big cats with severe clinical signs and in Gorillas, glucocorticoids (GC) were used successfully. Based on histopathological observation of overwhelming inflammation in lung and upper respiratory tissue with low to no viral presence, a daily low oral dose of prednisolone or dexamethasone was used, in order to counteract this part of SARS-Cov-2 pathogenesis. GC use in humans is still debated but does show clear benefits in individuals with over-reacting inflammatory response. Anecdotally a single dose of parenteral CG appeared effective in severely affected tigers.
- **Antiviral drugs:** some references and dosages of antiviral drugs can be found in experimental reports in Non-Human primates[99] or rodents[100], but administration of these treatments are not currently recommended outside of experimental settings.
- **Chloroquine efficiency** : largely controversial in human protocols, it was assessed in old world monkeys (macaques and green monkeys) with lack of success either to treat infected animals or as pre exposure prophylaxis protocol [101]. Not recommended.
- **Ivermectin**: its efficiency on SARS-Cov-2 is also very controversial in humans[102]. It was claimed to be able to interfere with the virus itself in vitro, and also to dock with ACE2 receptors and then impair virus attachment. However, in one recent study on Syrian Hamster, while it is not decreasing the viral load or fighting directly with the virus, it may show some immunomodulatory effect on type I Interferon pathway that could bring a more favourable outcome in inflammatory responses in some tissue, e.g. lungs[103]. The injected dosage in those animal studies is 400µg/kg. So far, some treatment of SARS-Cov-2 animals added it to the drug panel, with no clear results if it was positive or not.
- **Monoclonal antibodies** (Mabs) were used in one gorilla out of the San Diego outbreak. This silverback had previous medical heart conditions and displayed pneumonia, so he received antibiotics and monoclonal antibodies, with clinical success. The use of these Mabs can be indeed done across different species with well-designed protocols. But it should be clearly assessed from both ethical and availability point of view beforehand, and that will vary with time, country and even local constraints.
- **Heparin.** Used in human medicine to counter pulmonary thrombosis[104,105]. Anecdotally a single dose of heparin appeared effective in severely affected tigers.

Animal Vaccination

- Vaccines against several other coronaviruses are already available in veterinary medicine for some species[106] :
    - **Canine coronavirus:** inactivated and live modified vaccines exist. They do not protect from infection but are aiming to reduce signs of disease (mostly diarrhoea).
    - **Bovine coronavirus:** vaccines are known to greatly reduced signs intensity and duration in case of infection.

  - **Porcine coronavirus:** one vaccine exists against Transmissible GastroEnteritis (TGE), but the prevalence of this form is declining everywhere, so that vaccine is not really used anymore.
  - **Feline coronavirus:** FIP vaccine, unlike the other animal coronavirus vaccine, is not designed to produce antibodies, as it has been proven that the IgG antibodies are actually more harmful to the animals than the virus, leading to more severe signs and increased mortality. Thus, this vaccine is intended to provoke a local IgA protection (intranasal) in order to prevent virus invasion only.
  - **Avian coronavirus:** vaccine against Infectious Bronchitis. Reversion of virulence from the live attenuated strains used is a risk and therefore should use in wild birds is not recommended.
- Although there is a current debate in human medicine about the relevance (or its absence) of cross immunity between SARS-CoV-2 and other coronavirus[107,108], this is yet not applicable to wild animals. The use of one of the existing animal corona-vaccines is not recommended and can present a disease risk for wild species as most of the labelled vaccine are live attenuated ones for domestic animals.
- Human vaccines against COVID-19 are still under development in several countries. As of December 2021, more than 90 vaccine candidates were still under development. An excellent graphical guide to the diverse types of vaccines being developed can be found here. At the time of writing, they are more than 24 approved / authorized vaccines already applied in human populations all over the world. Among them:
  - 2 are mRNA vaccines
  - 10 are inactivated vaccines
  - 5 are adenovirus-based vaccines: one using a chimpanzee adenovirus, the others are using recombinant human adenovirus
  - 3 are based on a synthetic peptide

  Although several of these vaccines were applied on NHP during the course of their development, human vaccines should not be used in zoo animals, for both legal and ethical reasons. Several animal vaccines are now available.
- Few veterinary vaccine are on the market worldwide and they're mostly designed to target carnivores : dog, cats, and fur industry carnivores (mink, foxes). Russia registered the "Karnivac-Kov" in 2021 (inactivated vaccine), Zoetis developed his own vaccine quite early (recombinant based on S peptides), and Finland is currently injecting some captive minks with "FurcoVac". None of the vaccines are labelled for zoo species, their recorded target species are carnivores, based on OIE recommendation that suggest vaccination as one of the biosecurity tool in order to decrease virus circulation in farmed fur animals.
- Several US zoos started vaccination campaigns in 2021, mainly in carnivores and NHP species, using the Zoetis recombinant vaccine.
  - **Protocol**: primary vaccination is done with two injections 21 days apart. If the animal was previously infected, the recommendation is to wait at least 60 days after remission.
  - **Safety**: first results shows good safety, with minor side effects such as transient lethargy or vomiting after the 2nd injection.
  - **Efficacy**: it is too early to assess the true real protection of animals. Serological titres are starting to be collected and already show that carnivores seroconvert with higher titres than NHPs. Some apes still show weak seroneutralisation titres few weeks after the 2nd booster, while *Panthera* species seroconverted as expected in dogs and cats (titres >1:512) (*B.Nevitt, EAZWV/AZV joint meeting, 2021*).
  - In one zoo, there has been evidence of SARS-Cov-2 infection in big cats occurring during the course of primary vaccination and leading to disease, likely demonstrating that, like in humans, immunity status reached after the first injection is not protective.
- It should be noted that the veterinary vaccines mentioned so far are not complying to the DIVA (Differentiate Infection from vaccinated animals) strategy which is for instance required for avian influenza vaccines. Thus, serologic surveillance of viral circulation may be impaired by implementing vaccination. Theoretically, one could probably differentiate as all vaccines are targeting the Spike protein, so presence of antibody directed against Nucleocapsid elements would mean real infection, but this dichotomy has not been assessed yet[109].
- As for all other infectious diseases for which vaccine is available, the use of vaccine tool should be decided based on a throughout local risk assessment: even if safety appeared to be good, vaccination implies manipulation of animals, especially when non trained, with 2 to 3 injections and serological monitoring: this could represent a lot of risk, stress and restraint hazards that should be weighted compared to the real viral exposure risk, comorbidity factors of individuals, etc.
- Based on the currently available knowledge, in a European setting, the authors do not consider Covid-19 a sufficient

threat to zoo housed animals to warrant vaccination of zoo animals. The possible exception at this point might be snow leopards, but this is still under investigation (see above).

Free Ranging Wildlife

While there are several published papers about predictive[44,110,111] or experimental[73] sensibility or hypothetic scenarios regarding wildlife, so far proven involvement of wild animals in the epidemiology of this human disease is minimal, with the exception of the current US study on white tail deer.

- In Summer 2020, there was one report of a wild American mink in Utah, found with high serologic titter against SARS-Cov-2, but likely escaped from nearby mink farm[112]
- A review of Danish mink farms outbreaks[39] included a survey of wildlife surroundings the positive farms. All sampled wild carnivores (foxes, polecats, badgers), including feral cats were negative. In birds, RNA from SARS-CoV-2 were found on the feet of seagulls, while feathers and cloaca were negative. All other birds were negative as well. Interestingly, pooled sample from flies caught in flytraps revealed very low level of viral RNA.. The variant RNA was exactly the same that the one found in the nearby mink farms. Considering known escape abilities of this species, there is a strong suspicion that this animal is actually a captive released one.
- A recent publication titled, 'Exploring the potential effect of COVID-19 on an endangered great ape' Colchero et al. 2021, in Scientific Reports failed to meet basic scientific criteria. It grossly misrepresented what we know about great apes as a biological system and what we know about COVID-19 in humans. Extrapolating from humans to any other species is extremely difficult. It is also not really helpful as the susceptibility and the immune profile of a wild gorilla population is entirely unknown. However, the simple modelling exercise presented in this paper with multiple solutions is woefully inadequate and the conclusions misleading, by disease modelling standards and for the high-quality research expected of a peer-reviewed nature.com article. The paper has been retracted by the authors after being made aware of the shortcomings as it can be read on this link
- Regarding felids in the wild, and especially tigers, several warning messages were issued since April 2020, in order to reduce human / felid interface, in national parks and sanctuaries. Although fake news on wild tigers being dead with respiratory signs due to SARS-Cov-2 in India could be read online. While one dead tiger, named "T21" in Pench National Park, India, was suspected to be SARS-CoV-2 positive, it was eventually confirmed negative and died from bezoar occlusion followed by secondary pneumonia. To date, there is only one felid case recently (12th of January 2022) reported in the wild, a leopard in India described as found dead and infected with Delta variant in a paper currently in preprint[86]. In this report, animal died because of trauma, but SARS-Cov-2, antigens were found into lung tissue, spleen and also glial cells & endothelium of brain tissue.
- A screening within free ranging wildlife species in Croatia was performed from June 2020 to February 2021[113] and lead to 2.8%) positive ELISA results were detected; in wild boars (3.9%), red foxes (2.9%) and jackals (4.6%). However, the positive findings were not confirmed by sVNT. No viral RNA was found

Focus on Cervidae

At the time of writing, one wildlife deer species is raising concern: the White tail Deer (WTD) *Odocoeilus virginianus* in North America. The north American wild population for this species is more than 30 million animals, with a really strong anthropic population management.

- The sensitivity of this species to SARS-Cov-2 has been experimentally assessed, showing that fawns could be infected, could show clinical signs and organ lesions, and that they could transmit the virus to contact animals[114]
- Moreover, vertical transmission from dam to foetus was also experimentally produced, with an additional noticeable effect of Alpha VOC able to outcompete the historical Wuhan strain in this species [115].
- In feral population of WTD living in peri urban settings, first seroconverted animals were reported from a survey including 385 animals from 4 states (New York, Michigan, Pennsylvania and Illinois), with antibody to SARS-Cov-2 were found into 40% of the samples collected from January to March 2021[116]. Another study ran on Texas on 54 deer sampled between January and February 2021 revealed 37% of seropositive animals. In both studies, samples of the pre-pandemic periods were also assessed with no positive results[117].
- Eventually, RT-PCR positive animals[87] (on nasal swab samples) were reported in retrospective screening of samples taken during the September 2020 - February 2021 period in Ohio. 35.8% of swabs were PCR positive, and several variants were retrieved, with authors bringing the hypothesis of 6 different spatiotemporal event of human to deer passage. Sequencing of animal virus showed some amino acid substitutions within the Spike protein, that could suggest deer to deer transmission.

- Another study[118], still in preprint, is now reporting virus detection by RT-PCR in retropharyngeal lymph nodes of animals collected **between April 2020 and January 2021 in Iowa**, from both captive and wild settings. These samples were collected as part of a monitoring strategy on Cervid Wasting Disease. Again, the ratio of positive animals was around **33%,** but it was noticed to be higher (82%) when selecting sample between November 2020 and January 2021. A similar study was conducted in **Ohio** on WTD samples collected between **January and March 2021**[87], leading to the same prevalence of **35.8%** of RT-PCR positive animals. In this study, 3 different strains were found in deer, matching the current strains widespread in humans in this region at this time (mainly B1.2) and authors are also strongly supporting a deer-to-deer transmission according to profile of retrieved strains.

Several other countries thus initiated similar studies on their native Cervidae species, e.g. roe deer, fallow deer, red deer and reindeer for several European countries (UK, Sweden, Germany, Austria…). First unpublished raw results show no positive animals from the retrospective pandemic and pre pandemic periods. At this stage, this could be related to different factors such as specific ACE2 binding profile, population distribution into strongly anthropogenic environment, etc.

Zoo Context

EAZA public statements relating to SARS-CoV-2 can be found here: https://www.eaza.net/latest-news

Operational best practice documents for zoos are being continually updated and are available on the EAZA Member Area of their website: here

Is there any risk of transmission between animals?

- It has been proven experimentally in a lot of species that transmission can occur by direct contact [74,94,119] (e.g. orofecal route), but also without direct contact, by aerosol[120] / droplets, with distant contamination between individuals separated by a mesh fence [94,121]. However, the only intra specific efficient transmission example seems to be the mink in farm and hamsters in experimental settings, with animal density far higher than any zoo settings.
- In zoo settings, transmission between animals has been suspected but not yet proven, because the animals belonging to the same outbreak were usually exposed to the same infectious source (e.g positive keeper).

Is there a risk of transmission from visitors / keepers to animals?

- According to the current knowledge, between 50 and 75% of known zoo animal infections were linked to a positive caretaker (sick or asymptomatic). Therefore, close contact between high profile animals (i.e., carnivores and Non-human primates) and infected / suspect humans with COVID-19 should be prevented. The same social-distancing guidelines as between humans should be applied between human and animals (now recommended at 2 meters min. since variants occurrence).
- Individuals handling or caring for animals should implement the following basic hygiene measures, applying to both visitors and keepers:
    - Prevent contact with animals when ill.
    - Wash hands thoroughly before and after handling animals, their food, or supplies (e.g. enrichment items)
    - Avoid any close contact like “kissing” or petting (especially without gloves).
    - Wear FFP2 mask and appropriate PPE when minimal distance cannot be achieved (e.g. clinical exam under anaesthesia or medical training events).
- Regarding great apes, there are already a number of guidance documents:
    - One from EAZA great Ape TAG Vet advisors.
    - One from AZA / ZAHP Fusion Center.
    - Great apes, COVID-19 and the SARS CoV-2: Joint Statement of the IUCN SSC Wildlife Health Specialist Group and the Primate Specialist Group, Section on Great Apes.
    - The Ape Emerging Disease Management HUB: https://umnadvet.instructure.com/courses/324

Reassuring Statements about risk of transmission from zoo animals to visitors / keepers (for Media)

- Zoo animals are under veterinary care, including ongoing monitoring of infectious diseases. For some particular species, screening for some coronaviruses is already part of entry requirements (e.g. FIP in some Felidae) or readily looked for when any clinical signs are noted (e.g. diarrhoea in young bovids).

- Of the 1200 to 1400 extant **chiropteran** species, less than 30 are found in EAZA zoos. The species of chiropterans that are **mostly** involved with coronavirus (like Asiatic horseshoe bats or other small insectivorous species) are not kept within European zoo collections, which focus mostly on flying foxes. Egyptian fruit bats were able to be infected experimentally (see Table A.) but were asymptomatic and were not able to infect their cage mates.
- The environmental, sanitary and welfare conditions of zoo settings cannot in any way be compared to conditions in wildlife markets. Zoos employ exemplary hygiene and sanitation practices, excellent holding conditions adapted to the species' needs and daily monitoring of all animals in their care.
- One may be scared that animals infected by keepers could spill-back transmission to keepers/visitors. According to the few examples of viral load excreted by domestic animals naturally infected by human begins (domestic cats), the subsequent dose of excreted virus appears very low and are likely lower than the minimal infective dose. This zoonotic risk is considered as very low by several national health agencies (SciCom in Belgium, ANSES in France, USDA in USA..), FAO[122] and OIE, and even 4 months after the first positive cat and dog discovery, there is still a scientific consensus that carnivorous pets, despite their proximity to humans, are not playing a role of reservoir or spill over [123].
- Hence, the risk of incidentally infected wild captive animals shedding enough virus to infect keepers and visitors must be considered as even lower still in view of the greater distance between humans and zoo animals when compared to pets.

How to prevent transmission in a zoo context?

- Infective media are mainly saliva, aerosol and faeces. Urine, tears and blood were also found to contain virus at certain stages both human and animal disease, but with lower virus load compared to oronasal fluids. In experimentally infected ferrets, virus was found in urine until day 8, but with lower loads than nasal washes or faecal samples[94]. Therefore , prevention measures should focus on saliva, aerosol and feves, whether coming from human, animal or environment.
- American colleagues noticed that more than half of the reported cases are directly link with a positive keeper, sick or asymptomatic, that was working close to the infected animals. However, in one out of 4 cases, all staff was screened as negative around the time of infection. This should raise a question on other routes of infection or transmission that maybe not yet described.
- Special attention should be paid to enrichment, especially made out of anthropogenic items (e.g. plastic bottles, shipping boxes etc.) that were in prolonged contact with human beings particularly outside of the zoo. Disinfection should be applied, and if not applicable, a resting period of several days (see below "Stability of virus") is recommended, to allow the surface viral load to decrease.
- Caution should be paid to the fact that some references refer simply to RNA or genome detection, whereas other focus on actual tissue culture infective dose. Obviously, the latter are more relevant. With now two years of feedback on transmission studies in human, aerosol and droplets seems to be the main route of transmission while surface/fomite contamination role is likely very low[124]. Surface or hand became relevant routes when in contact with fresh contaminated droplets and the in contact with face (nose and eyes).
- Apart from keeping the staff informed and promoting social distancing, additional tools that can be used in zoo setting to increase level of monitoring, include wastewater analysis: if the zoo has one only sewer collecting output draining all human and animal wastewater before it is mixed with other effluent and before any secondary treatment, then wastewater can be sampled and screened for viral RNA. Any detection would mean circulation and shedding and then prompt further screening.
- Building / indoor ventilation is utterly important, lessons learned from hospital and other crowded human indoors space (planes, gymnasiums etc.). There has been a great amount of science produced about modelling fate and survival of SARS Cov2 contaminated droplets according to air changes per hour, fraction of outside air imported, quality of filters, etc… From different sources, the renewal volume should not fall under 7-10 vol. / hour and the fraction of fresh air should be kept over 0.3.[125] Use of filters are really relevant only when adding HEPA types (or even greater). UV treatment incorporated into air support system is also beneficial to reduce viral load and viral survival time.

Stability of virus in environment and disinfection

- Coronavirus are known to be able to survive and remains infectious in environment for hours and days[126].
- Like SARS-COv-1 and MERS-CoV [127], SARS-Cov-2 is likely inactivated by heat after **10 minutes above 56°C** [128] or within **less than 5 minutes at 70°C.** On the opposite, cold and negative temperatures are **not** a mean to decrease viral load as the virus survives to -14 to -18°C for 2 to 3 weeks[129]. Freezing was thought to play a role in spreading virus from infected meat plants to distant retailers / consumers; even if not proven, this long cold survival should be taken into account when reviewing contamination risk assessment at work with zoo animals (food preparation / storage)

- SARS-CoV-1 and SARS-CoV-2 seem to share the same propriety of stability on surface and in aerosols [128,130–132]
    - remaining viable in aerosol droplets for up to 3 hours.
    - remaining detectable on metal or plastic surface for up to 4 days, but their titres reduced a lot (e.g. from 10 to $10^{0.6}$ Tissue Culture Infective Dose / mL over 72h).
- The most efficient disinfectant are alcoholic compounds, but with appropriate contact time: propanol (100% or 70%) or ethanol (70%) for a minimum of **30 sec**. For other compounds such as quaternary ammonium or phenolic compounds, efficient contact time regarding coronavirus is usually **10 minutes.** Then, useful disinfectants are sodium hypochlorite (0.1% for 1 minute) and hydrogen peroxide (0.5% for 1 minute). Other usual disinfecting veterinary compounds like povidone-iodine 7.5% or chlorhexidine 0.05% are also inactivating the virus, but with longer exposure time (5 minutes) [128,130].
- Internal "control" validating disinfection protocols and their application in the zoo could be employed ensure efficiency. Assessing effect on virus persistence is not straightforward, but certain tools such as 'ATPmeter' could help evaluate the level of sanitization of surfaces and reduce viral load on fomites[133].
- Standard disinfection routines using sodium hypochlorite (0.5% on heavily touched surface, 0.1% on floor) in hospital rooms with positive patients were enough to obtain negative environmental samples in one study [134]. However, it should be noted that uncovered shoes were positive, as were ventilation exhaust outlets. Sodium hypochlorite stand out as an efficient and useful compound to decrease fomites epidemiological roles, as it is relatively safe for the environment and cheap to synthetize (NaCl electrolysis).

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