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 pre-COVID-19 scientific literature about coronavirus.

A massive amount of new science is becoming available daily [more than 97000 papers and counting at this date] but be aware to check 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). A lot of very relevant information such as species susceptibility or immunity across taxa is not yet available and will not be for months or years.

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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. Up to now, the virus spread globally with more than 115 million human cases in more than 192 countries at the time of writing this text. More than 2.5 million deaths are reported. 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.[1] One month later, it was also declared as a notifiable disease by O.I.E. when SARS CoV 2 was found infecting an animal.

Questions / Answers

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

Coronaviruses in general

Is coronavirus usual in wild species / Zoo animals?

- Yes, coronaviruses are very common in Mammals and Birds [2]. They are not always associated to 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 [3]
    - Alphaconoravirus: mainly found in bats, but this group also contains:
        - The Feline Coronavirus FeCov with its two forms (FeCV and FIP) [4]
        - The canine coronavirus type I and II [5]
        - Human viruses like HCov 229-E, often a component of the common cold
    - Betacoronavirus: most represented in mammals, from carnivores[6] to hoofstock[7891011] from hedgehogs[12] to bats. It also contains the 3 more recent emerging coronaviral diseases:
        - subgenera Merbecovirus; MERS CoV[13]
        - 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[14]
    - Deltacoronavirus: mostly avian species specific coronaviruses[14] , and some porcine one, recently recovered from leopard cats [15]
- Chiropterans are well known to be host of many viruses, including various coronaviruses at the same time [16,17]. 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.

What kind of disease does coronavirus provoke?

- Coronaviruses can infect several categories of somatic cells, but they often invade epithelial cells, especially those of the digestive mucosa and/or respiratory tract. Because of this tropism, the resulting diseases mainly fall into two groups:
    - Diarrhea and intestinal disorders (example seen in bovine calves, sometimes in association with rotavirus)
    - Respiratory syndromes, either from upper tract (like common cold) or deeper like bronchopneumonia.
- SARS-Cov-2 seems to have additional tropisms in humans:
    - a neuroinvasive potential, e.g. leading to the signs of anosmia and dysgeusia in humans, and rarely encephalitis.
    - cutaneous manifestations like skin rashes.
    - Other miscellaneous signs, rarer, like hairs loss, conjunctivitis, discoloration of toes or fingers.

- In animals, there are usually large difference regarding severity of signs according to the age. Neonates and young animals are prone to exhibit more heavy forms of disease, sometimes fatal, while adults are often showing less intense signs and able to recover faster.[5]

Can the coronaviruses be transmitted from Animal to Human?

- Generally, coronaviruses are species-adapted, and transmission from one species to another is rare. Only a few described species of coronaviruses have shown a broad host species range that includes humans:
    - SARS-CoV (Human, civet cats, racoon dogs, horseshoe bat, swine)
    - MERS-CoV (Human, bats, hedgehogs, camels)
    - Bov-CoV (Cattle, wild ruminants, camelids, dogs, and occasionally humans)[2]
- Transmission does not necessarily mean disease. Most of the time, when transmission to another species occurs, only subclinical disease in seen in the new hosts (unlike COVID-19 in humans).
- Viruses in general lack the regulation mechanisms avoiding / fixing copy errors of the genome in animal cells. Hence, mutation rates are of larger magnitude which explains that they can adapt to new host in (relatively little) time. However, it has recently been shown that some coronaviruses are capable of some replication regulation under certain environmental circumstances, which make them more complex adaptors.
- Coronavirus mutation rates are not greater than in most other viral families. However,
    - RNA viruses are more susceptible to mutation than DNA viruses.
    - Coronavirus RNA is longer than that of other RNA viruses, increasing the likelihood of copy incidents compared to viruses with shorter nucleic acids.
- Recombination ability is also an important feature of coronaviruses, well studied under the SARS outbreak in 2002. Coupled with mutation, this allows adaptation to occur (e.g., receptor binding ability, temperature adaptation enzymes) in a shorter time period, than for other viruses. Addition of these mechanisms is thought to be one of the main drivers of selection for this new coronavirus,[18] but also may be a future threat in case of recombination between two sarbecovirus like SARS-Cov-2 and MERS-Cov.[19]

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 was previously detected in the intermediate horseshoe bat (*Rhinolophus affinis*) from southwest China's Yunnan Province [20]. Recent papers revealed other very close sarbecovirus, that were found in 2020 from stored frozen sample of other bat species out of China:
    - from 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 Thaliand[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.
- There is 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: the SARS-CoV-2 RBD is adapted to receptors ACE2 which allows it to enter human cells, while Bat-CoV-RaTG13 is not. However, bats betacoronaviruses with ability to link human ACE2 and enter human cells are known from fields screening back in 2013 as shown in[24(p2)].
- 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 [25]. 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 [26,27].

- A reviewed hypothesis [28] 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, contrasting suggested preadaptation to the human host.
- As horseshoe bats were hibernating at the time when COVID-19 appeared in China, there is general consensus that the SARS-CoV-2 did not come directly from bats, but is of ancestral Bat-CoV-RaTG13 origin[29], requiring an intermediate / amplification host with reassortments in the RBD region to invade human cells. Due to lack of data, all this is speculative at this stage.
- Spillover events that occurred in mink farms in many countries recently raised the hypothesis of *Neovison* being the missing intermediate host, because of apparent high sensitivity of this species. This hypothesis is now investigated as there are strong evidence of the virus circulating inside and maybe outside before the Wuhan outbreak[30], at least in fall 2019 or even earlier. A WHO delegation in China is supposed to explore this scenario of an earlier origin of the virus, and the likelihood of links with farmed animals in Asia (American minks, racoon dogs etc.).
- New studies are becoming available that further attempt to predict the zoonotic capacity of mammal species for SARS-CoV-2. Particularly interesting are those studies that combine multiple approaches such as ACE2 sequences, 3D structural binding analysis with experimental data. While still at a preprint stage the recent work by Fischhoff et al.[31] is worthwhile for those who want to dig into the details of SARS-CoV-2 susceptibility across species.

Why did COVID-19 break through the species barrier? Can it happen in the Zoo?

- For a virus to make this kind of leap, a number of factors have to line up: Infected animal, infectious secretions, very close contact and possibly repetition in time [32].
- Time is also a very important factor: several genetic retrospective and phylogenetic studies agree that SARS and MERS emergence are linked to several decades of continuous proximity[33], allowing several mutation and recombination event to occur consecutively. Regarding SARS-CoV-2, there are different hypothesis about the amount of time needed for all recombinations to occur from Bat-RaTG13, some analysis considering it as not recent[34], while others states that strong selection in different host species and frequent recombination can lead quickly to new emergences of host adapted lineage[35].
- Through recombination, the new SARS-CoV-2 has acquired the molecular abilities to enter human cells, while the ability to infect other animal species under certain circumstances is not yet elucidated.
- Wildlife markets provide a unique occasion for interspecific transmission[36]:
    - Poor hygiene – slaughter.
    - Stressed animals likely to shed a lot of virus.
    - Continuous close and crowded contact between multiple live species unlikely to meet in the wild.
    - Close proximity to livestock, poultry and domestic animals.
    - Wildlife used as small household pets or slaughtered on-site and subsequently eaten, sometimes raw, promoting intimate contact between virus and host ‘s intestinal tract.
    - Increase of viral load along the food value chain from capture to restaurant.
- Conditions within zoo settings are very different:
    - Good hygiene practice.
    - Welfare of animals minimizing stress.
    - Monitoring and active surveillance of animal health, veterinary observation, screenings.
    - Predominantly captive bred animals.
    - No human consumption of wildlife.

COVID and domestic carnivores

Table A; Extant knowledge about domestic Carnivore species sensitivity to SARS-CoV-2 from [25,37–39]

N/A= not yet assessed

Species	**In Vitro Viral Particle entry**	**Computer & molecular prediction of ACE2 receptor binding**		**In vivo experimental infection success** *(blank: no data yet)*	**Natural transmission (Human > Animal)**
		From [25]	**From** [37,38]		
Domestic Dog *Canis familiaris*	YES	Likely (3/5)	Low (19/25)	No positive PCR but seroconversion Beagle dogs infected with the same viral load than cats showed neither clinical signs nor viral RNA in any organs or tissue. Only rectal swabs were positive [40]	Yes
Domestic Cat *Felis catus*	N/A	Likely (3/5)	Medium (21/25)	Yes + transmission to other cats	Yes
Ferret *Mustela putorius*	YES	Likely	Very low (17/25)	Yes + transmission to other ferrets[41,42]	Within one domestic setting, no infection occurred from infected humans to domestic ferrets ; [43] More recently (same author), one infection was confirmed in household

Domestic Dogs, Cats & Ferrets

- Several somewhat detailed case reports of "positive" **domestic** carnivores were described since March 2020. One common feature is that in all cases, the pets were usually kept and cared for by positive and shedding owners.
- First dog in Hong Kong (Pomeranian, 17-year-old), living with COVID-positive and sick owner, had weakly positive PCR results on nasal and oral swabs (repeated 5 times), while fecal samples remained negative. At first serology was negative, but a second one was reported as positive by the Hong Kong Health Dept[20]. The dog died from geriatric renal and cardiac failure that was reported as unrelated to SARS-Cov-2, but the owner denied necropsy.
- Second dog (German shepherd, 2 years old), living with COVID-19 positive and sick owner. Only one test in which nasal and oral swabs were PCR positive. No symptoms. This animal was placed in quarantine with another 4-year old dog, that remained negative. No further information on serology.
- Cat in Belgium (March 2020) After one week living with in infected owner (who had returned from Italy), the cat showed signs of illness compatible with coronavirus signs: anorexia diarrhea, vomiting and cough. RT PCR was positive for SARS-Cov-2 on gastric lavage and feces, with rather high viral RNA copies. Nine days after onset of clinical signs, the cat's health started to improve, until the condition resolved.
- Cat in Hong Kong: (March 2020) a domestic short-haired cat, when owner was confirmed with COVID-19, the cat was sent for quarantine at a state **animal-keeping facility**. Oral, nasal, and rectal samples tested positive for the virus. The cat has not shown any signs of disease.
- Several additional cases of positive cats (Germany, France, USA, Moscow, Hong Kong, Spain and U.K.) have been described. All these cases showed mild digestive and respiratory signs and recovered uneventfully. A very limited number dogs and cats are reported as dead when they tested positive (Antibody a,d/or PCR) but on almost all cases, another cause was the real lethal etiology (e.g. lymphoma, brain tumor..).
- More positive cats were found on a study near infected mink farms in the Netherlands. Out of 24 cats sampled surrounding the 2 first infected farms, 7 were detected as seropositive and only one cat was PCR positive for viral RNA. However, feral cats surveyed around recent mink farms outbreaks in Denmark were found negative[44].

- Among all positive reported cats and dogs, the majority of animals did not show any clinical signs [45]. When present, the signs are mainly digestive (diarrhea, vomiting, dysorexia) and/or respiratory (cough, sneezing, conjunctivitis, pneumonia), and usually self-resolving after few days.
- In a study [46] from Wuhan, China which examined 39 pre-COVID-19 outbreak [serum bank] and 102 post-outbreak domestic cat serum samples [animal shelters or pet hospitals] with an ELISA targeting the receptor binding domain (RBD) of SARS-CoV-2, 15/102 post outbreak sample were positive. Of the 15 samples, 11 also had SARS-CoV-2 neutralizing antibodies with titers ranging from 1/20 to 1/1080. No serological cross-reactivity was detected between the SARS-CoV-2 and type I or II feline infectious peritonitis virus (FIPV). Three cats owned by COVID-19 positive owners had the highest titers, indicating that the high neutralization titers could be due to the close contact between cats and COVID-19 patients.
- More than 4000 dogs, cats and horses were screened in infected areas in South Korea and the United States by RT PCR produced by Idexx Lab. None were found positive.
- At the time of writing, there are less than 90 cases notified to national agencies all over the world for domestic carnivores. Their role in epidemiology and circulation of virus is still considered negligeable by national and world expert groups. Pets may play a role in the local persistence of SARS-Cov-2 in domestic households in which case measures to prevent contact and handling must be implemented at the domestic level of family core; but at a larger scale, since there is no viral adaptation to these species, and they are not acting as reservoirs or major spillover hosts.
- Three papers [41,42,47] show that ferrets and domestic cats are at least somewhat susceptible species as they are able to be experimentally infected, shows clinical signs from mild (cats) to more severe (ferrets), but also to excrete enough virus for efficient transmission to cage mates. In contrast, dogs seemed to allow minimal replication, while chickens, ducks and pigs were apparently not susceptible [42].
- Susceptibility of ferrets and cats has been proven, but much remains to be determined about what makes them more susceptible than dogs. Actually, ACE2 sequence homology with human one is thought to be one major factor for feline susceptibility (85.2% sequence identity. On the other hand, this criteria cannot explain ferret susceptibility as its sequence identity (82.6%) is the same as the non-susceptible rat (82.5%)[48]. Other factors such as respiratory anatomy and physiology, as well as immunity pathways must then also play a great role.
- Another factor that could explain the higher susceptibility of cats compared to other species is that SARS-Cov-2 target cells are widespread in nearly all organs when compared to other species like chickens or pigs[49]
- The difference between experimental and natural infection should be noted. Although ferrets are easily infected and can transmit virus between each other in experimental settings (with a high viral load infective dose), the epidemiological pattern in domestic settings seems very different, with recued contamination of ferrets housed as pets in a human contaminated family household[43]
- Sampling domestic pets should be done according to context and national official veterinary recommendations, as it remains very hard to differentiate between a passive carriage from pets acting like fomites, and actual infection. A real epidemiological role of dogs and cats is unknown, but likely minimal. Viral loads were always found transiently, resuming to zero with days/ weeks, and they were found in anatomical location compatible with passive contamination (animals with nose near owner, licking and swallowing virus from sick owner skin or environment). The very low number of documented cases despite massive pet-ownership and ample interest likely indicates that pets play a no role in the current pandemic, except a very anecdotal one.

COVID and non-domestic carnivores

Table B; Extant knowledge about non domestic Carnivore (order) species sensitivity to SARS-CoV-2 from [2537–39]

N/A= not assessed yet

Species	In Vitro Viral Particle entry	Computer & molecular prediction of ACE2 receptor binding		In vivo experimental infection success *(blank: no data yet)*	Natural transmission (Human > Animal)
		From [25]	**From** [37,38]		
Tiger, Lion *Panthera leo* *Panthera tigris*	N/A	N/A	Medium (21/25)		Yes [50–52]
Puma *Puma concolor*	N/A	N/A	Medium (21/25)		Yes (OIE, 12.08.2020) USDA 2021

Snow Leopard *Panthera uncia*	N/A	N/A	Medium (21/25)		Yes (OIE, 18.12.2020)
American mink *Neovison vison*	N/A	N/A	Very low (14/25)		Yes[44,53,54] + Animal to Human transmission[55]
Polar bear *Ursus maritimus*		Low[56]			
European mink *Mustela lutreola*	N/A	N/A	Very low (17/25)		Reported but likely mismatched with European farmed American mink
Meerkat *Suricatta suricata*	N/A	Unlikely (2/5)	Very low (15/25)		
Civet cat *Paradoxyrus hermaphoditus*	N/A	Likely			
Masked palm civet *Paguma larvata*	N/A	Very low	Very low (13/25)		
Racoon *Procyon lotor*	?	Unlikely (2/5)			
Racoon Dog *Nyctereutes prycionides*	?	ACE2 matching also proven in [57]		Yes	
Fossa *Cryptoprocta ferox*	N/A	N/A	Very low (16/25)		
Red panda *Ailurus fulgens*	N/A	N/A	Very low (13/25)		
Sea otter *Enhydra lutris*	N/A	N/A	Low (17/25) [37,38] Very high [56]		
California SeaLion *Zalophus californianus*		Low[56]			
Harbour Seal *Phoca vitulina*		High[56]			
Walrus *Odobenus rosmarus*		Very High[56]			

- Carnivores are the mammal Order showing the highest number of non-experimental proven infections. Among them, mainly 3 species stand for most of the reports: domestic dogs, domestic cats and American mink. However, sampling bias has not yet been investigated but can be assumed due to the preponderance of cats and dogs in close contact to humans.
- It should be noted that in the tables above, some information concerning species sensibility such as ferrets or mink may be contradictory between molecular receptor prediction ("*in silico*" studies) modelling and actual observed infections from the field (in captivity). Thus, interpretation of taxonomic sensibility to SARS-CoV-2 at this point in time should be very cautious. Regarding captive wildlife, deer species and anteaters seem to be species to be particularly aware of ("high profile" species in [37]), especially as there are opportunities for close encounters between humans, deer (in children farms), and anteaters (keepers training/feeding).

Details regarding non-domestic Felids:

- On the 5th April 2020: The Bronx Zoo announced that one Malayan tiger had tested positive for SARS-CoV-2. Another Malayan, 2 Amur Tigers and 3 African lions had mild respiratory symptoms and developed a dry cough. The result of qPCR for the tiger samples (respiratory, fecal and serum) was confirmed by USDA's National Veterinary Services Laboratory, based in Ames, Iowa. At the time, New York City was experiencing massive human circulation and transmission of COVID-19. At the Bronx Zoo PPE for the keepers was implemented and the use of pressure hoses for cleaning stopped. The use of PPE to protect animals in a zoo setting must be very carefully weighed against the needs of front-line human medical staff. On the 22nd of April, fecal rRT-PCR testing identified viral RNA in the feces of symptomatic tigers (3 animals) and lions (3 animals); as well as an additional asymptomatic Amur tiger in the same facility as the other tigers. The academic partners will continue rRT-PCR testing of fecal samples to help understand how long the RNA can be detected. This test detects viral RNA and does not confirm shedding of infectious virus. All eight cats are doing well. They are behaving normally, eating well, and only the original affected tiger still has an occasional cough. Similarity of sequences are confirming the hypothesis of infected keeper (either asymptomatic carrier or pre-symptomatic) as a source virus transmission for the first tiger[50,52].
- On the 24th April 2020, Miami Zoo performed RT PCR tests on two Sumatran tigers showing ocular, nasal discharge and loss of appetite. Results were negative.
- During the 2nd wave of human contamination (starting in fall 2020), several other positive felids from genus *Panthera* were reported in USA (Tiger in Knoxville Zoo, Snow Leopard in Lousville Zoo) and in Europe (Lions in Barcelona Zoo, Lion in Tallin Zoo, Tigers and Lions in Swedish zoo, Prague Zoo). Captive Mountain lion cases were reported in South Africa, and more recently in Texas. In some French zoos, circulation of virus in lions and tigers was demonstrated by opportunistic positive serum samples and positive antigenic rapid test from nasal and rectal swabs, mostly associated to non-symptomatic animals. There was one case of euthanasia of a positive tiger, but attention should be paid to the real cause of euthanasia of this 17 year-old female, which is welfare because of respiratory and neurological signs were adding to current age related pathologies and compromising recovery chances according to the initial report.
- At the time of writing , there's only one case of positive juvenile wild cats, with 2 white tigers cubs reported dead with lung lesions alleged link to SARS-Cov-2 at Lahore Zoo in Pakistan, because they were hand reared by staff declared as positive, and because necropsy revealed lung and tracheal hemorrhages. However, no PCR test were performed.

Details regarding 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 analyzed 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. Initially three countries reported mink farm contamination:
  - **Netherlands**; on the 18th of June ,15 farms were contaminated. One month later, the number increased to 25 with only 8 reporting clinical signs. At the time of writing, there are now 33 infected farms. All animals from infected farms are culled (=1.5 millions 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 t 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 tocontain 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[55].
  - **Spain**; 1 farm was contaminated, at which 7 staff members were found PCR positive. All 93.000 minks were culled, and the positive proportion of samples was reported as high as 80%.

- In the second half of 2020, a lot of other European countries reported positive mink farms; 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 got outbreaks in farms: Utah (with 6 different farms between September and November), Michigan, Oregon, Wisconsin (3 farms). The strategy regarding culling differs between countries.
- 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 10-15%. 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. Some member state "used" this occasion to definitively terminate mink fur farm business, that was already scheduled for forthcoming years (e.g: Netherlands).

Details regarding Pinnipeds 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[58] , even free chlorine concentration of 0.5mg/L may not be enough to remove SARS-Cov-2 from water.

COVID and non-human primates (NHPs)

Table C; Extant knowledge about Non-Human Primates species sensitivity to SARS-CoV-2 from [2537–39]

N/A= not assessed yet

Species	**In Vitro Viral Particle entry**	**Computer & molecular prediction of ACE2 receptor binding**		**In vivo experimental infection success** *(blank: no data yet)*	**Natural transmission (Human > Animal)**
		From [25]	**From** [37,38,59]		
Coquerel sifaka *Propithecus coquereli*	N/A	N/A	High (24/25)		
Blue eyed black lemur *Eulemur flavifrons*	N/A	N/A	High (22/25)		
Aye Aye *Daubentonia madagascarensis*	N/A		High (24/25)		
Cheirogalus *Cheirogalus medius*	N/A		Low[59]		
Cynomolgus monkey *Macaca fascicularis*	YES	Likely (5/5)		Yes[60]	
Rhesus macaque *Macaca mulatta*	N/A	N/A	Very High (25/25)	Yes[61–63] and reinfection could not occur at T0+28 days new challenge	
Anubis baboon *Papio anubis*	N/A	Likely (5/5)	Very High (25/25)		
African Green Monkey *Chlorocebus aethiops*				Yes[63–65] Cytokine storm is seen in this species, like human and unlike Rhesus Macaques	**Yes**[66] Prescreening procedure in experimental animals revealed one monkey already exposed to European SARS Cov2 strain
Orangutan *Pongo pygmaeus*	N/A	Likely (5/5)	Very High (25/25)		

Chimpanzee *Pan troglodytes*	N/A	Likely (5/5)	Very High (25/25)		
Gorilla *Gorilla gorilla*	N/A		Very High (25/25)		**Yes** Animals signs; from transient lethargy to pneumonia. Monoclonal antibodies used in a male.

While non-human primates (NHPs), 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 NHPs is remarkably low, and include one African green monkey[66] and a group of nine gorillas at the San Diego Zoo, USA. Moreover, several primates have been tested in a few European zoos, mostly by fecal 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 recent paper in preprint [67] is describes the theoretical sensibility of prosimians. 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 Madagscar in April 2020 were assessed for SARS-Cov-2 by Pasteur Institute and were negative.

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 C.)
- 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[68].

New World Monkeys

- Based on New World primate sensibility to SARS-CoV-1 [69], 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 predictions 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.

Apes

- Coronavirus transmission was previously proven from Humans to apes with HCoV OC43, one of the human coronavirus involved in the common cold [70], 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 fecal samples [71]. 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 (ivermectin reported with in vitro and in vivo anti SARS Cov 2 effect[72])

Sensitivity of other mammals and of birds

Table D: Extant knowledge about miscellaneous (non NHP, non-carnivore) species sensitivity to SARS-CoV-2 from [2537–39]
N/A= not assessed yet

Species	In Vitro Viral Particle entry	Computer & molecular prediction of ACE2 receptor binding		In vivo experimental infection success (blank: no data yet)	Natural transmission (Human > Animal)
		From [25]	From [37,38]		
Horseshoe bat *Rhinolophus sp.*	YES	Likely	Very low (17/25)		
Daubenton's bat *Myotis daubentoni*	NO	N/A			
Vampire bat *Desmodus rotundus*	N/A	Likely (4/5)	Very low (13/25)		
Egyptian Fruit Bat *Rousettus aegyptiacus*				Yes	
European Bats					
Swine *Sus scrofa domesticus*	Yes[73]	Likely (5/5)	Low (19/25)	On study failed to get positive PCR and seroconversion Infected animals and sentinel [73] One recent study succeed to infect animals[74]	
Cattle *Bos taurus*	NO	Likely (4/5)			
African elephant *Loxodonta africana*	N/A	Unlikely (3/5)	Low (18/25)		
Camel *Camelus bactrianus*	N/A	N/A	Medium (21/25)		
Giraffe *Giraffa sp.*	N/A	N/A	Medium (21/25)		
Hippopotamus *Hippopotamus hippopotamus*	N/A	N/A	Medium (20/25)		
Alpaca *Vicugna pacos*	N/A	N/A	Medium (20/25)		
Reindeer *Rangifer tarandus*	N/A	N/A	High (21/25)		
White Tail Deer *Odocoileus virginainus*			High (21/25)	Yes[75] 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	
Manatee *Trichechus manatus*	N/A	N/A	Low[56]		

Giant anteater *Myrmecophaga*	N/A	N/A	High (21/25)		
Mouse *Mus musculus*	NO	Unlikely (2/5)	Very low (16/25)		
Rat *Rattus rattus*	N/A	Unlikely (3/5)	Very low (16/25)		
Chinese hamster *Cricetulus griseus*	NO	Likely (4/5)	High (22/25)		
Syrian hamster *Mesocricetus auratus*	N/A	N/A		Yes [76] Older hamsters exhibit more weight loss. Young animals launch earlier and stronger immune response.	
Deer Mouse *Peromyscus maniculatus*	N/A	N/A		Yes[77] Respiratory and intestinal viral invasion, but also presence of virus within the brain	
Guinea pig *Cavia porcellus*	N/A	Unlikely (2/5)			
Chinese Tree Shrews *Tupaia bellangeri chinensis*	N/A	N/A		Yes [78] Enter upper resp. tract, lungs, intestines and brain. No fatalities	
Chicken *Gallus gallus*	?	Unlikely (3/5)		No. Failed to get positive PCR and seroconversion	
Mallard duck *Anas platyrynchos*	?	?		No. Failed to get positive PCR and seroconversion	

- The ability of SARS-CoV-2 to infect other species has mainly been assessed by ***in vitro*** infection trial on various mammalian cells or by **computer simulated** prediction according to RBD / ACE2 receptors binding abilities / amino acid composition. Combination of these two approaches in 4 different studies provide the report in Table C above. However, the following should be noted:
- Great caution should be paid to all new papers and information released about animal species susceptibility to the virus[31,79]
  - 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 are performed on previously non receptive species , transformed in models like transgenic mice [80] 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 extreme viral loads).
- 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 harbor multiple other coronaviruses[81], mice and rats seem to be poor hosts for the SARS-CoV-2, as they lack the ACE2 receptor matching amino acids [25].

- 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 spillover, source of perpetuation and risk of reverse zoonosis[77,82]

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[83]. However, SARS-Cov-2 is a 30.000 bases long genome[84], making it the longest of the known RNA viruses.
- There are currently more than 400.000 SARS-Cov-2 genome sequences[85] uploaded on various public repositories platform, such as GISAID (Global Intuitive on Sharing Avian Influenza Data), and it should be outlined that nearly half of them are coming from U.K as they do have an intense activity in sequencing and reporting.
- 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, or PANGOLIN.
- 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 attribution to *Mustela lutreola* because of the blurry denomination "European farmed mink"), 7 from cats and 2 from dogs.

Table E. Strains or variants isolated from wild species.

Species	Strain and/or Clade and or Variant	Comment
Gorilla	B.1.429	Variant very widespread in humans in California
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[52]
Lions	Clade V in Bronx cases	
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.

- 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.

About testing in animals

- **Antigen Testing;**
  - **RT-PCR;** In human beings, testing relies on a large variety of tests, but the core tool remains RT-PCR detection of viral RNA mainly from nasopharyngeal and oropharyngeal swabs, and more recently from rectal swabs or feces. These tests are qualitative (positive/negative), they can be used to find presence of precise sequences such as in current variants. They are also quantitative to assess viral load (=value of the CT).
  - **RAPID TEST ;** Aside from these direct tests, more than 70 antigenic rapid test are also available on the market. They are build to detect the presence of protein from the Nucelocapsid (NP) through the use of lateral flow cassette system. One drop of serum or whole blood is enough for testing. In humans, the specificity of these test is often more than 98%, but their sensitivity is one of their major drawbacks[86], 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 in order to detect virus and shedding. If the animals are not trained for nasal swabs or anesthetized, testing feces remains a convenient option as fecal shedding is lasting for several days to week[51].

  - **Salivary test;** these tests have only very recently become available. Nasopharyngeal swab for RT-PCR reminds the gold standard for testing, but saliva looks promising as it is less invasive and quicker to collect (especially on a repeated rate) and some studies report a better sensitivity and constancy over infection course than nasopharyngeal samples. In animals, saliva excretion was found in several species such as macaques[87,88] and ferrets[41,89]. It could probably be a test for animals infected with SARS Cov 2, but with one caveat: it appears that there are two kinds of "salivary test" on the market: one detecting viral antigens, and another designed to reveal salivary immunoglobulins towards SARS Cov 2. The latter should not be used, as it is too human species specific.
- **Serological Antibody test**: There are different techniques included in this category; Rapid flow tests, different kind of ELISAs, different targeted antigens, etc. Some tests may have further potential in other animal species such as non-human primates or carnivore:
  - **Double antigen sandwich ELISA** based on recombinant viral protein that could detect both IgM and IgG antibodies [90]. 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%[86]. 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. [91]
- As many animals already harbor other species-specific coronaviruses, the SARS-CoV-2 specificity of the test must be precisely monitored [90].
  - RT-PCR test does not cross-react with other coronavirus (e.g. feline coronavirus), granting a good specificity.
  - available commercial tests for feline or canine coronavirus (ELISA) do not cross-react with SARS-COV-2.
- In humans, it seems that Ig A and M could be detected as early as few days post infection, while Ig G are seen later, and are currently reported to last at least for 3 to 4 months days[92]. Moreover, recent emerging results[93,94] demonstrate encouraging signs of strong, lasting immunity based both on B- and T-cells, **that cannot be detected** by the serological rapid tests described above. Duration of immunity in wild animals is currently unknown, but further serology of previously positive zoo animals may aid in building knowledge on duration of humoral immunity.
- 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 request by 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.

Issue and Potential treatment of positive animals

- According to the mild signs seen in animals so far, treatment could rely on general supportive care based on anti-inflammatory (NSAID) and antibiotic to control secondary infection, supplemented with miscellaneous intestinal / respiratory treatments.
- Some references and dosages of antiviral drugs can be found in experimental reports in Non-Human primates[95] or rodents[96], but administration of these treatments are not currently recommended outside of experimental settings.
- Chloroquine efficiency , largely controversial in human protocols, 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 [97]. Therefore, 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[98], 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[99]
- In one gorilla out of the San Diego outbreak, monoclonal antibody therapy was used. This silverback had previous medical heart conditions and displayed pneumonia, so he received antibiotics and monoclonal antibodies, with clinical success.

Potential for vaccination in animals

- Human vaccine research is currently under development in several countries, leading to more than 165 projects in progress. An excellent graphical guide to the diverse types of vaccines being developed can be found here. At the time of writing, they are nine approved / authorized vaccines already applied in human populations;
    - 2 are mRNA vaccines
    - 4 are inactivated vaccines
    - 2 are adenovirus-based vaccines: one using a chimpanzee adenovirus, the other one using a human recombinant adenovirus
    - 1 is based on a synthetic peptide
- Vaccines against several other coronaviruses are already available in veterinary medicine for some species[100];
    - Canine coronavirus; inactivated and live modified vaccines exist; they do not protect from infection, but are aiming to reduce signs of disease (mostly diarrhea).
    - 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 those IgG are actually more harmful to the animals in case of infection, 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 these live attenuated strains used is a risk, and therefore should restrained its use in wild birds.
- 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[101,102], 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.
- Linked with the current human vaccine research and development, several vaccines were experimentally applied in animals. Among them, one recent experiment mixing Region Binding Domain (RBD) of the spike protein together with hybrid ferritin nanoparticles lead to a very efficient vaccine in ferret; intramuscular injections and Intranasal instillation provided protection to vaccinated ferrets, that presented no clinical signs after viral challenge, even with high viral loads[103].
- At the time of writing, vaccination in animals is not implemented in any species, either domestic or wild. In a Virulence editorial, researchers recently wrote that vaccination of pets could become part of strategy plan against variants[104]. Here are local initiative about vaccination projects:
    - USDA recently produced a notice to clearly announce that they agree to consider biologic licenses and permit applications for vaccine in mink against SARS CoV 2.
    - Following the Gorilla cases, San Diego Zoo veterinary team is considering the use of a recombinant vaccine in their group Gorilla, with a spike protein-based vaccine. The R&D service from ZOETIS provided the product experimentally and announced its use could be extended to other species like mink.
    - The Federal Center for Animal Health in Russia is currently considering a vaccine for cats, rabbits and mink, with the clear objective of helping the fur trade industry.
    - The USFWS National BFF Conservation Center already vaccinated 120 black footed ferrets in the fall of 2020. There are not yet any scientific paper about this study, but ferrets were injected with a purified pike protein + adjuvant, in the same way that it was previously done in the same species for *Yersinia pestis* vaccine [105]
- OIE *adhoc* group on Covid-19 Animal-Human interface is currently discussing the opportunity of vaccinating wildlife. On their 11th Call of the 15th December, the group declared that vaccine of animals can be positively considered under circumstances: if the animal can become reservoir, if it can be a cause of viral recombination, or in case of high conservation values of its species.

Wildlife

- While there are a lot of published or in-review papers about predictive[37,106,107] or experimental[108] sensibility or hypothetic scenarios regarding wildlife, so far proven involvement of wild animals in the epidemiology of this human disease is minimal.
- A recent review of Danish mink farms outbreaks[44] includes a survey of wildlife surroundings the positive farms. All 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 only positive reported case was a wild American mink, found near a mink fur farm in Utah. 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.
- In Brazil, where two variants P.1 and P.2 are now circulating, some vampire bats *Desmodus rotundus* are being trapped and screened for SARS-Cov-2 antigens, but study is still in progress as indicated in last OIE adhoc call.
- Even if there are almost no reported cases, a precautionary approach is paramount whenever procedures involving wildlife are performed. The major stakeholders about wildlife conservation produce guidelines to prevent transmission risk; WCS, OIE or IUCN.
- 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 agonic pneumonia. To date, there are no positive wild felid reports outside of captivity.
- Among the order of carnivores, some authors propose that pinnipeds should be monitored closely because of their exposure, like cetaceans, to human wastewater leaked into the sea, that has been reported to contain some viral RNA[48]. Except from marine mammals, concerns about rodents in sewage are lowered by their very poor sensitivity to SARS-Cov-2. All other species, mainly poikilothermic like fishes and aquatic invertebrates are considered to play no role in transmission and to be at very low risk[109], especially when entering the food chain.

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 here

Is there any risk of transmission between animals?

- In the few reported zoo cases so far, a common source of contamination (infected and shedding keepers) is highly suspected and transmission between animal could not be neither confirmed nor excluded.
- It has been proven in cats, ferrets, deer fawns and hamsters that transmission can occur by direct contact [41,47,75] (e.g. orofecal route), but also without direct contact, by aerosol[110] / droplets, with distant contamination between individuals separated by a mesh fence [41,111]. 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.

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

- According to the current knowledge, SARS-CoV-2 demonstrates the ability to enter cells of several animal species such as bats, cats, ferrets and some primates. Therefore, close contact between these genera (i.e., felids, mustelids) and infected / suspect humans with COVID-19 should be restricted. 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 mask and appropriate PPE when minimal distance cannot be achieved (e.g. clinical exam under anesthesia).
- 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
- 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.

Reassuring Statements about risk of transmission from zoo animals to visitors / keepers

- 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. diarrhea 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 of animals being infected by keepers and 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[112] 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 spillover [113].
- 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.

Stability of virus in environment and disinfection

- Coronavirus are known to be able to survive and remains infectious in environment for hours and days[114].
- Infective media: SARS-CoV-2 could be excreted through oral cavity (saliva), respiratory tract (breath / aerosol) and also intestinal tract (feces), ocular conjunctiva (tears) and blood during some stages of the human disease. In experimentally infected ferrets, virus was also found in urine until day 8, but with lower loads than nasal washes or fecal samples[41].
- Like SARS-COv-1 and MERS-CoV [115], SARS-Cov-2 is likely inactivated by heat after **10 minutes above 56°C** [116] or within **less than 5 minutes at 70°C.** On the opposite, cold and negative temperatures are not a mean to decrease SARS Cov 2 viral load as the virus survives to -14 to -18°C for 2 to 3 weeks[117], and could even play a role in spreading virus from infected meat plants to distant retailers / consumers[118]
- SARS-CoV-1 and SARS-CoV-2 seem to share the same propriety of stability on surface and in aerosols [116,119–121]
    - remaining viable in aerosol droplets for up to 3 hours.
    - remaining detectable on metal or plastic surface for up to 4 days, but their titers 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) [116,119].

- 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[122].
- 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 [123]. 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).
- 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 1 year 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.

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