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 available daily [7900 and counting at this date] but be aware to check the source [e.g. pre-print server vs. peer-reviewed].

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

Moreover, the real information we need about the susceptibility and possible involvement of various animals 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 3 million human cases in 185 countries at the date of writing this text. 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 as pandemic.[1]

Questions / Answers

These are selected questions that either visitors or directors or 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 occur 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
        - Human viruses like HCov 229-E, often a component of the common cold
    - Betacoronavirus: most represented in mammals, from carnivores[5] to hoofstock[6][7][8][9][10] from hedgehogs[11] to bats. It also contains the 3 more recent emerging coronaviral diseases:
        - MERS CoV[12]
        - SARS CoV
        - SARS Cov-2
        - Additionally: HCoV-OC43, one 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
    - Deltacoronavirus: mostly avian species specific coronaviruses , and some porcine one., recently recovered from leopard cats [13]
- Chiropterans are well known to be host of many viruses, including various coronavirus at the same time [14,15]. 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.

Could 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 why they can adapt to new host with (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.

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 [16].
- However, there is a difference within the **R**eceptor **B**inding **D**omain RBD of the spike (S) protein between the two viruses: the SARS-CoV-2 RBD is adapted to receptors ACE2 which allows it to enter human cells, while Bat-CoV-RaTG13 is not.
- 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 [17]. 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 SAR-CoV-2 [18].
- 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[19], requiring an intermediate / amplification host with reassortments in the RBD region to invade human cells. Obviously, all this is speculative at this stage.

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 [20].
- Wildlife markets provide a unique occasion for interspecific transmission:
    - 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.
- 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, allowing several mutation and recombination event to occur consecutively. SARS-CoV-2 is likely to have emerged after a comparable amount of time, and not recently[21].

What about the positive domestic carnivores?

- 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.
- 5 somewhat detailed case reports of “positive” **domestic** carnivores are outlined below. One common feature is that in all four cases, the pets were 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[16]. 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: 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: 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.
- Two cats in New-York recently tested positive, exhibited respiratory signs and were housed with different COVID-19 positive patients. Please note there is possible disagreement between the Promed notification on 17.04 and the CDC confirmation as to the status of sickness of the owners.

- 5/1/2020 3:20:00 PMA non-yet peer reviewed study [22] details a study 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.
- Two recent papers [23,24] 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 [24].
- At this stage, it remains very hard to differentiate between a passive carriage from pets acting like fomites, and a real epidemiological role of dogs and cats from these 4 reported cases. 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 minor role in the current pandemic.

What about sensitivity to other wild carnivores?

- **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.
- 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.
- 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.

What about the sensitivity of non-human primates?

- 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 macaque (see table A.)
- Coronavirus transmission was previously proven from Humans to apes with HCoV OC43, one of the human coronavirus involved in the common cold [25], 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.
- Based on New World primate sensibility to SARS-CoV-1 [26], 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 A 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.
- 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. 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, and recently some ringtail lemurs were tested as negative in the context of sudden morbidity.

What about sensitivity of other mammals and of birds?

- 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 A below. 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:
    - While the objective is sometimes to assess a potential role of animal species in transmission, it **mostly is to identify potential animal models for further treatment and vaccination testing**. As labs rush to test SARS-CoV-2 in animal models the first results are emerging: teams in China have reported initial findings from infecting Rhesus macaques [27] and transgenic mice [28] 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, gulls) are very unlikely to be vectors for the SARS-CoV-2. Even if rodents can harbor multiple other coronaviruses[29], mouse and rat seem to be poor hosts for the SARS-CoV-2, as they lack the ACE2 receptor matching amino acids [17].
- It should be noted that in the table A, some information concerning species sensibility such as ferrets or mink may be contradictory between molecular receptor prediction modelling and actual observed infection. Thus, interpretation of taxonomic sensibility to SARS-CoV-2 at this point in time should be very cautious. Deer species and anteaters seem to be species to be particularly aware of ("high profile" species in [30]). This especially as there are opportunities for close encounters between humans, deer (in children farms), and anteaters (keepers training/feeding).

Table A : Extant knowledge about species sensitivity to SARS-CoV-2 from [17][30–32] **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 [17]	From [30,31]		
Horseshoe bat	YES	Likely	Very low (17/25)		
Daubenton's bat	NO	N/A			
Vampire bat	N/A	Likely (4/5)	Very low (13/25)		
Coquerel sifaka	N/A	N/A	High (24/25)		
Blue eyed black lemur	N/A	N/A	High (22/25)		
Cynomolgus monkey	YES	Likely (5/5)		Yes[33]	
Rhesus macaque	N/A	N/A	Very High (25/25)	Yes, and reinfection could not occur at T0+28 days new challenge	
Anubis baboon	N/A	Likely (5/5)	Very High (25/25)		
Orangutan	N/A	Likely (5/5)	Very High (25/25)		
Chimpanzee	N/A	Likely (5/5)	Very High (25/25)		
Gorilla	N/A		Very High (25/25)		
Swine	NO	Likely (5/5)	Low (19/25)	No: Failed to get positive PCR and seroconversion	
Cattle	NO	Likely (4/5)			
African elephant	N/A	Unlikely (3/5)	Low (18/25)		
Camel	N/A	N/A	Medium (21/25)		
Giraffe, Okapi	N/A	N/A	Medium (21/25)		
Hippopotamus	N/A	N/A	Medium (20/25)		
Alpaca	N/A	N/A	Medium (20/25)		
Reindeer	N/A	N/A	High (21/25)		
Giant anteater	N/A	N/A	High (21/25)		
Mouse	NO	Unlikely (2/5)	Very low (16/25)		
Rat	N/A	Unlikely (3/5)	Very low (16/25)		
Chinese hamster	NO	Likely (4/5)	High (22/25)		
Guinea pig	N/A	Unlikely (2/5)			
Dog	YES	Likely (3/5)	Low (19/25)	No positive PCR but seroconversion	Yes
Domestic cat	N/A	Likely (3/5)	Medium (21/25)	Yes + transmission to other cats	Yes
Tiger, Lion	N/A	N/A	Medium (21/25)		Yes
Puma	N/A	N/A	Medium (21/25)		
Ferret	YES	Likely	Very low (17/25)	Yes + transmission to other ferrets[23,24]	
American mink	N/A	N/A	Very low (14/25)		Yes
European mink	N/A	N/A	Very low (17/25)		
Meerkat	N/A	Unlikely (2/5)	Very low (15/25)		
Civet cat	N/A	Likely			
Masked palm civet	N/A	Very low	Very low (13/25)		
Racoon	?	Unlikely (2/5)			
Fossa	N/A	N/A	Very low (16/25)		
Red panda	N/A	N/A	Very low (13/25)		
Sea otter	N/A	N/A	Low (17/25)		
Chicken	?	Unlikely (3/5)		No. Failed to get positive PCR and seroconversion	
Duck	?	?		No. Failed to get positive PCR and seroconversion	

What about testing in animals?

- In human beings, testing initially relied on RT-PCR of viral RNA, mainly from nasopharyngeal and oropharyngeal swabs, but also from feces. These tests are either qualitative (mainly used for quick result) or quantitative to precisely assess viral load. Aside from these direct tests, a lot of serological assays are now being employed, mostly using a "Rapid Lateral Flow Test" format, testing for the presence of Ig G, A or M. In human beings, it seems that Ig A and M could be detected as early as few days post infection, while Ig G are seen later, and may last at least 28 days[34].
- While in many countries, veterinary laboratories were needed to perform human tests in order to increase daily test capacity, there are still options to get animals tested when relevant, i.e. animals with clinical signs and a history of contact with infected humans. This possibility must be endorsed and facilitated through national veterinary authorities.
- Regarding veterinary labs, Idexx has developed its own RT-PCR. Initial studies on dogs and cats showed:
    - that this RT-PCR test does not cross-react with other coronavirus (e.g. feline coronavirus), granting a good specificity.
    - that the available commercial tests for feline or canine coronavirus (ELISA) do not cross-react with SARS-COV-2.
- To date, only Idexx laboratories plan to release a commercial animal test in the USA during week 17/18. Requirement will be a validation of the test request by an official state veterinarian. The EU officially disapproves such an initiative and has asked member states to not encourage testing of animals at this stage.
- Several human serological tests are about to be released, especially to support the de-confinement phase in multiple countries. There are different techniques embedded in these test systems: 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:
    - Double antigen sandwich ELISA based on recombinant S1 protein that could detect both IgM and IgG antibodies [35].
    - Those based on lateral flow immunochromatography, using *Staphylococcus aureus* proteins A and/or G conjugate to reveal Ig G and Ig M. Those conjugates may work for numerous animal species test, but not all. The Literature must be consulted before trying to apply any kind of non-validated test [36].
    - As many animals already harbor other species-specific coronaviruses, the SARS-CoV-2 specificity of the test must be precisely monitored [35].
- 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.

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 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 (1.5-2m).
- 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.
    - Avoid any close contact like "kissing" or petting (especially without gloves).
    - Wear mask and other kind of 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

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).
- From 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 note 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..) and OIE
- 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.

What about stability of virus in environment? What can I use in the zoo as an efficient disinfectant?

- Coronavirus are known to be able to survive and remains infectious in environment for hours and days[37].
- 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[23].
- Like SARS-COv-1 and MERS-CoV [38], SARS-Cov-2 is likely inactivated by heat after **10 minutes above 56°C** [39] or within **less than 5 minutes at 70°C.**
- SARS-CoV-1 and SARS-CoV-2 seem to share the same propriety of stability on surface and in aerosols [39–42]
    - 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.** Other disinfectants that could be used include wine vinegar (1 minute), sodium hypochlorite (1-2 minutes), hydrogen peroxide (usually 2 minutes). Other usual disinfecting veterinary compounds like povidone-iodine 7.5% or chlorhexidine 0.05% are also inactivating the virus within 5 minutes [39,40].
- 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 [43]. However, it should be noted that uncovered shoes were positive, as were ventilation exhaust outlets.
- 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.

Online live references:

1. WHO: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
2. John Hopkins Univ
   : https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6
3. LitCOVID-19 database: https://www.ncbi.nlm.nih.gov/research/coronavirus/
4. BioOne Wildlife & Coronavirus Database : https://complete.bioone.org/COVID-19
5. Ku Leuven Institute "Living Paper" : https://rega.kuleuven.be/if/corona_COVID-19

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