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 [3011] 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 won't 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 1,300,000 human cases in 180 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.

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

Are coronavirus usual in wild species / Zoo animals?

- Yes, coronaviruses are very common in Mammals and Birds. 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: (2)
  - Alphaconoravirus : mainly found in bats , but this group also contains

    - The Feline Coronavirus FeCov with its two forms (FeCV and FIP) (16)
    - The canine coronavirus
    - Human viruses like HCov 229-E, often a component of the common cold
  - Betacoronavirus: most represented in mammals, from carnivores to hoofstock (11) (15), from hedgehogs to bats. It also contains the 3 more recent emerging coronaviral diseases:
    - MERS CoV
    - SARS CoV
    - SARS Cov
    - 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: (35) mostly avian species specific coronaviruses , and some porcine one., recently recovered from leopard cats
- Chiropterans are well known to be host of many viruses, including various coronavirus at the same time (30). 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 are able to 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 a neuroinvasive potential as well, e.g leading to the signs of anosmia and dysgeusia in humans

Could the coronaviruses be transmitted from Animal to Human?

- Generally, coronaviruses are rather 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:
  - SARS-CoV (Human, civet cats, racoon dogs, horseshoe bat, swine)
  - MERS-CoV (Human, bats, hedgehogs, camels)
  - Bov-CoV (Cattle, wild ruminants, camelids, dogs, occasionally humans) (1)
- Transmission does not necessarily mean disease. Most of the time, when transmission to another species occurs, there's only subclinical disease 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 is 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

To which animal species is this SARS-CoV-2 associated?

- SARS-CoV2 is showing 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

- However, there is a difference within the Receptor Binding Domain RBD of the spike (S) protein between the two viruses : the SARS-CoV-2 RBD is adapted to receptors the ACE2 which allows it to enter human cells, while Bat-CoV-RaTG13 is not.
- Pangolin coronaviruses have been described from Malayan pangolins 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. However, it is important to note that pangolins or any other species have not been identified as intermediary or amplification host in this SAR-CoV-2 outbreak. On a whole genome basis, the CoV from pangolins are very dissimilar to the SAR-CoV-2.
- As horseshoe bats were hibernating at the time when COVID-19 appeared in China, there is general consensus that the SARS-CoV-2 is of ancestral Bat-CoV-RaTG13 origin, but that an intermediate / amplification host with reassortments in the RBD region was necessary 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 possibly repetition in time.
- Wildlife markets are therefore 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 species unlikely 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.
- 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.
    - Typically, captive bred animals
    - No human consumption of wildlife
- Time is also 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.

What about the positive carnivores ? What about sensitivity to other species?

- Through recombination, the new SARS-CoV-2 has acquired the molecular abilities to enter human cells, and also the ability to infect other animal species under certain circumstances not yet elucidated.
- 4 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 (https://www.news.gov.hk/eng/2020/03/20200326/20200326_210657_932.html). 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 f**acility. Oral, nasal, and rectal samples tested positive for the virus. The cat has not shown any signs of disease.

  (source : https://www.news.gov.hk/eng/2020/03/20200331/20200331_220128_110.html?type=ticker)

- A non-peer reviewed study posted on the bioRxiv preprint server [01.04.2020] (44), 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 Ior 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. None were found positive (https://www.idexx.com/en/veterinary/reference-laboratories/idexx-sars-cov-2-COVID-19-realpcr-test/).

- 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 ability of SARS-CoV-2 to infect other species has mainly been assessed by ***In vitro*** by infection trial on various mammalian cells, or by computer simulated prediction according to RBD / ACE2 receptors binding abilities (2, 23). Combination of these two approaches in 4 different studies provide the report in Table A below.

- Additionally, 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 (https://www.researchsquare.com/article/rs-15756/v1) and transgenic mice (https://www.biorxiv.org/content/10.1101/2020.02.07.939389v3) that have the human ACE2 gene. Labs working on ferrets say they should also have initial results soon: a team led by virologist S. S. Vasan at the Australian Animal Health Laboratory in Geelong has found that the animals are susceptible to SARS-CoV-2 (https://www.nature.com/articles/d41586-020-00698-x#ref-CR1)

Table A : Extant knowledge about species sensitivity to SARS_CoV-2 from (16), (25), (36)

Species	In Vitro		Computer prediction of receptor binding Score /5: matched Amino acid	In vivo experimental infection success	Natural transmission seen
	Infected Cell	Viral Particle enter			
Horseshoe Bat	YES	YES	Likely	Not Yet	
Daubenton's bat	?	NO	?	Not Yet	
Vampire bat	?	?	Likely (4/5)	Not Yet	
Cynomolgus monkey	?	YES	Likely (5/5)	Not Yet	
Rhesus Macaque	?	?	?	Yes, and reinfection could not occur at T0+28 days new challenge	
Anubis baboon	?	?	Likely (5/5)	Not Yet	
Orangutan	?	?	Likely (5/5)	Not Yet	
Chimpanzee	?	?	Likely (5/5)	Not Yet	
Swine	YES	NO	Likely (5/5)	No. Failed to get positive PCR and seroconversion	
Cattle	?	NO	Likely (4/5)	Not Yet	
Elephant	?	?	Unlikely (3/5)	Not Yet	
Camel	?	?	?	Not Yet	
Mouse	NO	NO	Unlikely (2/5)	Not Yet	
Rat	?	?	Unlikely (3/5)	Not Yet	
Chinese Hamster	?	NO	Likely (4/5)	Not Yet	
Guinea Pig	?	?	Unlikely (2/5)	Not Yet	
Dog	?	YES	Likely (3/5)	No positive PCR but seroconversion	Yes
Domestic cat	?	?	Likely (3/5)	Yes + transmission to other cats	Yes

Tiger	?	?	?	Not Yet	Yes, likely in Lion too
Ferret	YES	YES	Likely	Yes + transmission to other ferrets	
Meerkat	?	?	Unlikely (2/5)	Not Yet	
Civet cat	YES	?	Likely	Not Yet	
Racoon	?	?	Unlikely (2/5)	Not Yet	
Chicken	?	?	Unlikely (3/5)	No. Failed to get positive PCR and seroconversion	
Duck	?	?	?	No. Failed to get positive PCR and seroconversion	

- **5th April 2020:** The Bronx Zoo revealed that one Malayan tiger had tested positive for SARS-CoV-2 with a qPCR (https://www.aphis.usda.gov/aphis/newsroom/news/sa_by_date/sa-2020/ny-zoo-COVID-19). Another Malayan, 2 Amur Tigers and 3 African lions had mild respiratory symptoms and developed a dry cough. The result was confirmed by USDA's National Veterinary Services Laboratory, based in Ames, Iowa. At this point in time, New York City is experiencing massive human circulation and transmission of COVID-19. At the Bronx Zoo PPE for the keepers has been 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.

- Two recent papers still under review (22,33) 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.
- Great caution should be paid to all new papers and information released about animal species susceptibility to the virus:
  - the objective sometimes is to assess a real role of animal species in transmission, sometimes to identify potential animal models needed for further treatment and vaccination testing.
  - the methods to get to conclusions may vary a lot like the following examples:
    - only in vivo assays (where immune system effects of hosts are mostly not considered)
    - raw computer models (prediction of molecular binding abilities)
    - experimental infection using high infective dose of SARS-CoV-2 injected directly injected in nose, trachea or blood
  - Hence, while all these studies provide valuable information, that may not be directly applicable to daily life (e.g. where animals are not exposed to extreme viral loads)

What about testing in animals?

- In human beings, testing so far relies on RT-PCR research of viral RNA, mainly from nasopharyngeal and oropharyngeal swabs, but also from feces. These tests are either qualitative (mainstream used one for quick result) or quantitative to precisely assess viral load. Aside from these direct tests, a lot of serological assays are under development, mostly using the form of "Rapid Test".
- Regarding veterinary labs, Idexx has developed its own RT-PCR. Initial studies on dogs and cats showed:
  - that this RT-PCR test is not cross-reacting with other coronavirus (e.g. feline coronavirus), granting a good specificity
  - that the usual commercial test for feline or canine coronavirus (ELISA) are not cross-reacting with SARS-COV-2 either.
- So far, there are no PCR or serological test available on the market for animals by veterinary labs.
- Several serological tests are about to be released, especially to support the de-confinement phase in a lot of countries. Review of techniques embedded in those test (Rapid flow ones, ELISA...) show that some of them may have further potential for other animal species such as non-human primates, but this use is not yet assessed properly and needs further studies.

The detection of COVID-19 virus in animals meets the criteria for reporting to the OIE through WAHIS, in accordance with the OIE Terrestrial Animal Health Code as an emerging 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 and recommendations for testing:
https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV19/Guidance_for_animal_health_laboratories.pdf

Zoo Context

The EAZA statement on SARS-CoV-2 can be found here:

https://www.eaza.net/assets/Uploads/Mailing-uploads/2020/2020-03-Corona-Virus-statement.pdf

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

- According to the current knowledge, SARS-CoV-2 is showing abilities to enter cells of several animal species such as bats, cats, ferrets and some primates. Therefore, close contact between these species families (i.e., felids, mustelids) and infected / suspect human with COVID-19 should be restricted and at any time. The same distance restriction than between human begins 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 or 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 : https://zahp.aza.org/wp-content/uploads/2020/03/COVID-19-and-Great-Apes_3.12.2020.pdf
  - 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. https://www.eazwv.org/resource/resmgr/files/transmissible_diseases_handbook/resources/Final_-_SARS_CoV-2_and_Great.pdf

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)
- 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 collection, mostly focusing on flying foxes that are not known hosts of the high-profile zoonotic coronaviruses.
- 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 of back transmission to keepers/visitors.. According to the few examples of viral load excreted by domestic animals naturally infected by human begins (dogs and cats), dose of excreted virus seems very low and are likely to be lower than the minimal infective one. This zoonotic risk is considered as very low by several national health agencies (SciCom in Belgium, ANSES in France, USDA in USA..) and OIE

  (https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/)
- Hence, risk of any incidentally infected wild captive animals shedding enough virus to infect keepers and visitors could be considered as even lower, regarding the greater distanciation compared to pets.

What about stability of virus in environment?

- Infective media: SARS-CoV-2 could be excreted through oral cavity (saliva), respiratory tract (breath / aerosol) and also intestinal tract (faeces), ocular conjunctiva (tears) and blood in some stages of the human disease. In experimentally infected ferrets, virus was also found in urine until days 8, but with lower loads than nasal washes or fecal samples (21).
- SARS-Cov-2 is likely inactivated by heat after **10 minutes above 56°C** (6) 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 (6,34)

  - 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 remains 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 chlorite (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 (6, 34)
- Caution should be paid that some references are refereeing 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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