

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

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

Preliminary note: the scientific content of this factsheet was collected from reliable sources such as OIE, European National references laboratories, WHO, and pre-Covid19 scientific literature about coronavirus.

A massive amount of new science is available daily [1087 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 of December 2019. Up to now, the virus spread globally with more than 180.000 human cases in over 150 countries at the date of writing this text. The virus name is SARS-COV-2, and 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. The purpose of this document is **not** to support a decision of opening/closing of all your zoo per se as this decision is mostly taken at the level of government in most of the European countries.

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, sometimes a component of the common cold
- Betacoronavirus : most represented in mammals, from carnivores to hoofstock (8) (14), 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.

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)
- 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 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 can't do it.
- Pangolin coronavirus was discovered in 2019. 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 the pangolin or any other species has not been determined as intermediary or amplification host in this SARS-CoV-2 outbreak.
- 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 dog in China? What about sensitivity to other species?

- Positive dog reported in Hong Kong in late February: repeated RT-PCR low-level SARS-CoV-2 viral RNA was detected in oro-nasal and fecal swabs. So far, the most credible hypothesis that this is actually a passive transfer of virus from its infected owner, without any infection of viral shedding of the dog. The dog showed no symptoms and to date has not sero-converted [15 March 2020] (<http://www.promedmail.org/post/7081842>)
- So far, the ability for 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). Combination of these two approaches in 3 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), (30) and (37)

Species	Infected Cell	Viral Particle enter	Computer prediction of receptor binding
Horseshoe Bat	YES	YES	Likely
Daubenton's bat	?	NO	?
Civet cat	YES	?	Likely
Non-Human Primates (no precise species)	?	YES	Likely
Orangutan	?	?	Likely
Swine	YES	NO	Likely
Mouse	NO	NO	Unlikely
Rat	?	?	Unlikely
Hamster	?	NO	?
Cattle	?	NO	?
Dog	?	YES	?
Cat	?	?	Likely
Ferret	?	?	Likely

- The Friedrich-Loeffler-Institut (FLI) (Germany) is currently testing experimental sensitivity of poultry and swine for SARS-CoV-2 but the results of this study won't be available before end of April

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. Therefore, close contact between chiropterans and infected / suspect human with CoViD19 should be forbidden. Keepers taking care of bats colony should be especially assessed.
- 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)
- Regarding great apes, there are already two official documents:
 - One from EAZA great Ape TAG Vet advisors
 - One from AZA / ZAHF Fusion Center : https://zahp.eaza.org/wp-content/uploads/2020/03/COVID-19-and-Great-Apes_3.12.2020.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.

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 (feces).



- SARS-CoV-1 and SARS-CoV-2 seems to share the same propriety of stability on surface and in aerosols (27)
 - remaining viable in aerosols for up to 3 hours
 - remaining detectable on metal or plastic surface for up to 3 days, but their titers reduced a lot (e.g. from $10^{3.7}$ to $10^{0.6}$ Tissue Culture Infective Dose / mL over 72h)
- The most efficient disinfectant remains alcoholic compounds, but with appropriate contact time: propanolol (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). (19)

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 database: <https://www.ncbi.nlm.nih.gov/research/coronavirus/>
4. BioOne Wildlife & Coronativus Database : <https://complete.bioone.org/covid-19>

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