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Opinion paper: Severe Acute Respiratory Syndrome Coronavirus 2 and domestic animals: what relation?

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Introduction

In late December 2019, an outbreak of clustered cases of pneumonia associated with a novel coronavirus was reported by the Chinese authorities to the World Health Organization (WHO). Several initial confirmed cases were linked to a wet market selling live animals and seafood products in Wuhan (Hubei province), China (Huang *et al.*, 2020). On 30 January 2020, the WHO declared the outbreak a Public Health Emergency of International Concern. On 11 February 2020, the causative pathogen was officially named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), responsible for Coronavirus Disease 2019 (COVID-19) (Du Toit, 2020).

Severe Acute Respiratory Syndrome Coronavirus 2 is mainly transmitted from person to person, by direct or indirect contact, through infectious microdroplets emitted when infected individuals spit, sneeze or cough (Bernard Stoecklin *et al.*, 2020). The massive circulation of this new pandemic coronavirus with a probable zoonotic origin raised questions on its ability to spillover to animal species and on the potential consequences of such events on both animals and humans.

This public health concern came to the attention of animal health authorities given the close contacts between humans and domestic animals. Therefore, in France, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) established an Emergency Collective Expert Appraisal Group (*Groupe d'Expertise Collective d'Urgence*, GECU 'Covid-19'). The GECU 'Covid-19' urgently convened on 4 March 2020 and 8 April 2020 to conduct an evaluation of the potential role of domestic animals in the ongoing COVID-19 pandemic.

Phylogenetic relationship between Severe Acute Respiratory Syndrome Coronavirus 2 and animal coronaviruses

Coronaviruses (subfamily *Orthocoronavirinae*, family *Coronaviridae*, order *Nidovirales*) are enveloped, single-strand, positive-sense RNA viruses, with the second largest known RNA genome. Coronaviruses are divided into four genera, according to the International Committee on Taxonomy of Viruses (ICTV): *Alphacoronavirus* (alpha-CoV), *Betacoronavirus* (beta-CoV), *Gammacoronavirus* (gamma-CoV), and *Deltacoronavirus* (delta-CoV). Betacoronaviruses are highly diversified in animals and consist of five sub-genera (ICTV): *Embecovirus*, *Hibecovirus*, *Merbecovirus*, *Nobecovirus* and *Sarbecovirus*.

Coronaviruses are responsible for infections in birds and mammals, including humans. They can pose a heavy disease burden on livestock with a broad spectrum of diseases in domestic animals, ranging from mild to severe enteric, respiratory, neurological, renal or systemic disease (Table 1). Most of these infections remain host-specific, being confined to a single species or to closely related species.

To date, beta-CoVs usually found in livestock belong to the *Embecovirus* sub-genus which includes the bovine coronavirus (BCoV, bovine-like CoV), the porcine hemagglutinating encephalomyelitis virus and the rabbit coronavirus (RbCoV HKU14).

In humans, coronaviruses causing upper-respiratory tract illnesses belong to the alpha-CoV or the beta-CoV genus, among which two viruses originate from horseshoe bats (*Rhinolophus* spp.) belonging to the *Sarbecovirus* sub-genus, that is, the SARS-CoV described in 2003, and the most recent SARS-CoV-2 (Table 1). These two viruses can induce severe acute respiratory syndromes in human. *Sarbecoviruses* are very diversified in horseshoe bats in Asia, Europe and Africa (Tong *et al.*, 2009; Ar Gouilh *et al.*, 2018). SARS-CoV and another beta-CoV, the MERS-CoV, have been reported to infect civets and dromedary camels, respectively, as intermediary hosts. Phylogenetic analysis shows that there

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Table 1 Classification of coronaviruses (CoVs) in human and livestock

Host species	Alpha-CoV	Beta-CoV	Gamma-CoV	Delta-CoV
Human	Human CoV-229 E Human CoV-NL63 NA	Human CoV OC43 Human CoV-HKU1 SARS-CoV-2 ¹ SARS-CoV ¹ Middle East Respiratory Syndrome-related CoV ¹	NA	NA
Swine	Porcine epidemic diarrhea virus Porcine respiratory CoV Transmissible gastroenteritis virus	Porcine hemagglutinating encephalomyelitis virus		NA Porcine CoV HKU15
Bovines	NA	Bovine CoV	NA	NA
Avian	NA	NA	Turkey CoV Infectious bronchitis virus Guinea fowl CoV	Nine avian CoVs
Rabbits	NA	Rabbit CoV HKU14	NA	NA

NA = Non applicable.

¹Bat origin (outbreaks in humans).

is no proximal relationship between SARS-CoV-2 and beta-CoVs circulating in domestic animals.

Crossing of species barrier

To date, the most convincing species of origin for SARS-CoV-2 is bats (family *Rhinolophidae*). However, it remains elusive as to whether an intermediate host was involved in the transmission event that led to human infection. The SARS-CoV-2 genome shares a high percentage of identity (96.3%) with the genome of RaTG13/2013 virus detected in bats belonging to the *Rhinolophus* genus in China (Zhou *et al.*, 2020).

Many studies investigated the role of pangolins in the current pandemic, as an intermediate host. The complete genome of pangolin-CoV-2020 virus identified in three sick Malayan pangolins was assembled in a study conducted by Liu *et al.* (2020). Even if molecular and phylogenetic analyses showed that SARS-CoV-2 was genetically closer to RaTG13/2013 than to pangolin-CoV-2020 (96.18% *v.* 90.24%, respectively), these findings suggest that pangolin-CoV-2020 or a related virus might have partially contributed to the origins of the SARS-CoV-2. However, this study does not support that SARS-CoV-2 emerged directly from the pangolin-CoV-2020. Further studies are required to identify the intermediate host and to state on the pangolin's role in SARS-CoV-2 transmission to humans.

According to the GECU, crossing the species barrier is not a common phenomenon as it requires genetic innovations (e.g. mutations and deletions) potentially involving genetic exchange for adaptation to a new host species (e.g. recombinations). The question is whether its evolution potential will enable SARS-CoV-2 to spillover to another animal

species. Today, SARS-CoV-2 is well adapted to humans with an efficient person-to-person transmission. In the current pandemic context, the GECU considers the adaptation of SARS-CoV-2 to domestic animals unlikely to occur, except under specific high pressure of viral infection. Nevertheless, the question of its sporadic transmission from man to animal arises as a prerequisite to such an evolution.

Studies on potential infections with Severe Acute Respiratory Syndrome Coronavirus 2

Angiotensin-converting enzyme 2 receptor studies

The attachment of SARS-CoV-2 to the host cell is mediated by the *Spike* protein (S) and its receptor Angiotensin-converting enzyme 2 (ACE2). In humans, ACE2 is expressed on the surface of cells in the upper oesophagus, lungs, kidneys, testicles and the intestinal epithelium. ACE2 is well conserved in mammals, birds, reptiles and amphibians. Recently, homology models of animal ACE2 and dynamic computational models for interaction with SARS-CoV-2 S-protein were developed in different animal species. For example, a molecular study showed that SARS-CoV-2 was able to use a swine ACE2 homologue expressed by HeLa cells to induce infection (Zhou *et al.*, 2020). This result suggests that pigs may theoretically be able to be infected with SARS-CoV-2. However, another study showed no entry of pseudotype particles expressing SARS-CoV-2 S-protein in epithelial-like pig kidney cell line (LLC-PK1) (Wan *et al.*, 2020). For bovine, a study showed that in Madin-Darby Bovine Kidney Epithelial Cells, there was also no entry of SARS-CoV-2 S-protein pseudotype particles (Hoffmann *et al.*, 2020). Ongoing research is also carried out at Erasmus University Rotterdam on rabbits as

this species might be susceptible to SARS-CoV-2 infection (<https://promedmail.org/promed-post?id=7316646>, seen on 5 June 2020). No molecular prediction has been carried out yet for poultry. According to the GECU, additional studies need to be conducted to elucidate the interactions between SARS-CoV-2 and ACE2 homologues in other animal species. However, a virus does not rely solely on the presence of a cell receptor to enter cells and to carry out its full cycle but also on other cellular factors including various proteases such as type II transmembrane serine proteases. Further studies should also be undertaken to identify all of them.

Experimental infections in farm animals

To date, two *in vivo* experimental studies have evaluated the susceptibility of pigs, chickens and ducks to SARS-CoV-2 infection and the ability of these animals to transmit the virus to contacts (Schlottau *et al.*, 2020; Shi *et al.*, 2020). Results shows that even with high viral dose for inoculation (10^5 PFU or 10^5 TCID₅₀), neither inoculated nor contact animals were tested positive for SARS-CoV-2. However, it should be borne in mind that in the study conducted by Shi *et al.* (2020), a very limited number of subjects inoculated were used to draw these conclusions: 5 pigs, 5 chickens and 5 ducks. In the study of Schlottau *et al.*, (2020), 9 pigs and 17 chickens were subjected to inoculation. Furthermore, only three contact animals of each species were used in both studies.

No experimental data have been made available for ruminants and rabbits so far.

Natural infections in farm animals

On April 2020, respiratory problems and increased mortality were observed in four mink farms in the Netherlands (Oreshkova *et al.*, 2020). In two farms, one worker at least had been found positive for SARS-CoV-2. Organ and swab samples from minks tested positive for SARS-CoV-2 RNA by RT-qPCR. At the end of May, the animal Health service reported that carcasses from 3 (out of about 50 investigated) additional mink farms were confirmed positive for SARS-CoV-2. Another suspected new farm was still under investigation (<https://promedmail.org/promed-post?id=7420433>, seen on 5 June 2020).

No studies about ACE2 gene expression in mink cell lines have been carried out to date. However, Sun *et al.* (2020) observed ACE2 expression in the lungs of ferrets belonging to the Mustelidae family as minks. Furthermore, computational model of SARS-CoV-2 S-protein and ACE2 homologue in ferrets suggested that this species can theoretically be infected with SARS-CoV-2 (Wan *et al.*, 2020). Moreover, experimental infections show clearly that ferrets are susceptible to the virus, with clinical signs and lesions observed in the respiratory tract (Shi *et al.*, 2020). By analogy, therefore, ACE2 gene might also be expressed in mink and recognized by the SARS-CoV-2 S-protein but this should be confirmed. According to GECU, this species is susceptible to SARS-CoV-2 infection and mink can transmit the virus amongst each other.

To date, SARS-CoV-2 virus outbreak in mink farms is the only event that was reported in farm animals.

Study designs for Severe Acute Respiratory Syndrome Coronavirus 2 detection in animals

Such studies face several challenges in order to develop diagnostic tools and better understand the role of animals in the ongoing pandemic.

Tests used to detect Severe Acute Respiratory Syndrome Coronavirus 2 in a naturally infected animal. Recently, RT-PCR has been widely used to detect RNA in animals that might potentially be infected with SARS-CoV-2. The primers used to amplify viral sequences should be specific to SARS-CoV-2 and should not cross-react with other animal coronaviruses. A positive RT-PCR result indicates that SARS-CoV-2 RNA is present in the sample tested. However, RNA detection is not strongly associated with the presence of infectious viral particles or with productive infection. Therefore, it does not provide sufficient evidence to conclude that the animal was infected. Passive contamination cannot be ruled out. Negative RT-PCR results mean that viral RNA was not detected in the sample or it is below the detection limit. However, a negative result cannot differentiate between an animal with a past SARS-CoV-2 infection from one never infected. In addition to RT-PCR, serological surveys may also be conducted. However, serological surveys cannot be used to identify animals infected recently because their immune system has not yet respond by producing antibodies. Regarding clinical signs (respiratory or digestive), the GECU emphasizes that they are not sufficient to detect a SARS-CoV-2 infection because some infected animals can be free from these manifestations.

Tests used to detect Severe Acute Respiratory Syndrome Coronavirus 2 in an experimentally infected animal. Informations from comparative SARS-CoV-2 sequence analyses and molecular modeling of the SARS-CoV-2 S-protein and ACE2 receptors can help to narrow down the choice of species that will be used in experimental infection and transmission studies. Studies should be conducted on large numbers of animals for robust statistical data. Animals are usually inoculated intranasally with known viral titers. Replication dynamic of SARS-CoV-2 in animals is then monitored in organs or tissues by viral RNA quantification (RT-qPCR) and virus titration in Vero E6 cells. Seroconversion can be detected by collecting sera from infected animals for antibody detection using an ELISA or seroneutralisation. Histopathologic studies and lesions can also be performed on organ samples. These results give indication if SARS-CoV-2 replicates efficiently or not in animals. Results from experimental infection should be interpreted with caution as infected animals do not always show clinical signs. Finally, transmission studies can also be monitored by co-housing infected animals with naïve animals. Viral RNA detection and seroconversion of contact animals are also investigated to evaluate SARS-CoV-2 transmission in animal population.

Conclusion

Phylogenetic analysis shows clearly that SARS-CoV-2 and beta-CoVs circulating in domestic animals are distant phylogenetically as they belong to two distinct sub-genus. Molecular modeling of interactions between ACE2 receptor and *Spike* protein suggests, for example, that pigs may theoretically be infected with SARS-CoV-2. However, two experiments conducted to date in livestock suggest that pigs, chickens and ducks are not susceptible to SARS-CoV-2 intranasal infection, in the conditions of the trials. Nevertheless, it is necessary to remain attentive to future studies and information, likely to change this assessment.

Recently, cases of minks in Netherlands and pets (cats and dogs) in Hong-Kong, Belgium, USA, France, Spain, Russia and Germany, all naturally infected with SARS-CoV-2, have been reported. Animals were all in close contact with COVID-19 owners. These cases of infection remain sporadic and isolated, in view of the high circulation of the virus in humans and the magnitude of the pandemic today.

Although biological and virological characteristics of this virus demonstrate its ability to cross species barrier, SARS-CoV-2 is now well adapted to human, with an efficient person-to-person transmission. To date, and in a context in which common Standard Operating Procedures and classical sanitary recommendations are followed, the GECU considers adaptation of this virus to domestic animals unlikely to occur, except under specific high pressure of viral infection. Nevertheless, the question of its sporadic transmission from human to animal arises as a prerequisite to such an evolution and further risk of virus transmission from animal to human. The GECU still recommends that people who are suspected or confirmed to be infected with SARS-CoV-2 limit their close physical contact with animals. Basic hygiene measures should always be implemented when handling and caring for animals.

Since April 2020, clusters of COVID-19 cases have been found in workers at three French slaughterhouses and also in other countries (Germany, Spain, Australia, the USA and Brazil). The French ministry of agriculture did not order any recall of products from the three plants. In the ANSES opinion (2020-SA-0037), the GECU underlines that the possible contamination of foodstuffs of animal origin via an infected animal has been ruled out. Moreover, good implementation of hygiene practices can prevent infected humans from contaminating food.

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Declaration of interest

ANSES carefully assessed all the interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals. Declarations of interests of ANSES experts are made public via the website www.anses.fr.

Ethics statement

Not applicable.

Software and data repository resources

None of the data were deposited in an official repository.

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