

Third update on possible animal sources for human COVID-19

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1 | INTRODUCTION

Approximately a year ago, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in humans was described for the first time in Wuhan, China.¹ Since, SARS-CoV-2 and its clinical manifestation, known as coronavirus disease 2019 (COVID-19), have dominated the news and varying restrictions to everyday life have been introduced in essentially all continents in an international effort to limit human-to-human spread as well as decrease hospitalization rates.² Updated information on confirmed high pathogenic CoV infections and fatalities in humans are provided in Table 1. This synopsis represents the third update on recent findings on animal sources that could pose a risk for human SARS-CoV-2 infection. The information provided is intended to update people working closely with animals on new evidence of cross-species transmission of SARS-CoV-2 from humans.

2 | ORIGIN OF SARS-COV-2: WHAT HAVE WE LEARNED SO FAR?

When assessing any new virus, it is essential to identify its origin as this could yield important data which could help in preventing future outbreaks. Further investigations into the origin of SARS-CoV-2 revealed that the virus itself likely originated from a bat sarbecovirus, a virus circulating in horseshoe bats.³⁻⁵ Horseshoe bats can be found

in tropical and temperate regions in Europe, Japan, Asia, and Africa. Divergence dates between SARS-CoV-2 and the bat sarbecovirus reservoir were estimated as 1948 and 1982, suggesting that the lineage which produced SARS-CoV-2 has been circulating unnoticed in bats for decades.^{3,6} Of note, the virus was introduced to humans via spillover or cross-species transmission but details still need to be established. SARS-CoV-2 adapted quickly to its new human host resulting in rapid human-to-human transmission, with a mean reproductive number (R) estimated to be 3.28 (median 2.79),⁷ which indicates that an infected person can potentially infect 3 to 4 others.

In theory, it is possible that SARS-CoV-2 in its current form evolved directly from horseshoe bats, but an intermediate host, such as pangolins or another species, is also plausible.³ SARS-CoV-2 emerged in Wuhan, China during the winter season, perhaps indicating that there was an intermediate host present at that time.⁶ As we outlined in our previous commentary,⁸ the pangolin has been proposed as the missing link bridging bats and humans in the context of SARS-CoV-2. Pangolins in some cases have developed natural disease associated with SARS-CoV-2 infection, perhaps indicating they may not be a natural reservoir.^{3,6} The current consensus is that more data is needed to conclusively determine the origin of SARS-CoV-2. Identifying intermediate host species capable of supporting SARS-CoV-2 replication is important as this could provide clues on future reservoir hosts. It has been determined that the likelihood of fish, birds, reptiles and amphibians to become a possible SARS-CoV-2 intermediate host in the future is low.^{9,10} Among livestock species including ruminants, pigs

and domestic poultry, reports of serious disease outbreaks, possibly suggesting a species jump of SARS-CoV-2, have not been reported to date. However, there is evidence that pigs¹¹ and ruminants¹² can be experimentally infected with SARS-CoV-2 at a low level, and livestock may pose a greater risk of serving as a reservoir in the future, when SARS-CoV-2 becomes more established in humans.¹³ A potentially important role in cross-species transmission has been suggested for rodents including squirrels, rats, mice, hamster and others.¹⁴ Rodents exist in sufficient numbers and densities for continuous transmission and are often in close proximity to humans, but so far experimental studies indicate a low probability or no risk of SARS-CoV-2 infection for mice and rats.¹³ Interestingly, it has been found more recently that Chinese tree shrews, a squirrel-like mammal with a wide distribution in Southeast Asia, could not only be infected with SARS-CoV-2 but also developed clinical signs analogous to COVID-19 in humans.¹⁵ Chinese tree shrews have been used as animal models in viral hepatitis, psychosocial and visual defect studies due to their phylogenetical closeness to primates.¹⁶

3 | VIRAL SPECIES JUMP OF SARS-COV-2 AND IMPLICATIONS: WHY IS IT IMPORTANT?

Many research efforts focus on the animal-human interface of SARS-CoV-2. With the high rate of infections and the overall high virus load present in the human population today, it is likely that SARS-CoV-2 may enter other new hosts. This process is known as species jump or spillover and requires some level of adaptation of the virus to the new host. Three stages of viral disease emergence leading to successful host switching have been defined previously.¹⁷

3.1 | Stage 1

During the first stage, a new host species becomes infected but there is no onward transmission. This scenario is likely true for dogs and cats: SARS-CoV-2 viremia or even clinical signs have occasionally been demonstrated in these pets, which were essentially always in close contact with COVID-19 infected humans and were the direct results of human-dog infection¹⁸ or human-cat-infection.¹⁹ However, to date, there have been no confirmed natural infections between

dogs, between cats or from cats or dogs to humans and companion animals are unlikely to spread COVID-19 at a larger scale.²⁰ Of note, naive cats kept under experimental conditions in close contact with SARS-CoV-2 infected cats can become infected, confirming a successful transmission between cats.^{21,22} However, under normal circumstances domesticated cats live solitary lives without socially structured groups and are not in regular close contact with other cats. The documented events so far suggest that pet cats and dogs can be considered dead-end hosts.

3.2 | Stage 2

The second stage of viral disease emergence are spillovers that go on to cause local chains of transmission in the new host population before the epidemic fades out (outbreaks). The authors are not aware of any SARS-CoV-2 infections in domestic or wild animals that fall into this category.

3.3 | Stage 3

The third stage is development of an epidemic or sustained endemic host-to-host disease transmission in the new host population. This stage has likely been reached in farmed mink populations, where all factors consistent with stage three have been observed, including confirmed human-to mink infections, mink-to-mink transmission with clinical signs in a large number of animals and mink-to human infection.²³ This has resulted in the culling of many commercial mink farms in the Netherlands,²⁴ Spain (<https://www.bbc.co.uk/news/world-europe-53439263>), the USA (https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2020/sa-08/sars-cov-2-mink) and Denmark²⁵ among other countries.

The species jump of viruses into a new host is in general of concern because of the potential introduction of genome mutations driven by inadequate replication in the intermediate or novel host. These changes can impact virus fitness in general and occasionally may result in increasing viral replication rate in the intermediate or novel host.²⁶ Unique SARS-CoV-2 mutations were identified in Dutch and also in Danish mink after the virus adapted to this species.^{25,27} Subsequently, the same mutated viruses were also detected in humans who were in close contact with

TABLE 1 Facts on high pathogenic human CoVs

Virus	Time of circulation	Laboratory confirmed cases	Deaths	Case fatality rate	Country distribution
SARS-CoV ^a	2002-2003	8096	774	9.6%	26
MERS-CoV ^b	2012-ongoing	2494	853	35%	27
SARS-CoV-2 ^c	2019-ongoing	67 210 778	1 540 777	2.3%	Global pandemic

^aSource: https://www.who.int/csr/sars/country/table2004_04_21/en/.

^bSource: <https://www.who.int/emergencies/mers-cov/en/>.

^cSource: <https://covid19.who.int> (Accessed 9 Dec 2020).

the mink.^{25,27} During vaccine development, it is crucial to monitor any viral changes which may occur at vaccine target sites, as these may render a novel vaccination product ineffective.²⁸ At this point, scientists suggest that the mink-specific SARS-CoV-2 mutations identified so far will not jeopardize the effectiveness of potential COVID-19 vaccines.²⁹

SARS-CoV-2 belongs to the RNA class of viruses, which are prone to high mutation rates (expressed as the number of substitutions per nucleotide per generation). Despite this fact, fortunately and similar to SARS-CoV, which is mutating at a slow rate ($0.80\text{--}2.38 \times 10^{-3}$ nucleotide substitution/site/year),³⁰ SARS-CoV-2 has an estimated annual substitution rate of 26 and an estimated evolutionary rate of approximately 0.90×10^{-3} substitution/site/year.³¹ In lay terms, this means that SARS-CoV-2 only has approximately two single mutations per month on average; this is half the rate seen in influenza viruses and a quarter of the mutations acquired by HIV.³²

4 | NEW INFORMATION ON SARS-COV-2 SUSCEPTIBLE ANIMAL SPECIES

At the time of our last update, it had been confirmed that *Felidae*, *Canidae*, and *Mustelidae* can become naturally infected with SARS-CoV-2.⁸ Under experimental conditions, *Cricetidae* and Macaques can also be infected but often only develop subclinical disease.⁸ The following information is an update on the current knowledge relevant to the susceptibility of different animal groups to SARS-CoV-2.

4.1 | SARS-CoV-2 in pets

Today pets often live in close contact with humans and are commonly considered part of the family. It comes as no surprise that SARS-CoV-2 has been detected in dogs and cats living in COVID-19 households.³³⁻³⁵ Often SARS-CoV-2 in cats or dogs was only detected by PCR assays, occasionally the pet in question seroconverted, and in only a few cases, mild clinical signs were described.⁸ Commonly, field assessments of the general cat and dog population using serology assays resulted in a low prevalence of antibody-positive animals.^{36,37} Overall, this has triggered a number of controlled experimental and observational studies. Since our last update, a few more experimental cat studies have been published (Table 2) further confirming that cats often remain asymptomatic while able to transmit SARS-CoV-2 to sentinel cats.^{21,22,38} Moreover, it was also reported that cats shed the virus for approximately 5 days with peak titres achieved from nasal shedding at day 3 and, when infected with SARS-CoV-2 twice 4 weeks apart, mounted an effective immune response and did not become reinfected.²² In contrast, SARS-CoV-2 shedding in experimentally infected dogs was not observed; however, seroconversion was reported.²² The overall data provided by research into canine and feline SARS-CoV-2 infection indicates these are end-stage hosts, there is no evidence of virus transmission to other dogs, but cats can infect naïve cats during the acute stage of infection if in close contact with each other.

Under experimental conditions, Golden Syrian hamsters^{39,40} as well as ferrets^{41,42} can be readily infected with SARS-CoV-2, which causes mild to no clinical signs with limited and often short-lived

TABLE 2 Summary of research studies demonstrating SARS-CoV-2 in pets

Species	Data type	Positive animals/total number of animals tested	Inoculation details		Reference
			Route	Dose ^a	
Domestic cats	Experimental	14/14	Intranasal	1.0×10^5 PFU	43
		6/6	Oral/intranasal	1.0×10^6 TCID ₅₀	38
		6/6	Intranasal/oral	5.2×10^5 PFU	21
		5/5	Intranasal	3.0×10^5 PFU	22
	Surveillance	6/60	NA ^b	NA	44
		0/87	NA	NA	45
		1/131	NA	NA	36
		15/39	NA	NA	37
Domestic dogs	Experimental	1/5	Intranasal	1.0×10^5 PFU	43
		3/3	Intranasal	1.4×10^5 PFU	22
	Surveillance	8/180	NA	NA	44
		0/497	NA	NA	45
		13/172	NA	NA	36
Ferrets	Experimental	10/10	Intranasal	1.0×10^5 TCID ₅₀	46
		9/9	Intranasal	1.0×10^5 PFU	43
Golden Syrian hamster	Experimental	Frequently used as animal models for human SARS-CoV-2			

^aMedian tissue culture infectious dose (TCID₅₀) per animal or plaque-forming unit (PFU).

^bNA, not available.

virus shedding. To the authors' knowledge, there have been no reports of SARS-CoV-2 naturally infecting pet hamsters or ferrets.

In general, it would appear pets are not easily infected. Confirmed reports of cats and dogs naturally infected with SARS-CoV-2 are very limited, while confirmed human infections have reached over 67 million cases, as outlined in Table 1. Therefore, pets do not pose a major threat to humans at this point and human infection from cats, dogs, ferrets or Golden Syrian hamsters has not been reported.

4.2 | SARS-CoV-2 in livestock species

Fortunately, studies investigating the susceptibility of livestock species to SARS-CoV-2 have rarely resulted in finding viral infectivity (Table 3). SARS-CoV-2 experimental infection trials in poultry using chickens, ducks, turkeys, quail and geese demonstrated these animals lacked susceptibility to the virus.^{46,47}

For pigs, most available data points towards this species not being susceptible to SARS-CoV-2; however, there are some recent conflicting reports. A US study found no evidence of clinical signs, viral replication or SARS-CoV-2-specific antibody responses in 9 5-week-old pigs when infected through the oral, intranasal and intratracheal routes; however it was also found that porcine cell lines including a porcine kidney cell line and swine testicular (ST) cell line could be readily infected.⁴⁸ In a Spanish study, 20 5-6-week-old pigs were divided into groups of 5 pigs and infected with SARS-CoV-2 using the intranasal (IN), intratracheal (IT), intramuscular (IM), or intravenous (IV) routes.⁴⁹ Pigs in the IN group were euthanized at 1 or 2 days post infection (dpi) while all other pigs were euthanized at 2 or 22 dpi. Nasal or rectal shedding of viral RNA was not detected in any of the pigs. Proximate trachea from a single IN-inoculated pig was SARS-CoV-2 RNA positive at 1 dpi. All other tissues from this pig and all tissues from all other pigs were SARS-CoV-2 RNA negative. No productive infection was observed in any of the pigs. Evidence of seroconversion against

TABLE 3 Summary of research into SARS-CoV-2 infection in livestock species

Species	Data type	Positive animals/total number of animals tested	Inoculation/study details		Reference
			Route	Dose ^a	
Pigs	Experimental	0/9	Intranasal	1×10^5 TCID ₅₀	46
		0/5	Intranasal	1×10^5 PFU	43
		0/9	Oral/intranasal/ intratracheal	1×10^6 TCID ₅₀	48
		0/20	Intranasal, intratracheal, intramuscular or intravenous	$1 \times 10^{5.8}$ TCID ₅₀	49
	Surveillance	3/16	Oronasal	1×10^6 PFU	11
		0/187	NA ^b	NA	45
Swine cell lines	Infection readily possible	Swine testicle (ST) cell line Porcine kidney (PK) cell line	0.05 multiplicity of infection (MOI) of passage 3 of a VeroE6- passaged SARS-CoV-2	48	
Cattle	Experimental	2/6	Intranasal	1×10^5 TCID ₅₀	12
	Surveillance	0/107	NA	NA	45
Sheep	Surveillance	0/133	NA	NA	45
Chickens	Experimental	0/17	Oculo-oronasal	1×10^5 TCID ₅₀	46
		0/5	Intranasal	$1 \times 10^{4.5}$ PFU	43
		0/10	Intrachoanal	$1 \times 10^{5.4}$ TCID ₅₀	47
	Surveillance	0/153	NA	NA	45
Ducks	Experimental	0/5	Intranasal	$1 \times 10^{4.5}$ PFU	43
		0/10	Intrachoanal	1×10^6 TCID ₅₀	47
	Surveillance	0/153	NA	NA	45
Turkeys	Experimental	0/10	Intrachoanal	$1 \times 10^{5.4}$ TCID ₅₀	47
Japanese quail	Experimental	0/10	Intrachoanal	$1 \times 10^{5.4}$ TCID ₅₀	47
White Chinese geese	Experimental	0/10	Intrachoanal	1×10^6 TCID ₅₀	47

^aMedian tissue culture infectious dose (TCID₅₀) per animal or plaque-forming unit (PFU).

^bNA, not available.

TABLE 4 Update on SARS-CoV-2 in farmed mink

First report					
Country	Date	Farm characteristics	Farm staff/owner COVID-19 status	Reference	Additional reports
Netherlands	26-April	Two farms	Positive	https://promedmail.org/promed-post/?id=20200427.7272289	14-Oct: 67 farms affected; approximately 440 000 mother animals and 2.2 million young mink have been culled (https://promedmail.org/promed-post/?id=20201019.7873326)
Spain	16-July	Single farm 93 000 mink Culling	Positive	https://promedmail.org/promed-post/?id=7584560	
Denmark	17-June	Single farm 11 000 mink Slaughter, quarantine	Not reported	https://promedmail.org/promed-post/?id=20200617.7479510	13-Oct: 63 farms affected (https://promedmail.org/promed-post/?id=20201014.7861560) 5-Nov: All mink, approximately 17 million animals, will be culled (https://promedmail.org/promed-post/?id=20201122.7963766)
USA, Utah	17-August	Two farms Quarantine	Positive	https://promedmail.org/promed-post/?id=20200818.7692815	2-Oct-20: Death of 7000-8000 mink within 10 days, 9 sites are in quarantine (https://promedmail.org/promed-post/?id=7847704)
Italy	27-October	Single farm	Not reported	https://promedmail.org/promed-post/?id=7897986	
Sweden	29-October	Single farm No culling	Positive	https://promedmail.org/promed-post/?id=20201103.7912846	6-Nov-20: Nine additional farms (total of 10/40) infected (https://promedmail.org/promed-post/?id=7924269)
USA, Wisconsin	5-November	Two farms 5000 mink Depopulation	Not reported	https://promedmail.org/promed-post/?id=7923387	
Greece	13-November	Two farms Culling	Positive	https://promedmail.org/promed-post/?id=7944705	
France	22-November	Single farm 1000 mink Culling	Not reported	https://promedmail.org/promed-post/?id=7965554	
Poland	24-November	Single farm	Not confirmed	https://promedmail.org/promed-post/?id=7976927	
Lithuania	26-November	Single farm 169 dead mink Culling of selected cages	Positive	https://promedmail.org/promed-post/?id=7976927	

the SARS-CoV-2 spike glycoprotein was detected at 14 and 22 dpi while neutralizing antibodies were detected at 22 dpi in pigs inoculated by parenteral routes (IM or IV). It was suggested that pigs may be a good model for SARS-CoV-2 immunogenicity studies.⁴⁹ In contrast, a Canadian study using 16 8-week-old pigs inoculated with SARS-CoV-2 via an oronasal route did find low susceptibility to infection in these pigs due to detection of viral RNA in nasal wash (2/16 pigs at 3 days post-challenge) and pooled oral fluids from another room (1/2 at 3 days post-challenge), as well as the successful isolation of virus from a pig. Furthermore, 2/16 pigs, unrelated to the SARS-CoV-2 RNA positive pigs, developed low neutralizing antibody titres against SARS-CoV-2 between 11 and 15 days post-challenge.¹¹

Little research has been done in ruminants so far; however, a recent study using six 4 to 5-month-old cattle intranasally inoculated with SARS-CoV-2, found low level virus replication and antibody development in 2 of the 6 animals.¹² A sentinel control animal did not become infected. While this may seem concerning, to date there is no indication that cattle play any role in the human pandemic nor are there any reports of naturally infected bovines.¹² For a natural human-to-cattle SARS-CoV-2 infection to happen, cattle need to be in close contact with an infected human and this may not occur frequently in today's cattle-raising facilities. However, further confirmation of this data is needed and the susceptibility of other ruminant livestock species such as sheep and goats needs to be investigated.

4.3 | SARS-CoV-2 in farmed mink

SARS-CoV-2 in mink behaves differently compared to other animal species. It is commonly associated with severe clinical outbreaks including high morbidity and mortality in infected farms; however, subclinical disease can also occur. So far, outbreaks have been reported in several European countries and in the USA. As a consequence of the various outbreaks seen in mink farms, several culling interventions have been carried out, as outlined in Table 4. Recently, a Chinese research group investigated the biological properties of SARS-CoV-2 in experimentally infected mink.⁵⁰ It was determined that SARS-CoV-2 replicates efficiently in the respiratory tract, as expected, and is transmitted among mink via respiratory droplets. As lesions in mink are similar to humans suffering from COVID-19, the mink model was proposed as a useful animal model to evaluate COVID-19 therapeutics or vaccines.⁵⁰

4.4 | SARS-CoV-2 in wildlife species

Investigations into wildlife species are perhaps under represented at this point in time. In large cats living in zoos, SARS-CoV-2 has been identified on several occasions, including in tigers and lions in New York, NY, USA in April,⁵¹ in a puma in Pretoria, South Africa in July (https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/

[Review?page_refer=MapFullEventReport&reportid=35399](https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/?page_refer=MapFullEventReport&reportid=35399)) and in tigers in the Knoxville, TN, USA in October (https://www.zooville.org/wp-content/uploads/2020/10/028-Zoo-Knoxville-Tiger-Tests-Positive-for-SARS-CoV-2-.pdf?_ga=2.16313462.1707933573.1604353641-1319189766.1604071942). All large cats that were confirmed as infected displayed mild respiratory signs, which promoted investigation, and had been in contact with COVID-19 positive animal handlers. In addition to large cats, research macaques can be readily infected under experimental conditions and also appear to present with mild clinical signs.^{52,53} No data is available on macaques or wild cats living in their natural habitats or any other wild animals.

The search for possible SARS-CoV-2 animal reservoirs in wildlife species is akin to the search for a needle in a haystack. In sub-clinically infected animals, SARS-CoV-2 viremia and shedding would be very short-lived and may not be detected even if recently infected animals were tested. Other alternative approaches to investigate the SARS-CoV-2 human-wildlife interface likely need to be pursued and may need to rely on mass sequencing on water,⁵⁴ air^{55,56} or pooled faecal samples from common areas such as freshwater reservoirs or feeding areas. Interestingly, in August 2020, China announced regular coronavirus tests at wholesale markets (weekly for major markets, monthly for smaller operations), with a focus on knives used at major stands, workers' clothing, surfaces, freezers, meat, seafood, sewage, restrooms, garbage trucks and offices (<https://promedmail.org/promed-post/?id=20200801.7635820>).

5 | SUMMARY

SARS-CoV-2 emerged in the human population towards the end of 2019 and has been spreading at an alarming rate and cases in humans continue to increase. This is predicted to continue until commercial vaccines, which recently became available in selected countries (<https://www.bbc.co.uk/news/uk-55227325>), are approved and have been distributed to a larger number of people, ensuring that a certain proportion of the global population is protected. Pet animals such as cats and dogs do not currently appear to pose a risk to humans; however, continuous monitoring of these animals is warranted. SARS-CoV-2 spillover into farm animals has not been reported to date, but if it happens, it likely happens very sporadically and involves a low number of animals. An exception to this is farmed mink, where SARS-CoV-2 spreads quickly and causes clinical disease in infected animals. As a precaution, nearly all affected mink farms implemented immediate mass culling. The rapid identification of a human-animal spillover event and its removal or containment is critical in safeguarding humans and also other animal species. Careful consideration and attention should be given to other future SARS-CoV-2 spillover events into the animal population in order to effectively control the ongoing pandemic.

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