Literature review: SARS-CoV-2 and COVID-19 - The role of domestic animals in transmission and the associated risks in the context of reproductive technology

Updated 28 January 2021

Executive summary

In December of 2019, numerous cases of undiagnosed pneumonia in people became apparent in Wuhan city, Hubei region, China. It was determined that this emerging disease was caused by a novel coronavirus, SARS-CoV-2, causing the disease COVID-19. On 30 January 2020, due to the large scale of the spread of the disease, WHO declared it to be a Public Health Emergency of International Concern, and on March 11, 2020, as global spread became apparent, SARS-CoV-2 was labelled a pandemic (WHO, 2020).

Members of the International Embryo Technology Society (IETS) are consistently on the front line exposed to animals and their derivatives and given the suspected animal origin of the SARS-CoV-2, it is relevant to review all literature currently available relating to the role of animals in infection and transmission of SARS-CoV-2, particularly in the context of reproduction, and assess the potential role of reproductive material (germplasm, embryos) in transmission.

The current evidence shows that the only animals that have been demonstrated to develop infection with SARS-CoV-2 naturally are members of the families *Felidae, Canidae* and *Mustelidae* families. Of these, only *Felidae* and *Mustelidae* have demonstrated the ability to transmit SARS-CoV-2 horizontally. There have been relatively few cases of natural infection in companion animals (cats, dogs and ferrets) and zoo animals (big cats and gorillas), which all have evidence of contact with a COVID-19 positive human case, and it appears that horizontal intraspecies transmission in these cases is rare.

Experimentally, wild animals including rhesus macaques, Egyptian fruit bats, golden Syrian hamsters, specific rodents, racoon dogs and white-tailed deer demonstrated susceptibility to infection, with evidence of intraspecies transmission in some animals (Frueling et al., 2020; Bosco-Lauth et al., 2021; Palmer et al., 2021; Schlottau et al., 2020; Sia et al., 2020; Shan et al., 2020; Chan et al., 2020).

The most significant development since the previous version of this review has been the outbreaks in farmed mink on many farms in Europe, Asia and the USA, and the documented efficient transmission of SARS-CoV-2 from human to mink, mink to mink, and mink to human, with the emergence of a mink-related variant of SARS-CoV-2. This evidence highlights that particular care should be taken if any assisted reproductive technologies are undertaken in species of the *Mustelidae* family, and emphasises the importance of strict biosecurity protocols and the use of personal protective equipment. In accordance with the ECDC's rapid risk assessment (ECDC, 2020), medically vulnerable people (individuals with risk factors for severe COVID-19 disease, such as the elderly) should avoid handling species of the *Mustelidae* family.

The limited available evidence suggests that it is unlikely that domestic species including dogs, horses and chickens can be infected and transmit the virus. Two experimental studies in ruminants demonstrated that dairy calves developed low level infection but did not transmit the virus to other calves, whereas white-tailed deer developed asymptomatic infection with transmission to indirect contact deer (Ulrich et al., 2020; Palmer et al., 2021). Another experimental study in domestic pigs demonstrated low-level infection but no transmission (Pickering et al., 2021).

Text in blue added 1 February 2021, to update document in response to the availability of new relevant information, with no changes to the original document posted 23 June 2020.

Other than the limited evidence described, studies looking at infection and transmission in livestock, zoo animals (other than big cats), or wild animals are limited, and the paucity of data does not preclude the possibility for other animals to be susceptible to infection with SARS-CoV-2. Currently, there is no evidence of transmission from domestic animals to humans except from farmed mink.

Literature published in the human field suggests that it is unlikely that the virus could be transmitted through semen, and currently there are no studies examining the transmission of SARS-CoV-2 in animal germplasm.

The relatively limited literature about animals suggests the potential for SARS-CoV-2 to be present in respiratory secretions, faeces and urine. Despite the low likelihood that an animal presented for breeding could be infected with SARS-CoV-2, the nature of our work in animal reproduction involves exposure to secretions that may carry infectious agents. Therefore, it is recommended that vigilance be maintained, including exercising routine hygiene, wearing of standard personal protective equipment (mask, gloves, eye protection, disposable gown), and adhering to thorough biosecurity practices, such as regular hand washing and cleaning of contact surfaces.

Introduction

In December of 2019, numerous cases of undiagnosed pneumonia in people became apparent in Wuhan city, Hubei region, China. It was determined that this emerging disease was caused by a novel coronavirus, SARS-CoV-2, causing the disease COVID-19. Since then, the virus has spread to more than 213 countries around the world, with almost 3 million confirmed cases, and over 202 000 deaths attributed to COVID-19 worldwide, as of 28 April 2020 (WHO, 2020).

Since this virus emerged, global research has been hastily conducted to acquire, collate and disseminate rapidly as much information about this virus and disease as possible. This provides a valuable means to inform governments, scientists, public health professionals, medical professionals, and the general public. However, it should be noted that most of the literature currently available on this subject has not been subjected to peer review, and some reports have not yet been verified.

As the international collaborative organisation for advanced reproductive techniques in animals, members of the International Embryo Technology Society (IETS) are consistently on the front line exposed to animals and their derivatives. Given the suspected animal origin of the novel coronavirus, it is pertinent to review all literature currently available related to the role of animals in infection and transmission of SARS-CoV-2, particularly in the context of reproduction, which include domestic (livestock and companion animals), laboratory, zoological, and endangered species, and assess the potential role of reproductive material (germplasm, embryos) in transmission.

Background

- On 31 December 2020 the World Health Organisation (WHO) was notified by Chinese authorities of cases of pneumonia in people linked with a seafood and live animal ‘wet’ market in Wuhan city, Hubei region of China (WHO, 2020).
- On 9 January 2020, WHO identified that the causal pathogen for the cases of pneumonia was a new emerging coronavirus. This coronavirus was temporarily labelled 2019-nCoV (novel coronavirus) (WHO, 2020).
- On 11 February 2020, the novel coronavirus was officially labelled SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) by WHO. The subsequent disease was labelled COVID-19 disease (coronavirus disease) (WHO, 2020).

Text in blue added 1 February 2021, to update document in response to the availability of new relevant information, with no changes to the original document posted 23 June 2020.

- On 30 January 2020, due to the large scale of the spread of the disease, WHO declared it to be a Public Health Emergency of International Concern.
- On March 11, 2020, as global spread became apparent, SARS-CoV-2 was labelled a pandemic (WHO, 2020).
- Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses that infect a wide variety of species, including humans, livestock, and companion animals.
- Coronaviruses are classified in four genera – *Alphacoronavirus, Betacoronavirus, Gammacoronavirus, Deltacoronavirus.*
- *Alpha*- and *Betacoronaviruses* usually infect mammals, and *Gamma*- and *Deltacoronaviruses* usually infect birds and fish.
- SARS-CoV-2 is classified as a *Betacoronavirus*.
- Zhou and others (2020) reported that 2019-nCoV (now named SARS-CoV-2) shares 79.6% sequence identity with SARS CoV, and shares 96.2% sequence identity at a whole-genome level to Bat CoV RaTG13 which was previously detected in *Rhinolophus affinis* (horseshoe bats) from Yunnan province, China. To date, this provides the most substantial evidence that SARS-CoV-2 originated from an animal reservoir, a bat.
- Additionally, it was found that SARS-CoV-2 likely utilises the same cell entry receptor, angiotensin converting enzyme 2 (ACE2), as SARS-CoV (Zhou et al., 2020).

Evidence of infection in animals

At the time of writing the original article (1 May 2020), there were few isolated reports of natural infection with SARS-CoV-2 in animals which were all associated with close contact with a human diagnosed with COVID-19. The majority of reports of SARS-CoV-2 infection in animals have been experimental 'proof of concept' studies under laboratory conditions. At the time of review of this article (1 February 2021), there have been additional reports of natural infection in domestic and zoo animals, many outbreaks in farmed mink, and some experimental studies examining the susceptibility of various animal species. However, there has not been a dramatic shift in understanding of the epidemiological role of domestic animals in transmission of SARS-CoV-2.

Natural conditions

Reports from the World Organisation for Animal Health (OIE):

1) 29 February 2020 – Hong Kong, China – A Pomeranian dog owned by a confirmed COVID-19 patient showing no clinical signs of COVID-19 tested positive to SARS-CoV-2 with qRT-PCR on nasal and oral swabs. Virus isolation negative (OIE, 2020a).
2) 18 March 2020 – Belgium, Netherlands – One cat owned by a confirmed COVID-19 patient presented with clinical respiratory and gastrointestinal signs and tested positive to SARS-CoV-2 by PCR. (OIE, 2020b).
3) 20 March 2020 – Hong Kong – A German Shepherd dog, one out of two dogs owned by a confirmed COVID-19 patient, neither showing any clinical signs, tested positive to SARS-CoV-2 with qRT-PCR and virus isolation. The other dog in the household tested negative (OIE, 2020a).
4) 31 March 2020 – Hong Kong, China – One cat owned by a confirmed COVID-19 patient, showing no specific clinical signs, tested positive to SARS-CoV-2 with qRT-PCR (OIE, 2020a).
5) Note: As of 3 April 2020, Hong Kong has tested more than 29 dogs and 17 cats from households with human COVID-19 cases or close contacts of cases, and apart from the two dogs and one cat, no further animal cases have been found (AFCD, 2020).

6) 6 April 2020 – Bronx, New York, USA – Tiger – Three lions and five tigers presented with a dry cough and wheezing, and presumed exposure to asymptomatic zoo employee with COVID-19. One tiger and one lion were tested, both were positive for SARS-CoV-2 with qRT-PCR (OIE, 2020a). Subsequently, there is an unconfirmed report that the remaining four tigers and two lions also tested positive to SARS-CoV-2 using an unspecified faecal test (WCS, 2020). It is not known whether all animals contracted the virus from the same source or whether transmission between animals occurred.

7) 15 April 2020 – Richmond, New York, USA – Dogs – one out of two German Shepherd dogs from a household with known COVID-19 affected inhabitants showed respiratory illness and tested positive to SARS-CoV-2 with PCR. Virus neutralizing antibodies were detected in both the clinically affected and clinically unaffected dog (OIE, 2020a).

8) 19 and 20 April 2020 – Netherlands – Two mink farms had mink showing respiratory disease and increased mortality rates. Epidemiological investigations and viral sequencing revealed that the mink were infected with SARS-CoV-2 through two separate introductions into the two farms, most likely from contact with infected farm workers, followed by widespread horizontal transmission between mink (Oreshkova et al., 2020). Workers on the farms were reported to have previously shown symptoms of COVID-19, or have been hospitalized with confirmed COVID-19 preceding clinical signs in the mink. However, concerningly, preliminary sequencing results suggest that one person may have contracted the virus from the mink; the investigation is ongoing (Oreshkova et al., 2020). Following this initial outbreak in two mink farms, a further nine mink farms in the Netherlands have become infected (Oreshkova et al., 2020).

9) 22 April 2020 – New York, USA – Two cats from separate households owned by confirmed COVID-19 patients, presented with respiratory signs and tested positive to SARS-CoV-2 with qRT-PCR, and had virus neutralizing antibodies (reported on 29 April 2020) (OIE, 2020a).

10) 30 April 2020 – Paris, France – One cat owned by a suspected COVID-19 patient with mild respiratory and digestive signs tested positive to SARS-CoV-2 on rectal swab, but negative on nasopharyngeal swab (Zientara et al., 2020). Currently (3 May 2020), the number of cats that were tested has not been published.

11) 18 May 2020 – Russia – One cat tested positive to SARS-CoV-2 with RT-PCR of throat and nasal swabs. (OIE, 2020a).

12) 19 and 20 May 2020 – Illinois and Minnesota, USA – in two separate incidences, one cat from a household with known COVID-19 affected inhabitants showing respiratory signs and testing positive to SARS-Co-2 with PCR (OIE, 2020a).

Notifications since the previous version (published 23 June 2020) of this review:

There have been 17 immediate notifications to the OIE reporting SARS-CoV-2/COVID-19 in domestic and zoological animals from several countries in the Americas, Europe, and Asia. The notifications each reported one or more affected animals of a variety of species including domestic cats, domestic dogs, farmed mink, a Puma, Snow Leopards, and a pet ferret. In all the reported cases, there was known contact with a confirmed COVID-19 positive human case prior to presentation with clinical signs and/or being tested. In all cases, human to animal transmission was suspected to have occurred.

Additionally, from other media sources, human to animal infection under natural conditions has been reported in gorillas in a zoo in the USA, and tigers and lions in several different zoos in the USA and Europe.

Serological surveys under natural conditions

Cats

A serological survey of cats in Wuhan including 102 cats sampled after the COVID-19 outbreak from animal shelters and pet hospitals in Wuhan and 39 cat samples from the laboratory serum bank which were collected in Wuhan in 2019, prior to outbreak, were tested for SARS-CoV-2 (Zhang et al., 2020).

No positive samples for viral RNA using a SARS-CoV-2 specific qRT-PCR commercial kit were detected from nasopharyngeal and anal swabs (Zhang et al., 2020).

Using an indirect ELISA based on the SARS-CoV-2 receptor binding domain, 15 from 102 surveyed (14% of cats) were positive for antibodies against SARS-CoV-2. Of the 15, 11 had SARS-CoV-2 neutralising antibody (73% of cats) with titre ranging from 1/20 - 1/1080 (Zhang et al., 2020).

The positive ELISA and VN tests were from cats either in a veterinary hospital (6), strays (6), or owned by a person diagnosed with COVID-19 (3). The cats with the three highest VN titres were owned by COVID-19 patients, contrasting with relatively low titres in cats in animal shelters or pet hospitals, demonstrating that close contact with an infected human lead to higher VN titres (Zhang et al., 2020).

This study demonstrates that cats produced neutralizing antibodies under natural conditions (Zhang et al., 2020).

Cats, dogs, horses

During the validation process of Idexx SARS-CoV-2 RealPCR Test, more than 3500 canine, feline and equine specimens from the United States of America and South Korea were tested, with no positive results (Idexx, 2020). By mid-April more than 5000 samples from Europe, Canada, USA and South Korea have been tested, with no positive results (Idexx, 2020).

Other animals

A serological survey of 1914 samples from 35 different species of animals[1] using a double antigen sandwich ELISA all tested negative for antibodies against SARS-CoV-2, leading the authors to conclude that all of the animal species included in the study could not act as intermediate hosts (Deng et al., 2020). The ELISA test was validated for specificity using samples from pig, mouse, rabbit, and sensitivity using samples from ferrets but was not validated in all 35 of the animal species. It is not clear where some of the animals were sourced or whether they could have had contact with a COVID-19 patient, except for one dog, who was owned by a COVID-19 patient, and tested negative (Deng et al., 2020). This study does not necessary preclude the involvement of all of these animal species in the epidemiology of SARS-CoV-2.

Experimental conditions

Ferrets

Experimental studies conducted in ferrets demonstrate that ferrets are highly susceptible to SARS-CoV-2 infection and effectively transmit the virus by direct or indirect contact. Ferrets were intranasally inoculated with 10^5.5 TCID50 of NMC-nCoV02 strain isolated from a patient in South Korea (Kim et al., 2020). Ferrets subsequently developed mild clinical signs, viral RNA was detected in blood, nasal washes, saliva, urine and faeces for up to eight days and infectious virus was detected in urine and faecal specimens

[1]Species (number): Livestock – pig (187), cow (107), sheep (133), horse (18); poultry – duck (153), chicken (153), goose (25); lab animals – mouse (81), rat (67), guinea pig (30), rabbit (34), monkey (39); companion animal – dog (487), cat (87); wild animals – camel (31), fox (89), mink (91), alpaca (10), ferret (2), bamboo rat (8), peacock (4), eagle (1), tiger (8), rhinoceros (4), pangolin (17), leopard cat (3), jackal (1), giant panda (14), masked civet (10), porcupine (2), bear (9), yellow-throated marten (4), weasel (1), red pandas (3), and wild boar (1).

of the inoculated ferrets (Kim et al., 2020). Direct contact ferrets developed viral RNA in their blood two days after contact demonstrating effective transmission to naïve ferrets (Kim et al., 2020). Indirect contact naïve ferrets also tested positive to viral RNA in nasal washes and faecal samples suggesting airborne transmission (Kim et al., 2020).

Shi and others (2020) also concluded that SARS-CoV-2 replicates efficiently in ferrets, finding that for ferrets inoculated intranasally and intratracheally in separate studies, viral RNA and infectious virus was detected in intranasal washes, and viral RNA was detected in only one rectal swab. All ferrets developed antibodies detected by ELISA and neutralisation assay (Shi et al., 2020).

Cats

Shi and others (2020) concluded that SARS-CoV-2 replicates efficiently in cats. Two repeat experiments using five inoculated cats and one naïve exposed cat repeatedly demonstrated that the exposed cat became infected, apparently through respiratory droplets (Shi et al., 2020).

Other animals

Experimental infection under laboratory conditions demonstrated that SARS-CoV-2 replicates poorly in dogs, pigs, chickens and ducks. Animals were inoculated with 10^5 PFU of samples of SARS-CoV-2 intranasally (Shi et al., 2020). Five inoculated beagles housed with two uninoculated beagles resulted in detection of viral RNA in rectal swabs of the inoculated dogs but no infectious virus detected (Shi et al., 2020). Two inoculated dogs seroconverted, but the rest were antibody negative by ELISA, demonstrating that dogs were not efficiently infected and did not transmit the virus (Shi et al., 2020). The experiment was replicated for pigs, chickens and ducks, all of which tested negative for viral RNA and anti-SARS-CoV-2 antibodies on day 14, leading to the same conclusion (Shi et al., 2020).

Under experimental conditions, golden Syrian hamsters were shown to be infected and transmit the virus effectively and developed antibodies against SARS-CoV-2 (Sia et al., 2020; Chan et al., 2020). Rhesus macaques did not develop overt clinical signs, but viral RNA was detected in upper respiratory tract and anal swab samples (Shan et al., 2020).

To investigate the susceptibility of potential animal hosts and the risk of anthropozoonotic spill-over infections, Schlottau and colleagues (2020) inoculated 9 Egyptian fruit bats, 9 ferrets, 9 pigs and 9 chickens intranasally under experimental conditions, and exposed them to 3 naïve contact animals of the same respective species. They found that Egyptian fruit bats developed transient infection with no clinical signs, and one naïve contact bat became infected, showing characteristics of a reservoir host; ferrets developed infection with transmission to all 3 naïve contact animals, and pigs and chickens were not susceptible to infection by intranasal inoculation (Schlottau et al., 2020).

Experimental intranasal inoculation of 6 dairy calves with SARS-CoV-2 and exposure to 3 uninoculated calves demonstrated low-level replication and specific seroreactivity in 2 calves despite the presence of preexisting antibody titres to a bovine betacoronavirus, with no horizontal transmission (Ulrich et al., 2020). The authors concluded that cattle show a low susceptibility to SARS-CoV-2 infection and are unlikely to play an epidemiological role in the pandemic (Ulrich et al., 2020). Domestic pigs also showed low level infection but did not transmit the virus to naïve pigs (Pickering et al., 2021).

Racoon dogs were investigated in a similar manner with 9 racoon dogs intranasally inoculated with SARS-CoV-2 and exposed to 3 naïve racoon dogs (Frueling et al., 2020). It was found that the racoon dogs were experimentally susceptible to SARS-CoV-2 infection, showed no clinical signs, and transmitted infection to contact animals (Frueling et al., 2020).

A study of peri-domestic wildlife in the USA found that deer mice, bushy-tailed woodrats and striped skunks were susceptible to infection, whereas several small rodents, lagomorphs and racoons were not (Bosco-Lauth et al., 2021).

Experimental intranasal inoculation of 4 white-tailed deer found that all inoculated deer developed infection with transmission to 2 indirect contact deer, but showed no clinical signs (Palmer et al., 2021).

Currently, all of the experimental studies describing infection in animals used inoculations with exceptionally large amounts of virus and relatively few animals. Exposure to this viral load is unlikely under natural conditions and therefore these studies must be interpreted with caution.

Receptors

It appears that SARS-CoV-2 has similar structure to SARS-CoV and also utilizes the host receptor angiotensin converting enzyme 2 (ACE2) (Wan et al., 2020). Analyses based on modelling of the receptor binding protein (RBP) and ACE2 interaction using atomic level structures of SARS-CoV isolated from different animal species over 10 years suggests that the structure of the ACE2 receptor in a number of animal species including pigs, ferrets, cats and non-human primates, may indicate that these animals could act as intermediate hosts (Wan et al., 2020). However, it is noted that other factors affect the infectivity and pathogenesis of SARS-CoV-2.

Additionally, Sun and others (2020) describe high ACE2 expression levels in cats and dogs in various different tissues including the skin, testes, retina, and ear tips, suggesting the potential for these species and tissues to become infected. However, it may be unwise to extrapolate that these animals could be a source of transmission.

Additionally, the significance of ACE2 receptor expression in human reproductive organs (testes, uterus, ovaries) is being investigated with a number of studies suggesting the potential for vertical and sexual transmission, and fertility issues following COVID-19 infection.

Evidence of SARS-CoV-2 animal to human transmission

Since the previous version of this review, many more outbreaks in farmed mink have occurred around the world, resulting in the culling of millions of animals. It has also emerged that human-mink, mink-mink, and mink-human transmission can occur. Mink transmit the virus very efficiently and exhibit a range of clinical signs including respiratory signs, anorexia, weight loss and increased mortality. Mink to human transmission was first documented in April in the Netherlands where epidemiological investigations and genome sequencing identified that mink to human transmission had occurred (Koopman, 2020; Oude Munnink et al., 2021). A significant occurrence in Denmark at the end of 2020, has been the emergence of human infections with a new mink-related variant of SARS-CoV-2 carrying a Y453F mutation in the spike protein, which is a major target of the immune response. There is concern that this mutation could lead to changes in infectivity, antigenicity and transmissibility which prompted the ECDC to conduct a rapid risk assessment (ECDC, 2020). According to the ECDC risk assessment, the overall risk to human health posed by SARS-CoV-2 mink-related variants is:

- no different to other strains for the general population
- low for the general population in areas with a high concentration of mink farms and moderate-to-high for medically vulnerable individuals (individuals with risk factors for severe COVID-19 disease, such as the elderly) living in the same areas
- moderate for non-medically vulnerable individuals with occupational exposure and very high for medically vulnerable individuals with occupational exposure.

Based on this assessment, it would be advised that any members working closely with mink or members of this family take particular care when handling these animals, their excretions or genetic material. Additional biosecurity measures including personal protective equipment and strict cleaning and disinfection protocols should be implemented. It is recommended that individuals that are medically vulnerable do not handle species of the *Mustelidae* family.

Evidence of SARS-CoV-2 in semen or embryos

Currently, there is no literature published related to the presence of SARS-CoV-2 in animal germplasm, however, there are some studies in human COVID-19 patients assessing the potential effects of SARS-CoV-2 on sexual transmission and reproductive function.

Single cell RNA-seq profiling of human testes indicate that ACE2 is expressed in Leydig cells, Sertoli cells and spermatogonia which indicates that theoretically the testes are a potential target for SARS-CoV-2 (Wang and Xu, 2020). In contrast, using single cell transciptome analysis on single testicular cells from testes of three healthy young adults it was determined that there was sparse expression of ACE2 and serine protease TMPRSS2 in testicular cells, therefore the authors concluded that ACE2-mediated viral entry into target host cells was unlikely to occur in the testes (Pan et al., 2020).

It was theorized that high ACE2 receptor expression in the testes, specifically the Leydig cells, Sertoli cells and spermatogonia, may cause the testes to be vulnerable to damage and/or dysregulation from SARS-CoV-2 infection (Ma et al., 2020). A retrospective study of the sex hormone profiles of 81 male COVID-19 patients and 100 healthy male patients (age 20-54 years) detected some significant aberrant results in COVID-19 patients. However, this study did not address whether SARS-CoV-2 could be detected in semen and is therefore of less relevance to this review (Ma et al., 2020).

In another study, single ejaculate semen samples were collected from 34 male patients at a median of 31 days after diagnosis of COVID-19 and tested by qRT-PCR for identification of SARS-CoV-2, however no virus was detected on any of the semen samples. Six of the patients had experienced scrotal discomfort at the time of COVID-19 diagnosis (Pan et al., 2020).

Similarly, semen samples were obtained during the recovery phase from 12 patients diagnosed with COVID-19 (age 22-38 years) by ejaculation and from the testes a patient who died from COVID-19 (age 67 years). The deceased man had test results consistent with acute phase of infection. No SARS-CoV-2 RNA was identified in any of the semen samples or testes of the deceased patient, demonstrating that transmission of the virus via semen in the recovery phase is unlikely (Song et al., 2020). However, whether the virus could be shed in human semen during the acute phase of infection is still unknown in the absence of significant evidence.

In females, it also appears that sexual transmission of SARS-CoV-2 is unlikely. In 35 female patients diagnosed with COVID-19, vaginal discharge, cervical or vaginal residual exfoliated cells and anal swab samples all tested negative for SARS-CoV-2 RNA except for one anal swab sample (Cui et al., 2020).

A study of the potential for intrauterine vertical transmission of COVID-19 in nine pregnant women found that there was no evidence of transuterine infection caused by vertical transmission in women with COVID-19 in late pregnancy (Chen et al., 2020).

However, an analysis by Colaco and others (2020) of single cell RNA seq datasets of human embryos for a number of key cell entry mechanisms for SARS-CoV-2, concluded that human embryos express receptors for SARS-CoV-2 and also have the necessary machinery for virus internalization and replication. The analyses included single cell RNA-seq datasets of human embryos for SARS-CoV-2 receptors ACE2 (receptor for viral entry) and BSG (receptor for viral entry), serine protease TMPRSS2 for viral spike protein priming (entry to cell) and endosomal protease CTSL for spike protein activation via the endosomal route. The results were that ACE2 and TMPRSS2 are co-expressed in the proportion of epiblast cells and BSG and CTSL are expressed during embryonic development.

Infection/transmission during reproductive techniques

There is no literature that specifically relates to the transmission of SARS-CoV-2 in animal germplasm or the risks of exposure associated with veterinary reproductive techniques.

Available evidence suggests that SARS-CoV-2 virus may be present in nasal and ocular discharge, respiratory secretions, faeces, and urine (Kim et al., 2020) (OIE, 2020). Currently there is no conclusive evidence of transmission of SARS-CoV-2 by venereal contact, semen, embryos, or intrauterine transmission.

However, although there is no literature published that specifically investigates animals as fomites for transmission of the virus, as was also found by O'Connor and others (2020), following the recent outbreaks of SARS-CoV-2 infection in mink farms in the Netherlands and Denmark, there is evidence that transmission occurred amongst mink through fomites, infectious respiratory droplets, and dust particles potentially containing faecal matter. This presents a potential exposure risk for farm and animal health personnel. This could also include those involved with assisted reproductive technology in species of the *Mustelid* family, such as mink or ferrets.

Conclusion

Current evidence shows that the only animals that have been demonstrated to develop infection with SARS-CoV-2 naturally are members of the families *Felidae, Canidae* and *Mustelidae* families. Of these, only *Felidae* and *Mustelidae* have demonstrated the ability to transmit SARS-CoV-2 horizontally.

Experimentally, rhesus macaques, Egyptian fruit bats, golden Syrian hamsters, specific rodents, racoon dogs and white-tailed deer show susceptibility to infection, with evidence of intraspecies transmission in some animals (Frueling et al., 2020; Bosco-Lauth et al., 2021; Palmer et al., 2021; Schlottau et al., 2020; Sia et al., 2020; Shan et al., 2020; Chan et al., 2020). It is important to note that a positive viral RNA with PCR does not necessarily imply that the animals have produced infectious virus or that transmission could occur.

There are relatively few cases of natural infection in companion animals (cats, dogs and ferrets) and zoo animals (big cats and gorillas), which all have evidence of contact with a COVID-19 positive human case, and it appears that horizontal intraspecies transmission in these cases is rare. More evidence has emerged for SARS-CoV-2 infection in mink with human to animal, animal to animal, and animal to human transmission documented. Specifically, the emergence of a mink-related variant of SARS-CoV-2 highlights the importance of strict biosecurity protocols and the use of personal protective equipment when handling species particularly of the *Mustelidae* family. In accordance with the ECDC's rapid risk assessment (ECDC, 2020), medically vulnerable people should avoid handling species of the *Mustelidae* family.

The limited available evidence suggests that it is unlikely that domestic species including dogs, horses and chickens can be infected and transmit the virus. Two experimental studies in ruminants demonstrated that dairy calves developed low level infection but did not transmit the virus to other calves, whereas white-tailed deer developed asymptomatic infection with transmission to indirect contact deer (Ulrich et al., 2020; Palmer et al., 2021). A study in domestic pigs demonstrated low level infection but intraspecies transmission did not occur (Pickering et al., 2021).

Other than the limited evidence described, studies looking at infection and transmission in livestock, zoo animals (other than big cats), or wild animals are limited, and the paucity of data does not preclude the possibility for other animals to be susceptible to infection with SARS-CoV-2. Although some studies suggest the susceptibility of some wildlife species to infection, thus far this has been under experimental conditions. Currently, there is no evidence of transmission from domestic animals to humans except from farmed mink.

The very few studies that have been done in humans suggest that it is unlikely that the virus could be transmitted through germplasm; many of these have not been subjected to peer review. Currently there are no studies examining the transmission of SARS-CoV-2 in animal germplasm.

The still relatively small number of studies in animals suggests the potential for SARS-CoV-2 to be present in respiratory secretions, faeces and urine. The presence of viral RNA in environmental samples associated with mink farms demonstrates that fomites and dust must also be considered a potential route for transmission, although infectious virus was not isolated in these instances. Despite the low likelihood that an animal presented for breeding could be infected with SARS-CoV-2, the nature of our work in animal reproduction involves exposure to secretions that may carry infectious agents. Therefore, it is recommended that vigilance be maintained, including exercising routine hygiene, wearing of standard personal protective equipment (mask, gloves, eye protection, disposable gown), and adhering to thorough biosecurity practices, such as regular hand washing and cleaning of contact surfaces.

References

Agriculture, Fisheries and Conservation Department (AFCD) 2020, *Notice: Information for registered veterinary surgeons on the testing of novel coronavirus in pet animals*, Hong Kong Veterinary Association, viewed 4 May 2020, http://www.hkva.org/notice.asp?nid=21.

Bosco-Lauth, AM, Root, JJ, Porter, SM, Walker, AE, Guilbert, L, Hawvermale, D, Pepper, A, Maison, RM, Hartwig, AE, Gordy, P, Bielefeldt-Ohmann, H & Bowen RA, 2021, 'Survey of peridomestic mammal susceptibility to SARS-CoV-2 infection', pre-print, viewed 29 January 2021, https://doi.org/10.1101/2021.01.21.427629.

Chan, J, Zhang, A, Yuan, S, Poon, V, Chan, C, Lee, A, Chan, W-M, Fan, Z. Tsoi, H-W, Wen, L, Ronghui, L, Cao, J, Chen, Y, Tang, K, Luo, C, Cai, J-P, Kok, K-H, Chu, H, Chan, K-H & Yuen, K-Y 2020, 'Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility', *Clinical Infectious Diseases*. 10.1093/cid/ciaa325.

Chen, H, Guo, J, Wang, C, Luo, F, Yu, X, Zhang, W, Li, J, Zhao, D, Xu, D, Gong, Q, Liao, J, Yang, H, Hou, W & Zhang, Y 2020, 'Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records', *The Lancet*, https://doi.org/10.1016/S0140-6736(20)30360-3.

Colaco, S, Chhabria, K, Singh, N, Bhide, A, Singh, D, Singh, A, Husein, A, Mishra, A, Sharma, R, Ashary, N & Modi, D 2020, 'Expression of SARS-CoV-2 receptor ACE2 and the spike protein processing enzymes in developing human embryos', pre-print, viewed 25 April 2020, https://arxiv.org/abs/2004.04935.

Cui, P, Chen, Z, Wang, T, Dai, J, Zhang, J, Ding, T, Jiang, J, Liu, J, Zhang, C, Shan, W, Wang, S, Rong, Y, Chang, J, Miao, X, Ma, X & Wang, S 2020, 'Clinical features and sexual transmission potential of SARS-CoV-2 infected female patients: a descriptive study in Wuhan, China', pre-print, viewed 25 April 2020, https://www.medrxiv.org/content/10.1101/2020.02.26.20028225v2.

Deng, J, Jin, Y, Liu, Y, Sun, J, Hao, L, Bai, J, Huang, T, Lin, D, Jin, Y & Tian, K 2020, 'Serological survey of SARS-CoV-2 for experimental, domestic, companion and wild animals excludes intermediate hosts of 35 different species of animals', *Transboundary and Emerging Diseases,* accepted author manuscript, doi:10.1111/tbed.13577.

European Centre for Disease Prevention and Control, 2020, 'Detection of new SARS-CoV-2 variants related to mink', 12 November 2020, ECDC: Stockholm.

Freuling, CM, Breithaupt, A, Müller, T, Sehl, J, Balkema-Buschmann, A, Rissmann, M, Klein, A, Wylezich, C, Höper, D, Wernike, K, Aebischer, A, Hoffmann, D, Friedrichs, V, Dorhoi, A, Groschup, MH, Beer, M & Mettenleiter, TC, 2020, 'Susceptibility of Raccoon Dogs for Experimental SARS-CoV-2 Infection', Emerging Infectious Diseases, vol. 26, no. 12:2982-2985, doi:10.3201/eid2612.203733.

Idexx 2020, *Corona virus update – Emerging coronavirus strains and veterinary patients*, Idexx, USA, viewed 29 April 2020, https://www.idexx.com/en/veterinary/reference-laboratories/coronavirus-diagnostic-update/.

Kim, Y-I, Kim, S-G, Kim, S-M, Kim, E-H, Park, S-J, Yu, K-M, Chang, J-H, Kim, EJ, Lee, S, Casel, MAB, Um, J, Song, M-S, Jeong, HW, Lai, VD, Kim, Y, Chin, BS, Park, J-S, Chung, K-H, Foo, S-S, Poo, H, Mo, I-P, Lee, O-J, Webby, RJ, Jung, JU & Choi, YK 2020, 'Infection and Rapid Transmission of SARS-CoV-2 in Ferrets', *Cell Host & Microbe*,
doi.org/10.1016/j.chom.2020.03.023

Koopmans, M, 2021, 'SARS-CoV-2 and the human-animal interface: outbreaks on mink farms', *The Lancet Infectious Diseases*, vol. 21, issue 1, pp. 18-19, https://doi.org/10.1016/S1473-3099(20)30912-9.

Ma, L, Xie, W, Li, D, Shi, L, Mao, Y, Xiong, Y, Zhang, Y & Zhang, M 2020, 'Effect of SARS-CoV-2 infection upon male gonadal function: A single center-based study', pre-print, viewed 25 April 2020, https://doi.org/10.1101/2020.03.21.20037267

O'Connor, AM, Totton, SC & Sargeant, JM 2020, 'A rapid review of evidence of infection of pets and livestock with human associated coronavirus diseases, SARS, MERS, and COVID-19, and evidence of the fomite potential of pets and livestock', *SYREAF*, Michigan State University, viewed 19 April 2020, http://www.syreaf.org/.

Oreshkova, N, Molenaar, RJ , Vreman, S, Harders, F, Oude Munnink, BB, Hakze-van der Honing, RW, Gerhards, N, Tolsma, P, Bouwstra, R, Sikkema, RS, Tacken, MGJ, de Rooij,MMT, Weesendorp, E, Engelsma, MY, Bruschke, CJM, Smit, LAM, Koopmans, M, van der Poel, WHM , Stegeman, A 2020, 'SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020', Euro Surveillence, 25(23):pii=2001005. https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005.

Oude Munnink, BB, Sikkema, RS, Nieuwenhuijse, DF, Molenaar, RJ, Munger, E, Molenkamp, R, van der Spek, A, Tolsma, P, Rietveld, A, Brouwer, M, Bouwmeester-Vincken, N, Harders, F, Hakze-van der Honing, R, Wegdam-Blans, MCA, Bouwstra, RJ, GeurtsvanKessel, C, van der Eijk, AA, Velkers, FC, Smit, LAM, Stegeman, A, van der Poel, WHM, Koopmans, MPG 2021, 'Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans', *Science*, vol. 371, no. 6525, pp.172-177. doi: 10.1126/science.abe5901.

Palmer, MV, Martins, M, Falkenberg, S, Buckley, A, Caserta, LC, Mitchell, PK, Cassmann, ED, Rollins, A, Zylich, NC, Renshaw, RW, Guarino, C, Wagner, B, Lager, K & Diel, DG, 2021, 'Susceptibility of white-tailed deer (Odocoileus virginianus) to SARS-CoV-2', pre-print, viewed 29 January 2021, https://doi.org/10.1101/2021.01.13.426628.

Pan F, Xiao X, Guo J, Song Y, Li H, Patel DP, Spivak AM, Alukal J,P, Zhang X, Xiong C, Li PS & Hotaling JM 2020, 'No evidence of SARS-CoV-2 in semen of males recovering from COVID-19', *Fertility and Sterility*, doi: https://doi.org/10.1016/j.fertnstert.2020.04.024.

Pickering, BS, Smith, G, Pinette, MM, Embury-Hyatt, C, Moffat, E, Marszal, P & Lewis, CE, 2021, 'Susceptibility of Domestic Swine to Experimental Infection with Severe Acute Respiratory Syndrome Coronavirus 2. Emerging Infectious Diseases', vol. 27, no. 1, pp. 104-112, https://dx.doi.org/10.3201/eid2701.203399

Rijksoverheid 2020, *News: COVID-19 detected on two mink farms. Rijksoverheid, Netherlands*, viewed 30 April 2020, https://www.rijksoverheid.nl/actueel/nieuws/2020/04/26/covid-19-geconstateerd-op-twee-nertsenbedrijven.

Schlottau, K, Rissmann, M, Graaf, A, Schön, J, Sehl, J, Wylezich, C, Höper, D, Mettenleiter, TC, Balkema-Buschmann, A, Harder, T, Grund, C, Hoffmann, D, Breithaupt, A & Beer, M 2020, 'SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study', *The Lancet Microbe*, vol. 1, issue 5, pp. e218-e225, https://doi.org/10.1016/S2666-5247(20)30089-6.

Shan, C, Yao, Y-F, Yang, X, Zhou, Y-W, Wu, J, Gao, G, Peng, Y, Yang, L, Hu, X, Xiong, J, Jiang, R-D, Zhang, H-J, Gao, X-X, Peng, C, Min, J, Chen, Y, Si, H-R, Zhou, P, Wang, Y-Y & Yuan, Z-M 2020, 'Infection with Novel Coronavirus (SARS-CoV-2) Causes Pneumonia in the Rhesus Macaques', pre-print, viewed 30 April 2020, 10.21203/rs.2.25200/v1.

Text in blue added 1 February 2021, to update document in response to the availability of new relevant information, with no changes to the original document posted 23 June 2020.

Shi, J, Wen, Z, Zhong, G, Yang, H, Wang, C, Huang, B, Liu, R, He, X, Shuai, L, Sun, Z, Zhao, Y, Liu, P, Liang, L, Cui, P, Wang, J, Zhang, X, Guan, Y, Tan, W, Wu, G, Chen, H & Bu, Z 2020, 'Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2', *Science*, Epub ahead of print, doi: 10.1126/science.abb7015.

Sia, SF, Yan, L-M, Chin, AWH, Fung, K, Poon, LLM, Nicholls, JM, Peiris, M & Yen, H-L 2020, 'Pathogenesis and transmission of SARS-CoV-2 virus in golden Syrian hamsters, pre-print, viewed 24 April 2020, https://doi.org/10.21203/rs.3.rs-20774/v1.

Song, C, Wang, Y, Li, W, Hu, B, Chen, G, Xia, P, Wang, W, Li, C, Sha, J, Hu, Z, Yang, X, Yao, B & Liu, Y 2020, 'Detection of 2019 novel coronavirus in semen and testicular biopsy specimen of COVID-19 patients', pre-print, viewed 24 April 2020, doi:10.1101/2020.03.31.20042333.

Sun, K, Gu, L, Ma, L & Duan, Y 2020, 'Atlas of ACE2 gene expression in mammals reveals novel insights in transmission of SARS-Cov-2', pre-print, viewed 24 April 2020, https://doi.org/10.1101/2020.03.30.015644.

Ulrich, L, Wernike, K, Hoffmann, D, Mettenleiter, TC & Beer, M 2020, 'Experimental infection of cattle with SARS-CoV-2', *Emerging Infectious Diseases*, vol. 26, no. 12, pp. 2979-2981, https://doi.org/10.3201/eid2612.203799

Wan, Y, Shang, J, Graham, R, Baric, R & Li, F 2020, 'Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS', *Journal of Virology*, doi10.1128/JVI.00127-20.

Wang, Z & Xu, X 2020, 'scRNA-seq Profiling of Human Testes Reveals the Presence of ACE2 Receptor, a Target for SARS-CoV-2 Infection, in Spermatogonia, Leydig and Sertoli Cells', *Cells*, vol. 9, no. 4, 920, https://doi.org/10.3390/cells9040920.

Wildlife Conservation Society (WCS) 2020, *WCS Newsroom: Update: Bronx Zoo Tigers and Lions Recovering from COVID-19*, Wildlife Conservation Society, New York, viewed 4 May 2020, https://newsroom.wcs.org/News-Releases/articleType/ArticleView/articleId/14084/Update-Bronx-Zoo-Tigers-and-Lions-Recovering-from-COVID-19.aspx.

World Health Organisation (WHO) 2020, *Rolling updates on coronavirus disease (COVID-19),* World Health Organisation (WHO), viewed 25 April 2020, https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen.

World Organisation for Animal Health (OIE) 2020a, *World Animal Health Information System*, World Organisation for Animal Health (OIE), viewed 29 April 2020, https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/WI/index/newlang/en.
- Dog 1 Hong Kong: https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33455
- Dog 2 Hong Kong: https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33684
- Cat Hong Kong: https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33832
- Tiger New York, USA: https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33885
-Lion New York, USA: https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=34054

Text in blue added 1 February 2021, to update document in response to the availability of new relevant information, with no changes to the original document posted 23 June 2020.

- Two cats New York:
https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=34086
- German Shepherd dog New York:
https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=34525
- Cat Illinois:
https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=34590
- Cat Minnesota:
https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=34548
- Cat Russia:
https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=34443
- Updated immediate notifications:
OIE WAHIS – Weekly disease information, viewed 28 January 2021,
https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/WI/index/newlang/en
World Organisation for Animal Health (OIE) 2020b, *Information provided by the National Veterinary Services of Belgium*, World Organisation for Animal Health (OIE), viewed 29 April 2020,
https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/Belgium_28.03.20.pdf.

Zhang, Q, Zhang, H, Huang, K, Yang, Y, Hui, X, Gao, J, He, X, Li, C, Gong, W, Zhang, Y, Peng, C, Gao, X, Chen, H, Zou, Z, Shi, Z & Jin, M 2020, 'SARS-CoV-2 neutralizing serum antibodies in cats: a serological investigation', pre-print, viewed 24 April 2020, 10.1101/2020.04.01.021196.

Zhou, P, Yang, X-L, Wang, X-G, Hu, B, Zhang, L, Zhang, W, Si, H-R, Zhu, Y, Li, B, Huang, C-H, Chen, H-D, Chen, J, Luo, Y, Guo, H, Jiang, R-D, Liu, M-Q, Chen, Y, Shen, X-R, Wang, X, Zheng, X-S, Zhao, K, Chen, Q-J, Deng, F, Liu, L-L, Yan, B, Zhan, F-X, Wang, Y-Y, Xiao, G-F & Shi, Z-L 2020, 'A pneumonia outbreak associated with a new coronavirus of probable bat origin', *Nature*, 579, pp270–273, https://doi.org/10.1038/s41586-020-2012-7

Zhu, H, Guo, Q, Li, M, Wang, C, Fang, Z, Wang, P, Tan, J, Wu, S & Xiao, Y 2020, 'Host and infectivity prediction of Wuhan 2019 novel coronavirus using deep learning algorithm', pre-print, viewed 24 April 2020, 10.1101/2020.01.21.914044.

Zientara, S, Sailleau, C, Dumarest, M, Vanhomwegen, J, Klonjkowski, B, Caro, V, Manuguerra, J-C, Lepoder, S 2020, *COVID-19 (SARS-CoV-2), cat, Paris,* ProMED mail, viewed 4 May 2020,
https://promedmail.org/promed-post/?id=7289409.

Text in blue added 1 February 2021, to update document in response to the availability of new relevant information, with no changes to the original document posted 23 June 2020.