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Animal models of SARS-CoV-2 transmission

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SARS-CoV-2 emerged in China as a zoonotic virus in December 2019. The virus proved to be human-to-human transmissible and its global spread resulted in the ongoing COVID-19 pandemic, associated with high morbidity and mortality. Vaccines were developed at an unprecedented speed and proved to be efficacious in preventing disease, but it remains to be determined if vaccines are able to interrupt transmission. Moreover, virus variants of concern continue to emerge that appear more transmissible and/or less sensitive to virus-specific immune responses. Here, we briefly review the role of animal models in assessing prophylactic and therapeutic options to interrupt SARS-CoV-2 transmission.

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Introduction

In December 2019 a cluster of patients with severe respiratory tract disease was notified in Wuhan, China. A novel coronavirus was rapidly identified as the causative agent and due to the close relationship to severe acute respiratory syndrome (SARS)-coronavirus the virus was named SARS-CoV-2 [1]. Efficient transmission, even by pre-symptomatic and asymptomatic individuals, combined with domestic and international travel, resulted in a pandemic of coronavirus disease-2019 (COVID-19). Phylogenetic comparison with previously identified coronaviruses suggested a zoonotic origin of SARS-CoV-2 [2–4]. A wide diversity of closely related betacoronaviruses was detected in bats and pangolins across Asia [5,6,7••], but the exact source species and potential intermediate host remain elusive.

Human-to-human transmission

Since the majority of COVID-19 outbreaks occurred in household settings or events involving clusters of people in close contact, it is thought that SARS-CoV-2 is primarily transmitted via direct, indirect or close contact with infected individuals through contaminated secretions like saliva, respiratory droplets and aerosols. These droplets and aerosols can be expelled by coughing, sneezing, talking or singing and infect the subsequent host by reaching the respiratory tract or eyes [8–14]. Evidence for long-distance airborne transmission between humans is limited [15]. Despite consistent evidence that SARS-CoV-2 can survive on specific surfaces [16–18,19•], evidence of fomite transmission between humans is also limited. In addition to respiratory transmission, viable SARS-CoV-2 has been demonstrated in urine [20] and stool specimens [21,22] obtained from infected humans, but transmission via the fecal-oral route is thought to be of limited relevance in the spread of the virus among humans.

SARS-CoV-2 RNA can be detected by RT-PCR on nasopharyngeal swab material 1–3 days before symptom onset, with peak values around symptom onset, followed by a gradual decline over time [23–27]. Persistence of RNA for several weeks has been reported, especially in individuals with severe COVID-19 [23,24,28,29]. However, detection of infectious virus is a better measure for transmissibility [30••]. Shedding of infectious virus was initially not thought to occur for more than 8 days after symptom onset [24,31,32], but a recent study showed longer shedding of infectious virus in a selection of patients, which positively correlated with disease severity and viral load. In that study, infectious virus shedding became undetectable with the appearance of neutralizing antibodies in serum [30••]. Comparative studies have shown that the relationship between detection of viral RNA and infectiousness differs between persons with mild and severe disease [33]. The level of shedding of viral RNA differs greatly among individuals, in part explaining the observed highly skewed patterns of transmission towards a limited proportion of human-to-human contacts [34].

Transmission by individuals without symptoms was already suspected early in the pandemic. Two transmission 'types' can be distinguished: transmission by asymptomatic (infected people who never develop symptoms) or by pre-symptomatic (infected people who have not yet developed symptoms) individuals. The true extent of

asymptomatic infections is still unclear, but a recent systematic review and meta-analysis (that included several studies with limitations) estimated the proportion of truly asymptomatic cases to be 1 in 6 infections [35]. A study performed in close contacts of confirmed COVID-19 cases estimated the proportion to be 23% [36]. Both studies demonstrated transmission by asymptomatic individuals, albeit it to a lower extent compared to transmission by symptomatic patients. Pre-symptomatic transmission has often been demonstrated, and is in line with isolation of infectious virus as discussed above. Although frequently demonstrated, the estimated rates of pre-symptomatic transmission vary considerably: from 6.4% up to 44% [25,37,38]. Clearly such parameters are not fixed and may differ for emerging variants with increased transmissibility and/or disease severity.

Emergence of other zoonotic coronaviruses

Two other zoonotic coronaviruses recently emerged to cause outbreaks in humans. In 2003 SARS-coronavirus emerged from bats [39,40], most likely via infection of palm civets as intermediate host [41]. SARS-CoV caused a large outbreak that was initiated by several superspreading events and subsequently amplified in hospitals [42]. The outbreak was associated with high case-fatality rates. However, in contrast to SARS-CoV-2, SARS-CoV proved to be only moderately transmissible among humans. This was probably due to the fact that viral excretion peaked relatively late, around 10 days post symptoms onset [43–45], leading to a predominant occurrence of transmission in the second week of illness. At this stage infectious patients were often hospitalized and most cases of SARS-CoV human-to-human transmission occurred in healthcare settings, potentially due to aerosol-generating procedures. Transmission by pre- or asymptomatic individuals (as described above for SARS-CoV-2) proved to be limited [46]. This strongly facilitated case-based surveillance, contract tracing and isolation measures. Although SARS-CoV was spread to multiple countries, the outbreak was fully contained by non-pharmaceutical interventions [47,48].

In 2012 another zoonotic coronavirus was identified as the causative agent of a cluster of severe respiratory tract disease patients in the Middle East and was named Middle East Respiratory Syndrome (MERS)-coronavirus [49]. Dromedary camels were identified as intermediate host [50,51] and it is thought that the virus likely originated from bats (similar to SARS-CoV and SARS-CoV-2) [49,52]. Although MERS-CoV rapidly spread globally [53], it is generally regarded less human-to-human transmissible than SARS-CoV or SARS-CoV-2, with an early estimate of an R_0 below 1 in the general community and in households [54•]. In healthcare settings human-to-human transmission has led to large outbreaks with higher R_0, providing a warning sign for pandemic potential [55–57]. Continuous zoonotic events are being reported. The current consensus is that close and prolonged contact with an index case seems to be required for MERS-CoV transmission and adaptation would be required for efficient human-to-human transmission.

COVID-19 outbreak containment

Strategies to contain the COVID-19 pandemic were initially limited to non-pharmaceutical interventions, including lockdowns and physical distancing measures. Within a year after onset of the pandemic, several vaccines were developed and shown to be safe and effective in large double-blind placebo-controlled clinical studies [58••,59••,60••,61••]. Large-scale implementation of these vaccines will reduce morbidity and mortality, alleviate the pressure on health care systems and relieve non-pharmaceutical interventions. However, it remains unclear how well vaccines protect from upper respiratory tract shedding and subsequent viral transmission. A recent study found the first evidence of reduced incidence of infection in household contacts of vaccinated healthcare workers, but the level of protection from shedding may differ for different variants, with age, and with time since vaccination [62]. In addition, it remains to be determined what vaccination coverage will be reached eventually, given vaccine hesitancy and disparities in access. Therefore, for the months and years ahead, it is crucial to continue to explore potential additional countermeasures that inhibit SARS-CoV-2 transmission.

Animal models to study coronavirus transmission

Animal models have been instrumental to gain insight into SARS-CoV, MERS-CoV and SARS-CoV-2 replication kinetics, shedding, pathogenesis and medical countermeasures, and have been reviewed elsewhere [63•,64–66]. These models include non-human primates (NHPs), cats, dromedary camels, ferrets, hamsters, rabbits and (transgenic) mice. Since coronaviruses emerge after zoonotic introduction, it is essential to study coronavirus transmission potential and evaluate transmission prevention by medical countermeasures. Animal models of transmission are therefore crucial. For SARS-CoV-2 specifically, this includes assessment of transmissibility of emerging variants, or transmission in the presence of vaccine-induced immunity. In this review, we provide a brief summary of the currently employed animal models to study different aspects of COVID-19, with a focus on transmission in combination with therapeutics that could interrupt transmission.

Transmission studies in animal models are relatively scarce. Natural SARS-CoV-2 transmission in animals has been described in companion animals (e.g. cats and dogs) and on mink farms. Experimental animal transmission models remain limited to the cat, ferret and hamster model. Although it has been shown that rabbits are susceptible to experimental SARS-CoV-2 infection, given

Table 1

Overview of transmission models

	Mouse	Cat	Hamster	Ferret	Fruit bat	NHP[d]
Animal handling BSL3	Easy	Difficult	Easy	Intermediate	Difficult	Difficult
Species relevance	Limited	Limited	Limited	Sialic acids RT[a] resemble human	Close to original host	Close to human
Naturally susceptible	Only variants[b]	Yes	Yes	Yes	Not fully	Yes
Clinical presentation	Variable	Limited disease	Severe disease	Limited disease	Limited disease	Limited disease
Transmission efficacy[c]	Unknown	DC: efficient Aer: not efficient	DC: efficient Aer: efficient	DC: efficient Aer: possible	Limited	Unknown
Reagents availability	Widespread	Limited	Limited	Limited	Limited	Widespread
Transmission literature	Not available	Limited	Multiple publications	Multiple publications	Limited	Not available
References	N/A	[74,76,77]	[97••,104,108]	[87,89,91,94••]	[89]	N/A

[a] RT = respiratory tract.
[b] mouse susceptibility differs per model, dependent on the use of mouse strain / transgenic mice. Some models are susceptible to variants of concern [63•,117].
[c] DC = direct contact, aer = aerosol.
[d] NHP = non-human primates.

the high dose needed to establish productive infection, transmission of the virus from rabbit-to-rabbit is unlikely [67]. Ferrets thus far appear an ideal model to study transmission in the absence of clinical signs, whereas hamsters are more appropriate to study transmission in the context of pathology and severe disease. NHPs are rarely used for transmission studies, in part due to practical limitations in high-containment facilities. An overview of the characteristics of different animal models and their usability in transmission studies is shown in Table 1.

Human-to-animal transmission: companion animals

Naturally occurring human-to-animal transmission was already reported during the SARS-CoV outbreak in 2002/2003. Viral RNA was detected in oropharyngeal swabs obtained from cats after contact with infected individuals, and infections were confirmed serologically [68]. Susceptibility of cats was subsequently confirmed experimentally [69]. Because of these reports, several studies performed in Hong Kong describe swabbing of companion animals in close contact with confirmed COVID-19 cases. Here, household transmission to dogs [70] and cats [71] was observed, but in most cases infected animals remained asymptomatic [72]. This remains to be determined for emerging variants [73]. Experimental infections confirmed susceptibility of cats, but dogs proved poorly susceptible [74,75].

Cats were also evaluated as an animal model for SARS-CoV-2 transmission. A first study assessed airborne transmission among cats housed in adjacent cages (also allowing for respiratory droplet transmission) and demonstrated that experimental transmission is feasible, although this remained limited to infection of 1 out of 3 sentinel animals [75]. Direct contact transmission in cats was more robust, showing infection of all sentinel animals in several separate small studies [74,76,77].

Human-to-animal transmission: outbreaks on mink farms

Large outbreaks of SARS-CoV-2 infection have been reported on mink (*Neovison vison*) farms in the Netherlands, Spain, Italy, Sweden, Greece, the US and Denmark, initiated by a human-to-mink transmission [78]. Circulation and spread initially went unnoticed as mustelids usually develop asymptomatic or mild disease, and transmission on some farms was only confirmed after serological surveys. However, upper and lower respiratory tract involvement has been described [79,80]. Transmission on mink farms was rapid and widespread after initial introduction. Once introduced, it has proven difficult to stop transmission on a farm, and ongoing transmission between farms was described [81•]. Large-scale culling of mink was initiated when it was observed that ongoing SARS-CoV-2 transmission led to an accumulation of mutations in the S protein, and that these viruses could spill back from mink farms into the community in Denmark and possibly the Netherlands [81•,82–84].

Experimental infection or transmission studies in mink have not been performed to date. Mink belong to the family of mustelids (*Mustelidae*), together with ferrets. Ferrets were already shown to be highly susceptible to SARS-CoV in 2003 [69], and are now in widespread use to study SARS-CoV-2 transmission and pathogenesis.

Experimental SARS-CoV-2 transmission studies: ferrets

Ferrets (*Mustela putorius furo*) are an invaluable model to study the pathogenicity and transmission of respiratory viruses [85]. In SARS-CoV-2 infection studies, it was shown that ferrets rarely develop clinical signs of disease. Weight loss is not observed, some studies report a slight and transient fever. Additionally, mild respiratory symptoms (e.g. nasal discharge) and mild haematological changes (e.g. lymphopenia) have occasionally been reported [75,86–89]. However, although clinical symptoms are absent or mild, there is substantial shedding of SARS-CoV-2 observed in the respiratory tract, starting at day 2 and sometimes still detectable by RT-PCR two weeks post inoculation [87,90•,91]. Since ferrets develop no or relatively mild disease and histopathological changes, combined with RT-PCR detectable viral loads and shedding from the respiratory tract, ferrets are regarded an optimal model for asymptomatic or mild disease in humans and are often used in transmission studies [92]. Recently, it was shown that aged ferret (>3 years old) have higher viral loads, longer shedding and more severe lung inflammatory cell infiltration, accompanied by more clinical symptoms (fever and weight loss) compared to juvenile and young adult ferrets [93].

SARS-CoV-2 transmission via direct contact was found to be the most efficient route of transmission, since all naive contact animals became infected during co-housing in different transmission studies [87,89,91]. SARS-CoV-2 transmission via the air over short distance (i.e. animals were separated by a maximum distance of 10 cm) occurred in two out of six [87] and three out of four animals [91], whereas transmission via the air over more than one meter distance occurred in two out of four ferret pairs [94••]. Despite the similarities in transmission efficiencies between the studies, differences were observed in the robustness of infection of recipient animals and the time after exposure that animals became infected. Whereas in most studies infection by either route resulted in a similar duration and level of virus shedding in (in) direct recipient animals as compared to the donor animals [89,91,94••], Kim *et al.* detected only low levels of SARS-CoV-2 RNA in nasal washes of the indirect recipient ferrets, virus shedding was shorter and no infectious virus was isolated [87].

Because of efficient SARS-CoV-2 direct contact transmission, ferrets are a sensitive model to evaluate prophylactic and therapeutic intervention strategies aimed at preventing virus transmission. As such, the model was used to demonstrate that prophylactic daily intranasal administration of a stable fusion inhibitory lipopeptide, which interacts with the HRN region of S_2 and prevents conformational changes in the S protein, could completely prevent SARS-CoV-2 direct contact transmission during 24-hour co-housing with infected animals [90•]. In another study, a ribonucleoside analogue inhibitor initially intended as influenza treatment (MK-4482/EIDD-2801) was evaluated in the ferret model as a therapeutic treatment. Treatment with orally administered MK-4482/EIDD-2801, twice a day, significantly reduced the SARS-CoV-2 load in the upper respiratory tract of ferrets and prevented transmission to untreated contact animals that were exposed 30 hours after inoculation of donors [95•].

Experimental SARS-CoV-2 transmission studies: hamsters

Molecular docking studies on the binding between Ace2 and the S protein initially suggested that the Syrian golden hamster (*Mesocricetus auratus*) is a suitable small animal model for SARS-CoV-2 pathogenesis studies [96,97••]. Indeed, upon intranasal challenge hamsters develop acute but transient respiratory distress, peaking at 4–5 days post inoculation, and lose weight, followed by an eventual recovery [96,97••,98]. SARS-CoV-2 infection of hamsters is associated with high viral loads and prolonged detection of viral genomes in oral swabs and nasal washes by RT-PCR. Hamsters additionally show clear histopathological lesions [96,98,99]. Upon comparison of the course of SARS-CoV-2 infection in young and aged hamsters, it was found that replication kinetics are similar but aged hamsters exhibit more pronounced weight loss, combined with more histopathological lung damage [100]. Because hamsters recapitulate (part of) COVID-19 disease as observed in humans and they are a relatively easy to handle, hamsters are a valuable model to study severe COVID-19 in humans. Furthermore, hamsters can be used to screen antiviral agents in a disease model [101]. Finally, hamsters have been used in adoptive transfer and vaccination challenge studies [102,103].

Although viral RNA can persist in the hamster respiratory tract, infectious virus can be detected only for a short period after intranasal inoculation, leaving a potentially small window for transmission. However, transmission of SARS-CoV-2 to naive animals that were co-housed with infected animals in a direct contact setup is robust [96,97••,104]. It was determined that the minimal amount of contact time between infected and naive hamsters required for efficient transmission is 4 hours [105] or less [97••]. In addition, despite the prolonged detection of viral RNA in nasal washes and throat swabs for over two weeks, transmission only occurs during the first six days post inoculation [97••]. Aerosol transmission models for hamsters have also been developed, are robust, and more efficient than fomite transmission [97••,104]. Interestingly, hamsters infected through direct contact transmission develop no or only limited weight loss, in contrast to directly inoculated animals.

Prior exposure of hamsters to SARS-CoV-2 resulted in protection from re-infection, with significantly reduced virus replication in the upper respiratory tract [106]. Virus