Animal Models for COVID-19

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of coronavirus disease 2019 (COVID-19), an emerging respiratory infection caused by introduction of a novel coronavirus into humans in late 2019 in China's Hubei province. As of July 24, 2020, SARS-CoV-2 has spread to 215 countries, has infected more than 16 million people, and has caused more than 640,000 deaths. Since humans do not have pre-existing immunity against SARS-CoV-2, there is an urgent need to develop therapeutics and vaccines to mitigate the current pandemic and to prevent the re-emergence of COVID-19 in the future. In February 2020 the World Health Organization (WHO) assembled an international panel of experts to develop animal models for COVID-19 to accelerate testing of vaccines and therapeutics. This review summarizes the findings to date and provides relevant information for preclinical testing of COVID-19 vaccine candidates and therapeutics.

Although there are discrepancies in the estimated case-fatality ratio (CFR) of COVID-19 in humans, it is clear that severity is age-stratified and that the CFR in patients over 65 years of age is likely higher than 1% [1]. Initially, infection with SARS-CoV-2 is characterized by a range of mild symptoms, including fever, cough, dyspnea and myalgia [2]. Partly these are caused by the capacity of SARS-CoV-2 to replicate efficiently in the upper respiratory tract. While most patients subsequently resolve the infection, the disease may also progress to severe pneumonia. In severe cases, bilateral lung involvement with ground-glass opacities are the most common chest computed tomography (CT) findings. Disease progression can then involve acute respiratory distress syndrome (ARDS), and in some cases an inflammatory syndrome resembling septic shock. Histological examination of the lungs of patients showed bilateral diffuse alveolar damage (DAD), pulmonary edema and hyaline membrane formation

[3]. COVID-19 is also characterized by damage to other organ systems associated with coagulopathy and characterized by elevated fibrinogen and D-dimer levels, indicating increased thrombus generation and fibrinolysis [4]. Those at higher risk of severe COVID-19 are individuals with underlying conditions such as obesity, diabetes, hypertension, chronic respiratory disease and cardiovascular disease [1].

Through the 'Solidarity' trials, the WHO has launched a global campaign to test therapeutics and vaccines on an unprecedented scale [5]. In order to test these and other potential medical countermeasures, it is imperative to identify animal models for COVID-19 that provide measurable readouts for potential interventions and that utilize representative virus isolates [6]. To this end, the WHO R&D Blueprint team established an *ad hoc* expert working group focused on COVID-19 disease modelling (WHO-COM). In this review we provide a summary of the current literature on COVID-19 animal models (Supplementary Table 1), including studies generated by the WHO-COM group since February 2020, which we hope will serve to facilitate further preclinical analysis of vaccines and therapeutics.

Mouse Models

The main impediment for SARS-CoV-2 infection of murine cells is the lack of appropriate receptors to initiate viral infection. SARS-CoV-2, like SARS-CoV, uses the cellular surface protein angiotensin-converting enzyme 2 (ACE2) to bind and enter cells, and murine ACE2 does not effectively bind the viral spike protein [7]. To solve this problem some strategies have been developed as follows:

Virus adaptation to mACE2

The spike protein of SARS-CoV-2 can be modified to gain effective binding to murine ACE2. One strategy is sequential passaging of SARS-CoV-2 in mouse lung tissue [8]. This method is successful because RNA virus populations consist of a mutant swarm of closely related viral quasispecies. Rare viruses in the swarm containing spike mutations that increase their binding affinity to murine ACE2 are expected to be selected due to their higher levels of replication in murine lungs. Alternatively, mouse adaptation of SARS-CoV-2 can be achieved using reverse genetics to modify the receptor-binding domain (RBD) of the virus so that it can infect murine cells via the mouse ACE2 protein. Using these approaches, mice were sensitized for infection but developed very mild disease [9]. It is likely that additional efforts aimed at mouse-adaptation will result in the outgrowth of additional virus variants that can cause more severe disease. These mice will be useful for pathogenesis studies and for studies of antivirals and vaccines. One potential caveat is that the mutations in the SARS-CoV-2 spike protein that enhance affinity for the mouse ACE2 receptors are located in the receptor binding domain, the primary target for the neutralizing antibody response. These mutations could result in a wild type virus neutralizing mAb being falsely considered non-neutralizing.

Expression of human ACE2 in genetically modified mice

A different approach consists of modifying the mice to express hACE2. There are currently three transgenic models in which hACE2 is under the expression of a tissue-specific promoter (e.g., for epithelial cells, *Krt18* [10]), a universal promoter (CMV enhancer followed by the chicken beta-actin promoter [11]), or the endogenous *mACE2* promoter [12]. All of these mice are susceptible to infection by SARS-CoV-2, but the differences in hACE2 expression result in a

pathogenic range from mild to lethal disease. In particular, with the exception of the model in which hACE2 is controlled by the mouse ACE2 promoter, all other models develop encephalitis after infection with SARS-CoV [13] or SARS-CoV-2 [14]. However, while SARS-CoV infection of K18-hACE2 mice results in a highly lethal encephalitis, the neurological infection caused by SARS-CoV-2 infection in these mice is less severe. Some mice appear to succumb to severe pneumonia, at times when the brain infection is not substantial [15]. These mouse models provide a stringent test for vaccine and therapeutic efficacy and may be useful for studies of pathogenesis.

An alternative approach that mirrors tissue-specific expression of human ACE2 is to substitute the *mACE2* gene by the *hACE2* gene. Similar models expressing human dipeptidyl peptidase-4 (DPP4), the Middle East respiratory syndrome coronavirus (MERS-CoV) receptor, have been successfully developed [16-18]. One such hACE2 humanized mouse has now been reported, and supports replication of SARS-CoV-2 in respiratory and brain tissues, although mice do not develop severe disease [19]. However, more severe disease is expected to occur in hACE2 knock-in mice if virus is passaged serially through mouse lungs. Overall, these mice will probably be very useful models of human disease especially if combined with viral adaptation that increases virulence of SARS-CoV-2 in mice.

Finally, instead of permanent genetic modification, it is also possible to generate mice susceptible to SARS-CoV-2 infection by sensitizing the respiratory tract of these animals to SARS-CoV-2 replication through transduction with adenovirus (Ad5) or adeno-associated virus (AAV) expressing hACE2. This system, pioneered for MERS studies [20], allows transient replication of SARS-CoV-2 in lungs of mice for several days until immune clearance, and it

has the advantage that it can be applied quickly to different strains of mice. Upon infection with SARS-CoV-2, Ad5-*hACE2* mice develop a widespread infection of the lungs and histopathological changes consistent with a viral pneumonia. Mice developed clinical disease characterized by changes in body scoring (hunching) and weight loss. Virus is generally cleared by 7 days after infection, although not in some immunocompromised mice [21] [14]. Mice sensitized via AAV-*hACE2* delivery are also susceptible to SARS-CoV-2 infection, but virus replication seems to be lower than in Ad5-*hACE2* transduced mice [22]. Ad5-*hACE2* and AAV-*hACE2* sensitized mice are useful for evaluating vaccines and antiviral therapies, as well identifying SARS-CoV-2-specific antibody and T cell epitopes. A limitation with these mice, as well as in some of the transgenic hACE2 mice, is that hACE2 is expressed ectopically, which may change the tissue or cellular tropism of the virus.

Other mouse models and approaches

Additional ongoing efforts to develop mouse models for SARS-CoV-2 infection studies involve mice humanized with hACE2 and human hematopoiesis, and Collaborative-Cross (CC) mouse studies. Mice transplanted with human immune cells or human immune system (HIS) mice, have been widely used to study human-specific viral infections [23,24] and the combination of HIS and ACE2 expression could help to further explore the efficacy of vaccines and therapies, in particular those that modulate human immune cells. Similarly, previous studies using the CC model of genetic diversity, a panel of recombinant inbred mice with expanded susceptibility to viruses that normally do not cause disease in laboratory mice can be used to enhance virus disease susceptibility, however, infection remains heavily dependent on a functional entry receptor [25,26]. CC mice were previously used with mouse-adapted SARS-CoV to identify mechanisms of pathogenesis and genetic loci that determine

susceptibility [27]. Presumably, CC studies could enable exploration of an expanded range of SARS-CoV-2 phenotypes in mice that potentially better recapitulate human disease, as mouse adapted strains become available.

In summary, a variety of murine models for mild and severe COVID-19 have been described, or are under development. All will be useful for vaccine and antiviral evaluation and some share features with the human disease. No murine model at present recapitulates all aspects of human COVID-19, especially unusual features such as the pulmonary vascular disease and hyperinflammatory syndromes observed in adults and children, respectively [28,29]. However, continued refinement may eventually result in models for even these aspects of the human disease.

Syrian Hamster Model

Syrian hamsters (*Mesocricetus auratus*) are small mammals that have been used as animal models for other respiratory viruses, including SARS-CoV, influenza virus, and adenovirus [30-33]. *In silico* comparison of the angiotensin-converting enzyme 2 (ACE2) sequence of humans known to interact with the SARS-CoV-2 spike glycoprotein receptor-binding domain (RBD) with that of hamsters [34] suggested that Syrian hamsters might be susceptible to SARS-CoV-2 infection. Indeed, upon experimental intranasal infection, Syrian hamsters show mild to moderate disease with progressive body weight loss starting very early after infection (days 1-2 post-inoculation). All animals challenged by different groups and with different SARS-CoV-2 isolates consistently showed respiratory signs, including labored breathing [34,35]. Additional signs of morbidity included lethargy, ruffled fur and hunched posture [34]. After two weeks of

infection, hamsters typically recovered. Of particular interest is the fact that SARS-CoV-2 infection in hamsters reflect some of the demographic differences of COVID-19 in humans. Thus, aged hamsters as well as male hamsters seem to develop a more severe disease than young and female hamsters respectively [36,37] (Table 1).

SARS-CoV-2 disease in hamsters is associated with high levels of virus replication and histopathological evidence of disease, which included ground glass opacities and evidence of gas in the cavity surrounding the lungs [37]. These findings are similar to those previously reported for SARS-CoV infection in this model [31]. Viral RNA is readily detected in the respiratory tract and other tissues, such as the small intestine, which could be useful for the evaluation of therapeutics and vaccines. Virus transmission to cage mates has also been observed [34], suggesting that hamsters could be useful in transmission studies. Histologically, inflammatory infiltrates with abundant viral antigen expression and apoptosis were observed in the upper and lower respiratory tract starting at 2 days post-infection (dpi), being most severe at 4 dpi and resolving at 14 dpi. Among the non-respiratory tract tissues, only the intestine demonstrated viral antigen expression in association with severe epithelial cell necrosis, damaged and deformed intestinal villi, and increased *lamina propria* mononuclear cell infiltration. Lung disease was also demonstrated by CT. High-resolution micro-CT scans showed airway dilation and significant consolidations in the lungs of infected hamsters [35]. A quantitative analysis revealed an increase of the non-aerated lung volume in these hamsters. This method thus allows quantitative monitoring of disease without the need of euthanizing the animals.

Expression of chemokines/cytokines in the lungs of hamsters peaked at 4 dpi and then gradually resolved at 7 dpi. Interferon-γ and proinflammatory chemokines/cytokines were potently induced at 2 dpi and 4 dpi and dropped to the baseline level at 7 dpi. SARS-CoV-2 induced lung pathology in hamsters appears to be driven by immune pathology, as lung injury at 4 dpi is markedly reduced in STAT2 knockout hamsters while viral loads are massively increased and viral RNA disseminated in multiple peripheral tissues [35]. Serum neutralizing antibodies were detected as early as 7 dpi. Passive immunization of naïve hamsters with these convalescent serum samples resulted in significantly reduced respiratory tract viral loads but no obvious improvement in clinical signs and histological changes. Furthermore, SARS-CoV-2 can be transmitted between hamsters via close contact and non-contact routes [34,38]. Transmission via fomites on the other hand was possible, but not efficient [38].

Since studies in hamsters can be completed quickly and in a cost-effective manner, there is an increasing interest in the use of this model for screening of therapeutics. Until now, lack of/or limited efficacy was demonstrated for the re-purposed drugs hydroxychloroquine (with or without azithromycin) and favipiravir [39,40], whereas a YF17D-vectored SARS-CoV-2 vaccine candidate conferred efficient protection against SARS-CoV-2 challenge in hamsters [41]. Adoptive transfer of SARS-CoV-2 neutralizing antibodies protected hamsters from SARS-CoV-2-induced disease [42]. A putative caveat of the model is the lack of research tools for this species which are still scarce compared for example with those for mice.

Ferret Models

313 Ferrets (*Mustela putorius furo*) have been shown to be a highly valuable model to test
314 pathogenicity and transmission of human respiratory viruses, including influenza virus and
315 respiratory syncytial virus (RSV) [43,44]. It is thus not surprising that the ferret model has been
316 investigated for studies of COVID-19 pathogenesis and SARS-CoV-2 transmission. Despite
317 the use of different isolates of SARS-CoV-2, the results have been remarkably consistent
318 across all laboratories.
319
320 Following mucosal exposure with SARS-CoV-2, clinical alterations in ferrets are undetectable
321 or mild and may include lethargy, nasal discharge, wheezing, oropharyngeal mucus buildup,
322 sneezing, and loose stool [45]. Ferrets infected by small-particle aerosol had similar disease,
323 albeit at 100-fold lower doses. Peaks of elevated body temperatures have been observed in
324 some studies, although alterations in body weight are absent or minimal. Minor alterations in
325 hematological parameters such as mild lymphopenia and neutrophilia have also been
326 observed. SARS-CoV-2 virus shedding is observed in nasal and oropharyngeal swabs [46-49].
327 As with Syrian hamsters, virus replication is detected in the upper respiratory tract very early
328 after infection (day 2) and is detectable during two weeks of infection. Virus replication in
329 ferrets appears to be restricted to the respiratory and GI tracts (Table 1).
330
331 The predominant histopathology findings in SARS-CoV-2 infected ferrets euthanized at the
332 peak of virus replication include mixed (pyogranulomatous or eosinophilic and histiocytic)
333 inflammation within alveolar spaces and perivascular mononuclear inflammation. In addition,
334 in the larger airways of these animals, bronchial submucosal granulomatous foci with
335 eosinophilic material and collagen fragments (suggesting collagen degeneration) were

observed. Microscopic findings in euthanized animals were mild and included bronchoalveolar or alveolar inflammation.

Ferrets also are able to transmit virus efficiently to uninfected animals in experimental settings. Efficient transmission occurred from experimentally infected ferrets to naïve cage mates; transmission from exposed ferrets to companion animals that were separated by steel grids did occur but was not efficient [48,50]. These studies indicated that airborne transmission of SARS-CoV-2 can occur, and suggested that the ferret model may be useful for further transmission studies.

Studies performed to date strongly indicate that experimental SARS-CoV-2 infection in ferrets results in a predominantly upper respiratory tract infection. These findings make the ferret model especially suited to test the efficacy of mucosal vaccines and therapeutics aimed to prevent upper airway infection and/or transmission.

Non-Human Primate Models

Non-human primate models have been explored for COVID-19 in rhesus macaques, cynomolgus macaques, and African green monkeys. Studies from several laboratories have shown high levels of viral replication, including both viral RNA and infectious virus, in both the upper and lower respiratory tract, pathologic features of viral pneumonia, and variable induction of mild clinical disease [51-54]. Only mild clinical disease has been reported in nonhuman primates, and insufficient comparable data exists at this time to determine if there is more clinical disease in rhesus macaques, cynomolgus macaques, or African green

monkeys. Induction of innate, humoral and cellular immune responses as well as robust protection against re-challenge has also been reported, demonstrating induction of natural protective immunity in this model [53]. NHPs inoculated via multi-route mucosal, intrabronchial, or aerosol exposure routes showed radiographic abnormalities (chest X-ray, CT scan, or 18FDG-PET scan) within two days that tended to resolve by days 11-15 post-infection. Evidence of live virus shedding has been found in both the respiratory and GI tract. In addition, hematological changes with evidence of T cell activation, mild lymphopenia and neutrophilia may be observed in infected NHPs.

Infection with SARS-CoV-2 in the elderly is also associated with adverse clinical outcome. Currently, two NHP studies in rhesus and cynomolgus macaques have focused on the effect of age on SARS-CoV-2 infection [52,55]. Both studies showed that aged animals shed virus from nose and throat for longer compared to young adult animals. In rhesus macaques, higher viral loads were also detected in lung tissue of aged animals. In addition, advanced age in rhesus macaques was also associated with increased radiological and histopathological changes. These studies highlight the importance of including age in the selection criteria of animals, as testing treatment options for severe disease require animal models that recapitulate the disease seen in humans.

Recent studies have reported the immunogenicity and protective efficacy of prototype inactivated virus vaccines and DNA vaccines in the rhesus macaque model [56,57]. In these studies, the vaccines induced binding and neutralizing antibodies and resulted in substantial reductions of viral replication in the lower respiratory tract, and to a lesser extent the upper respiratory tract, following SARS-CoV-2 challenge. These findings raise the possibility that

vaccines may be more effective at blocking lower respiratory tract disease compared with upper respiratory tract disease. Anamnestic immune responses were observed following challenge, even in animals that had no virus detected after SARS-CoV-2 challenge, suggesting that protection with these particular vaccines is likely mediated by rapid immunologic control rather than true sterilizing protection. Vaccine-elicited neutralizing antibody titers also correlated with protective efficacy [56].

Additional Animal Models

Mink

The mink (*Neovison vison*), which is listed in the zoological family Mustelidae, was shown previously to be susceptible to SARS-CoV infection [58] and also mink lung epithelial cells and lung-derived cells could be infected with SARS-CoV [59]. Minks also turned out to be naturally susceptible for SARS-CoV-2 infection. In the Netherlands, an infection of mink with SARS-CoV-2 in two breeding farms was detected at the end of April 2020, most likely as the result of contact with a SARS-CoV-2-infected farm worker [60]. In contrast to ferrets, minks displayed moderate respiratory signs which included labored breathing, and some animals died as a result of infection. SARS-CoV-2 virus was found in the majority of throat and rectal swabs, collected from dead animals from both farms. Similar to ferrets, the viral loads in mink were higher in the throat swabs than in the rectal swabs. While minks may represent a suitable model for moderate to severe COVID-19, these animals are difficult to handle under laboratory conditions.

Cats

Three experiments so far have demonstrated that domestic cats (*Felis catus*) are highly susceptible to SARS-CoV-2 infection, and are able to transmit the virus to naïve contact cats [49,61,62]. For example, the inoculation of the Chinese SARS-CoV-2 isolate CTan-H through the intranasal route into juvenile (70-100 days old) and subadult cats (6-9 months old) resulted in virus replication in the upper and lower respiratory tract as well as the GI tract. Both experimentally infected and contact cats seroconverted. At necropsy, interstitial pneumonia, loss of cilia and epithelial necrosis as well as inflammation in nasal turbinates and trachea were observed. The authors did not describe clinical signs in any of the infected cats, except that two juvenile cats (out of 10 total) died on day 3 and 13 post-infection [49]. Virus antigen was found in epithelial cells of the nasal turbinates, necrotic debris in the tonsil, submucosal glands of the trachea and enterocytes of the small intestine. SARS-CoV-2 transmission by droplets was also demonstrated [49]. While cats may represent a suitable model for asymptomatic to moderate COVID-19, the benefits should outweigh the concerns of using companion animals for research; also, cats are rather difficult to handle in biosafety level 3 (BSL-3) containment and are not a standard animal model. However, due to their close contact to humans, additional studies e.g. on environmental contamination (cages, beds, food/water bowls, litterboxes, etc.) or transmission efficiency studies may be important to inform veterinary and public health authorities about the risk of cats as intermediate hosts/virus carriers in the SARS-CoV-2 human-animal interface.

Dogs

Dogs (*Canis lupus familiaris*) have been shown to be susceptible to SARS-CoV-2, but to a very mild degree. Two experiments so far have been published in this species, concluding that dogs have a low susceptibility to the SARS-CoV-2 infection [49,62].

Pigs

In silico data suggested that swine ACE2 should bind the SARS-CoV-2 spike protein. However, several experimental infections performed in pigs (*Sus scrofa domesticus*) by different research groups indicate that this species is not susceptible to SARS-CoV-2 infection in vivo [48,49]. No clinical signs and no clear evidence of virus replication have been observed in pigs. Therefore, pigs do not appear to represent a suitable animal model for COVID-19. Conversely, previous studies reported SARS-CoV infection in pigs [63]. Experimental infection of pigs with SARS-CoV resulted in detection of viral RNA in the blood and seroconversion, but not in clinical signs or virus isolation, which ruled out pigs as amplifying hosts for SARS-CoV [64]. In contrast, infection with another bat betacoronavirus, called swine acute diarrhoea syndrome coronavirus (SADS-CoV), has been demonstrated in swine [65]. Therefore, due to their importance as livestock species and the enormous global number of pigs, it may be still important for future studies to address the putative susceptibility of additional pig breeds to SARS-CoV-2 infection.

Chickens and Ducks

At least one in silico study using the informational spectrum methodology proposed chicken as a potential susceptible animal species for SARS-CoV-2 infection [66]. However, the limited experimental studies performed so far have suggested that chicken, including embryonated chicken eggs, and ducks are not susceptible to SARS-CoV-2 infection [48,49]. Neither chicken

nor ducks appear to represent suitable animal models for SARS-CoV-2 infection studies. These findings are similar to those previously reported for SARS-CoV infection, in which experimental inoculation of different bird species with SARS-CoV, including chickens, resulted in neither replication nor seroconversion [67].

Fruit bats

Bats are regarded as the natural reservoir of many coronaviruses including SARS-CoV and SARS-CoV-2 [68,69]. Intranasal inoculation of fruit bats (*Rousettus aegyptiacus*) with SARS-CoV-2 resulted in efficient replication in the upper respiratory tract and seroconversion in 7 out of 9 animals. Transmission occurred to one out of 3 direct contact animals. Clinical signs were absent, but rhinitis could be detected by immunohistology [48]. Conversely, previous studies showed that a SARS-like coronavirus did not replicate in fruit bats after experimental inoculation [70]. These findings suggest that, although *Rousettus* bats are not the original reservoir species of SARS-CoV-2, experimental infection of these fruit bats could help to model the physiopathology of the virus in its host.

Preclinical Alternatives to Animal Models

Historically animal alternatives for studying respiratory viruses have involved in vitro approaches such as cell lines (e.g. Vero cells, A549, MDCK) or primary tissue-derived human cells in conventional cell cull culture. However, over the past decade advances in engineering, cell biology, and microfabrication have come together to enable development of new human cell-based alternatives to animal models. In this regard, microengineered organs-on-chips and lung organoids have been shown to support key hallmarks of the cytopathology

and inflammatory responses observed in human airways after infection with SARS-CoV-2 and have served to study human disease pathogenesis and test new candidate COVID-19 therapeutics and expedite drug repurposing [71,72].

Conclusions

Since SARS-CoV-2 emerged in the human population in late 2019, it has spread via human-to-human transmission to most countries in the world, leading to a coronavirus pandemic of an unprecedented scale. Under the umbrella of the WHO, the WHO-COM is fostering the development of COVID-19 animal models through international exchange of protocols, unpublished data and ideas across many laboratories in the world. As discussed in this review, an important number of studies have been conducted, many of them by members of the WHO-COM, indicating that most of the animal models susceptible to infection with SARS-CoV-2 show mild clinical disease, while others do not support viral replication.

In studies based on the three dimensional X-ray structure of SARS-CoV-2 spike protein bound to human ACE2, Zhai et al discussed the variance observed between 19 different animal species as well as within three colonies of the same species of bat from different provinces within China. This analysis noted that many predicted ACE2 receptor affinities (especially dog and pig) did not match their relative natural resistance to SARS-CoV-2. This was proposed to be due to differences in the ACE2 expression levels between species in the respiratory epithelium [73]. Similarly, a recent study aimed to predict the host range of SARS-CoV-2 by a comparative structural analysis of ACE2 in more than 400 vertebrates. These data show discrepancies between the predicted susceptibilities and those experimentally

observed, with ferrets for example predicted to have very low susceptibility to infection [74]. These data suggest that susceptibility to infection may be a function of several factors, including genetic ACE2 composition, organ-specific ACE2 expression and other host factors such as additional receptors and host immune responses.

One immediate goal of the group is to evaluate whether mimicking human co-morbidities, co-infections or the immune senescence associated with age in these animal models may result in more severe disease phenotypes. The existing animal models have also been valuable for testing vaccines and therapeutics. Several vaccine candidates have shown protection in rhesus macaques [56,57], and both the cynomolgus and the rhesus macaque models have been useful for testing of therapeutics [75]. Many of the pathogenesis studies described in this review have also highlighted an important caveat in COVID-19 research, which are the methods utilized to measure virus replication. The group found that viral RNA/genome copy numbers measured by qPCR assays were three to four orders of magnitude higher than infectious virus titers measured by cell culture assays. These findings have important implications for the future evaluation of vaccines and therapeutics.

There have been concerns that coronaviruses might pose a risk of vaccine-associated enhanced respiratory disease (VAERD) or antibody-dependent enhancement (ADE) of virus entry and replication in Fc receptor-bearing cells [76]. These types of syndromes have been linked to vaccines that induced substantial levels of non-neutralizing antibodies or type 2 helper CD4 T cell (Th2) biased responses. Therefore, evaluating the relative potency of neutralizing activity to overall binding antibody and obtaining evidence for CD4 T cell subset biased responses through cytokine production or antibody subtype response patterns would

be informative. To ensure such models are able to provide these vital readouts, it is important to attempt to induce VAERD in COVID-19 challenge models using suboptimal doses of candidate vaccines or antigenic preparations specifically designed to induce the required detrimental immune profile and associated lung pathology. Such studies are of high priority for the WHO-COM group, which intends to provide some guidance on the potential for vaccine-elicited immunopathology. In summary, the work presented here demonstrates that there are a number of potential small and large animal models that investigators can utilize to explore important aspects of COVID-19, including mechanisms underlying the pathology, transmission, and host response to the infection as well as the safety and efficacy of potential therapeutics or vaccines.

Future studies should standardize challenge models to allow comparison of different vaccine candidates, and should establish animal models for assessing VAERD. In the context of VAERD, the establishment of a positive control allowing comparisons between different vaccine candidates in preclinical models is a high priority. Continued refinement and development of COVID-19 animal models will contribute to the development of vaccines, therapeutics, and other countermeasures.

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Table 1. SARS-CoV-2 infection in humans and in animal models. This table illustrates the main features of SARS-CoV-2 infection in animal models and whether these features are also present in human COVID-19. NHP: Non-human primates; CNS: Central nervous system; GI: gastro-intestinal; ARDS: Acute respiratory distress syndrome.

Virus replication	
Upper respiratory tract	Humans, mice, hamsters, ferrets, NHP, minks, cats, bats
Other organs	Humans, hACE2 mice (CNS), hamsters, ferrets (GI tract), NHP (GI tract)
Clinical signs	
Fever	Humans, ferrets
Nasal discharge	Humans, ferrets
Labored breathing	Humans, hamsters
Pneumonia	
Bilateral lung involvement	Humans, hamsters, NHP
Ground-glass opacities	Humans, hamsters, NHP
Focal edema, inflammation	Humans, hamsters, ferrets, NHP
ARDS	Humans
Transmission	
Humans, hamsters, ferrets, cats, bats	
Immunology	
Seroconversion	Humans, hamsters, NHP, ferrets, bats, mice
Neutralizing Ab titers	Humans, hamsters, NHP, ferrets, mice
Demographics	
More severe disease: males	Humans, hamsters
More severe disease: aged	Humans, hamsters, NHP