

## Review Article



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### Conflict of Interest

The authors declare no potential conflicts of  
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### Abbreviations

AAV, adeno-associated virus; AAV-hACE2,  
adeno-associated virus-mediated hACE2  
expression; ACE2, angiotensin-converting  
enzyme 2; AGM, African green monkey;

# Experimental Animal Models of Coronavirus Infections: Strengths and Limitations

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## ABSTRACT

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since the emergence of SARS-CoV-2 in the human population in late 2019, it has spread on an unprecedented scale worldwide leading to the first coronavirus pandemic. SARS-CoV-2 infection results in a wide range of clinical manifestations from asymptomatic to fatal cases. Although intensive research has been undertaken to increase understanding of the complex biology of SARS-CoV-2 infection, the detailed mechanisms underpinning the severe pathogenesis and interactions between the virus and the host immune response are not well understood. Thus, the development of appropriate animal models that recapitulate human clinical manifestations and immune responses against SARS-CoV-2 is crucial. Although many animal models are currently available for the study of SARS-CoV-2 infection, each has distinct advantages and disadvantages, and some models show variable results between and within species. Thus, we aim to discuss the different animal models, including mice, hamsters, ferrets, and non-human primates, employed for SARS-CoV-2 infection studies and outline their individual strengths and limitations for use in studies aimed at increasing understanding of coronavirus pathogenesis. Moreover, a significant advantage of these animal models is that they can be tailored, providing unique options specific to the scientific goals of each researcher.

**Keywords:** SARS-CoV-2; COVID-19; Animal models; Emerging infectious disease

## INTRODUCTION

Coronaviruses are pleomorphic, enveloped, single-stranded, positive-sense RNA viruses belonging to the family *Coronaviridae*. Further classification under the *Coronavirinae* subfamily includes division into 4 genera, alpha, beta, gamma, and delta, based on phylogenetic relationships and genomic structures (1). In general, coronaviruses infect a wide range of hosts. The alpha and beta coronaviruses are reported to infect only mammals. On the other hand, gamma and delta coronaviruses infect birds, but can infect mammals to some extent (2).

Coronaviruses cause respiratory and intestinal infection in animals and humans. While most human coronaviruses induce self-limiting respiratory tract infections (HCoV-NL63, HCoV-229E, and HCoV-OC43), some can cause severe infections in the elderly, infants,

BAL, broncho-alveolar lavage; COVID-19, coronavirus disease 2019; dpi, days post-infection; hACE2, human angiotensin-converting enzyme 2; HFH-4/FOXJ, hepatocyte nuclear factor-3/forkhead homologue 4; HIS, human immune system; IRF3, interferon regulatory factor 3; MERS-CoV, Middle East respiratory syndrome coronavirus; NAb, neutralizing antibody; NHP, non-human primate; S, spike; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; scRNA, single-cell RNA.

#### Author Contributions

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and young children (2). In November 2002, there was an emergence of the severe acute respiratory syndrome coronavirus (SARS-CoV), followed by Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, and the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late December of 2019, all of which are beta-coronaviruses (3). Of these 3 coronavirus outbreaks, only SARS-CoV-2 has caused an ongoing pandemic, with over 100 million confirmed cases and a 2.1% case mortality rate as of February 2021 (4).

Although several SARS-CoV-2 vaccines have already been licensed, the host immune components involved in generating a successful immune response to this virus have not yet been fully elucidated. Thus, further understanding of the immunopathologies that arise from aberrant host immune responses against SARS-CoV-2 is needed. To address the remaining questions and further understanding the detailed mechanisms underlying disease pathology, suitable animal models that recapitulate the human immune response to SARS-CoV-2 infection are needed. Thus, this review aims to discuss the use of different animal models for pathological and immunological studies on SARS-CoV-2 in order to generate a better and more diverse understanding of viral pathogenesis and the host immune response.

## RECEPTOR, VIRUS ENTRY, AND HOST IMMUNE RESPONSE

Previous studies on SARS-CoV lead to the discovery of the cell entry receptor, angiotensin-converting enzyme 2 (ACE2) (5,6). The ACE2 protein mediates several different functions including catalysis and amino acid transport, as well as acting as a viral receptor (7). ACE2 are expressed in many different cells of the body (8), however, ACE2 expression in the oral, nasal, and nasopharyngeal epithelium as well as on type II pneumocytes are believed to be important for SARS-CoV-2 transmission and respiratory manifestations (7). Due to the high nucleotide identity (79%) between SARS-CoV and SARS-CoV-2, ACE2 was hypothesized to be a common receptor for these viruses by computational studies (9-11), which was later proven by experimental studies (6,12). Cell entry is mediated by the SARS-CoV-2 spike (S) glycoprotein, which binds to the ACE2 receptor and subsequently activated via the transmembrane protease serine 2, a protease that operates as a cofactor enhancing SARS-CoV-2 entry by proteolysis of both viral S protein and the ACE2 receptor (13,14).

Studies of animal and human coronaviruses indicate that both the innate and adaptive immune responses are important mediators of host protection against these viruses. In general, innate immune cells express pathogen-recognition receptors, such as C-type lectin receptors, NOD-like receptors, RIG-I-like receptors, and TLRs, to recognize pathogen-associated molecular patterns (15). Coronaviruses are recognized by cytosolic and endosomal RNA sensors including RIG-I and TLR3, TLR7, and TLR8. Ligation of these cellular receptors results in activation of transcription factors including NF- $\kappa$ B and interferon regulatory factor 3 (IRF3), leading to expression of pro-inflammatory cytokines, chemokines, and type I IFN (16-18). Type I IFN activates JAKs and STAT via the IFN $\alpha/\beta$  receptor. JAKs phosphorylate STAT proteins, which then form a complex with IRF3 (19). These complexes stimulate the expression of IFN-stimulated genes, including antiviral proteins and important mediators of the innate immune response. However, most viruses have several strategies to antagonize the host's innate immune system by targeting viral sensors or blocking the downstream antiviral signaling molecules. For example, studies showed that non-structural proteins of coronaviruses impede the host innate immune response, and especially IFN signaling pathways (20-24). In addition, a recent study reported that SARS-CoV-2 induced delayed

type I IFN response (21). The virus immune evasion provides a window for uncontrolled viral replication, leading to a surge of hyperactive host immune response (cytokine storm) later during infection, which eventually resulting in detrimental outcomes.

Adaptive immunity to SARS-CoV-2 infection involves T and B cell immune response and generation of antiviral neutralizing Abs (NAbs) (25). In general, after T cell activation, CD4<sup>+</sup> and CD8<sup>+</sup> T cells differentiate into effector T cells and cytotoxic T cells, respectively. CD4<sup>+</sup> T cells function as a helper or effector producing cytokines and chemokines, while CD8<sup>+</sup> T cells specifically kill virus-infected target cells. The B cell immune response against SARS-CoV-2 is characterized by the production of mainly IgG and IgM Ab. During SARS-CoV infection, seroconversion was detected as early as 4 days and generally within 10–16 days (25). In contrast, MERS-CoV patients were found to seroconvert 2–3 weeks post-onset of infection (26), and an absent or delayed Ab response was strongly associated with severe and fatal cases. Recent studies reported that SARS-CoV-2 infection induces very low levels of NAbs in mild disease while high neutralizing titers were only detected in severe cases of coronavirus disease 2019 (COVID-19) (27-29). Correlation of Ab levels with disease severity of COVID-19 had been reported from several studies, however, not yet fully elucidated (30-32). Furthermore, given that adaptive immune response is critical for the control of infection and future re-infection, however, several studies reported that many patients with severe SARS-CoV-2 infection develop lymphopenia (33,34). Although the mechanism behind is still an open question. Therefore, understanding the immune responses, proper innate and adaptive immune response, elicited after SARS-CoV-2 infection is critical for early recognition and control of coronavirus infection and replication within the host and protection against reinfection.

## ANIMALS NATURALLY INFECTED WITH SARS-CoV-2

Several animal species, such as companion animals, captive tigers and lions in New York zoo, and minks, have been reported positive for SARS-CoV-2 (35-37). Whereby, transmission to these animals were primarily due to closed contact with infected humans. Among these animals, cats including domestic cats, tigers and lions showed to be more susceptible to SARS-CoV-2. While sustained SARS-CoV-2 infections within domestic setting is implausible, however, environment within intensive farms with susceptible animals may pose higher probability of zoonotic or reverse zoonotic transmission. In April 2020, minks from 2 separate farms in the Netherlands showed respiratory and gastrointestinal disorders, mortality rates of 1.2% to 2.4%, and lesions of interstitial pneumonia (37,38). The outbreak was presumably introduced by infected employee with a subsequent pervasive transmission between animals in the farm. Auspiciously, direct impact of SARS-CoV-2 to animals only cause transient clinical infections, and most companion and captive animals showed less susceptibility, relatively weak viral replication and showed no strong evidence of sustained transmission from animal-to-humans or animals-to-animals. However, further research is warranted to definitively determine probable role of these animals to community transmission of SARS-CoV-2.

## EXPERIMENTAL ANIMAL MODELS

### Mouse model

The mouse (*Mus musculus*) has been adopted as a primary animal model for various virological and immunological investigations due to several advantages, including cost-effectiveness,

ease of handling, and suitability for large-scale studies (39). However, wild-type and inbred mouse strains cannot be used for SARS-CoV-2 research due to the low affinity between the viral S protein and the murine ACE2 receptor, an interaction required to initiate virus infection (40). Advanced biotechnology has provided several excellent tools to resolve this issue. These include the generation of transgenic animals expressing human ACE2 (hACE2) and were used to elucidate by providing significant findings regarding SARS-CoV-2 pathogenesis.

Transgenic mouse strains expressing the hACE2 receptor have been developed using different promoters, allowing an avenue for precision research (41-45). Hepatocyte nuclear factor-3/forkhead homologue 4 (HFH-4/FOXJ) is a transcription factor with expression highly restricted in cells possessing motile cilia (39), hence, used as a promoter in developing HFH4-hACE2 transgenic mice. HFH4-hACE2 transgenic mice overexpress hACE2 under the control of the HFH4/FOXJ promoter and have been shown to be permissive to SARS-CoV-2 infection (37,38). Thus, using a ciliated cell-specific promoter, it was postulated that hACE2 will be expressed in the ciliated epithelial cells of the respiratory tract (46). HFH4-hACE2 mice exhibited only minimal weight loss during the course of infection; however, severe pneumonia was observed as characterized by extensive fibroplasia and alveolar necrosis. In addition, significant lymphopenia and neutrophilia in peripheral blood were also observed. The K18-hACE2 transgenic mouse (known as B6.Cg-Tg(K18-ACE2)2PrImn/J), in which hACE2 expression is driven by the epithelial cell cytokeratin-18 promoter, was originally developed for SARS-CoV and subsequently evaluated for SARS-CoV-2 infection (42). Both the HFH4-hACE2 and the K18-hACE2 transgenic mice exhibited virus replication in the lungs, lung inflammation, and body weight loss after infection (47,48). Disease severity in these TG mouse models correlates with hACE2 expression levels, however, hACE2 is also highly expressed in the brain leading to neurological-related mortality (42,43,48). This finding is in contrast to COVID-19 in humans as central nervous system infection is rarely observed (49). Therefore, there are some issues with the use of hACE2 transgenic mice for SARS-CoV-2 pathogenesis and immunological studies. Further, another limitation with this mouse model has been its limited availability and difficulty obtaining it in a reasonable timeframe in certain countries during the 2020 COVID-19 pandemic. To circumvent this, researchers developed mouse-adapted SARS-CoV-2 strains, avoiding the need for hACE2 mice and permitting use of more readily available mouse strains (50,51). In addition, researchers have also transferred hACE2 into mouse lung cells using adenoviral vectors to rapidly produce mice susceptible to SARS-CoV-2 infection (52,53). For example, Hassan et al. (53) rendered mice susceptible to SARS-CoV-2 infection through the use of a recombinant human adenovirus type 5-expressing hACE2, which resulted in the expression of hACE2 in the lower respiratory tract of wild-type mice. In these mice, SARS-CoV-2 replication was found to be restricted to the respiratory tract where it caused the development of mild to moderate interstitial and perivascular inflammation. However, with this approach, there is the possibility that an antiviral immune response might be elicited against the adenoviral vector. In addition, adeno-associated virus (AAV)-mediated hACE2 expression (AAV-hACE2) has also been developed. This is another vector-based delivery system for the expression of hACE2 in the mouse respiratory tract (54). SARS-CoV-2-infected AAV-hACE2 mice had inflammatory pulmonary infiltrates and developed SARS-CoV-2 S-specific IgG and NAbS at 4–7 days post-infection (dpi). However, studies in the transduced hACE2 mouse model have generated inconsistent results, possibly due to non-uniform transduction of the lung epithelium leading to variable hACE2 expression in the mouse lung (55).

Currently, most of what is known with respect to viral pathogenesis and host immune responses has been derived from studies using mouse models. However, the genomic

comparison has revealed significant variation between the human and murine immune systems, bringing the suitability of this animal model for the study of human physiology into debate (56,57). These disparities include differences in leukocyte subsets, TLRs, defensins, and Ab subsets, which may underlie the divergent immune response sometimes seen between the 2 species (39,58). To address this, Brumeau et al. (59) developed a human immune system (HIS)-humanized animal model expressing hACE2. This HIS mouse model mimics the human immune system making it an appealing for the study of the mechanisms of human-like anti-viral immune responses (59,60). In fact, the HIS mouse model is readily infected with SARS-CoV-2 at doses as low as  $2.8 \times 10^3$  pfu through the intranasal route, and importantly, recapitulates the main immunopathological events and immune responses seen in COVID-19 patients. In addition, the HIS mouse can also generate human IgM and IgG Abs that are specific for the SARS-CoV-2 RBD protein (59). Despite the limitations of humanized mice, this mouse model shows promise as a tool for studying SARS-CoV-2 pathogenesis and the resulting human immune response.

### Hamster model

The golden Syrian hamster (*Mesocricetus auratus*) has been used for many decades to study human-associated diseases and infections caused by other respiratory viruses such as SARS, adenovirus, and influenza virus (61). Analysis of the ACE2 gene showed that the Syrian hamster differs at only 4 out of 29 amino acids in the SARS-CoV-2 S-contacting regions of ACE2 in humans (62). In addition, previous animal studies showed that SARS-CoV, which also uses the ACE2 receptor for cell entry, can replicate robustly in Syrian hamsters (63). Thus, this suggested that the golden Syrian hamster would also be suitable as an experimental animal model for investigating SARS-CoV-2 infection. To this end, Sia et al. (64) recently reported that Syrian hamsters are susceptible to SARS-CoV-2 infection. Following inoculation, Syrian hamsters develop moderate interstitial pneumonia leading to transient mild to moderate disease. Infection with high titers of SARS-CoV-2 was also found to cause severe pathological lesions in the lungs of Syrian hamsters (63). In addition, induction of marked SARS-CoV-2 replication in the lungs, a strong inflammatory response with neutrophil infiltration, and edema were also observed (65). Boudewijns et al. (65) showed that the SARS-CoV-2-induced lung pathology observed in hamsters appears to be caused by the host immune response. Several studies showed significant evidences that the dysregulation of the innate immune system contributes to the clinical presentation of severe SARS-CoV-2 infection (66,67). The hyperactivity of the innate immune system, causing cytokine storm, has been postulated as pathological driver in severe SARS-CoV-2 infection (68,69). Furthermore, T cell immunosuppression due to adaptive immune dysfunction may increase the risk of SARS-CoV-2 systemic dissemination (70). Further, an immunological study in hamsters showed that the type I IFN response, and especially the STAT 2 signaling pathway, is key in the pathogenesis of SARS-CoV-2. A study in STAT2<sup>-/-</sup> hamsters showed that STAT2 signaling promotes severe lung injury caused by SARS-CoV-2, but restricts systemic viral dissemination (65). Furthermore, a study in RAG2 KO hamsters showed that the absence of functional B and/or T cells exacerbates pathogenesis at the early stage after SARS-CoV-2 exposure (71).

Studies showed that SARS-CoV-2-infected hamsters can mount a strong humoral immune response with the development of potent Nabs, which can confer protection from subsequent infection, within 14 days (63,72). Further, passive transfer of convalescent hamster sera has a protective effect and reduces viral loads in recipient hamsters after SARS-CoV-2 infection (62,63). It is noteworthy that NAb titers were higher in younger hamsters than in their aged

counterparts (72), a direct contrast to what has been observed in COVID-19 patients where lower NAb titers were reported in younger COVID-19 patients (<50 years old) compared to that in elderly COVID-19 patients. Several cohort studies postulated that disease severity was associated with the production of higher NAb titers against SARS-CoV-2 (30-32), though this correlation needs further verification. However, a higher viral load may lead to more severe disease, but may also trigger the generation of a stronger Ab response late during infection due to increased levels of viral antigen.

Compared to other animal models Syrian golden hamsters exhibit a more consistent lung disease phenotype during SARS-CoV-2 infections. Despite the fact that this animal model recapitulates the main immunopathological events and immune responses observed in COVID-19 patients, Syrian hamsters do not succumb to SARS-CoV-2 infection, which does not match the human clinical profile. Recently, another hamster model, the roborovski dwarf hamster (*Phodopus roborovskii*), was found to be highly susceptible to SARS-CoV-2 (73). These animals also exhibited a rapid onset of severe clinical disease, mimicking the clinical and pathological outcomes observed in severe human COVID-19. However, mortality was not observed even in roborovski dwarf hamsters, outside of humane euthanasia required at a bodyweight loss of 15% (in contrast to the requirement at 25% body weight loss in the murine model).

While hamsters have the advantage of the ease of handling, this model is less frequently used due to the limited availability of research reagents (74), such as immunologic reagents for examining host immune responses, particularly for protein expression, and transgenic disease models which are required for a more complete evaluation of the host response. Nonetheless, hamsters exhibit viral pathogenesis and immune responses consistent with those seen in humans and are capable of transmitting the virus. Hence, this strengthens the argument for use of hamsters as an animal model for SARS-CoV-2 studies.

### Ferret model

The ferret (*Mustela putorius furo*) has been well characterized as an animal model for infection with many respiratory viral pathogens due to their natural susceptibility to human respiratory viruses (75-77). In addition to the presence of the appropriate viral receptor, histo-anatomical features of the ferret respiratory tract are analogous to that of the human respiratory tract. These include the anatomical proportions of the upper and lower respiratory tract, glandular density in the bronchial wall, and the number of generations of terminal bronchioles (78-81). In addition, SARS-CoV-2 replicates efficiently in the respiratory tract of ferrets without prior adaptation (78,79). Unlike other small mammalian models, numerous clinical signs found in humans following SARS-CoV-2 infection are also present in ferrets after experimental intranasal inoculation. These include nasal discharge, wheezing, mucus build-up in the oropharynx, sneezing, and loose stool. Thus, this further supports the significance of this animal model for the investigation of viral transmission by measuring the capacity of SARS-CoV-2 to spread to naïve ferrets through direct contact or indirectly through respiratory droplets (78,79). Another advantage of the ferret model is its size. Compared to rodents their large size allows for more repeated blood sampling at volumes sufficient for immunological analyses throughout the course of infection (81). Thus, this increases the ability to monitor the progression of the host immune response.

Recently, Kim et al. (82) found evidence of reinfection with a heterologous variant of SARS-CoV-2 in ferrets, which occurred in a manner correlating with decreased NAb titers. Thus, ferrets with high NAb titers were found to show attenuated virus titers in both the respiratory

and digestive tracts following reinfection thereby leading to rapid viral clearance. This suggests Nabs play a critical role in cross-protection against heterologous SARS-CoV-2 reinfection. With the emergence of several virus variants, SARS-CoV-2 has been grouped into more than 6 different clusters (83). Thus, determining the potential for and consequence of reinfection with SARS-CoV-2 sequence variants is critical to further understand the development of protective immunity against this virus.

Although ferrets are outbred animals, they are considered specific-pathogen-free because they are bred under a controlled condition and raised in facilities following strict biosecurity protocols solely for medical research. Studies performed with ferrets, in contrast to non-human primates (NHPs), have the advantage of eliminated additional confounding factors that may affect the result of certain immunological or viral pathogenesis studies. Hence, they are considered a good animal model for assessing the efficacy of novel vaccines and therapeutic approaches (81). While ferrets provide usefulness and value as a comparative model in which to study the pathogenesis and transmission of human respiratory diseases, the greatest limitation of this model is the inadequate availability of ferret-specific immunological reagents. This impedes the in-depth determination of any immunological mechanisms underpinning viral disease transmission, protection, and immunopathology in this animal model. To overcome this limitation, researchers have employed a revolutionary approach using single-cell RNA (scRNA) sequencing to decipher the transcriptional signatures of immune cells and can be used to identify stochastic variations in gene expression within a single population (84-86). A study by Lee et al. (87) employed this scRNA technique and found an increase in response to IFN by certain subsets of natural killer cells and CD8<sup>+</sup> T cells, suggesting increased expression of the IFN-responsive genes might have a beneficial effect on the rapid clearance of SARS-CoV-2.

Therefore, ferrets represent a useful animal model not only for viral transmission and pathogenesis studies but also for the investigation of the safety and efficacy of vaccines and therapeutic approaches for emerging viruses. Thus, given the important contribution of the ferret model to virus research, additional ferret-specific immunologic, genomic, and proteomic tools are currently being developed.

### NHP model

The phylogenetic proximity of NHPs to humans makes these animals invaluable as models and as a result, they are widely recognized as ideal animal models for emerging and re-emerging diseases. Coronavirus studies, in particular, have greatly benefited from the use of NHPs as infection and disease models. NHPs, including rhesus macaques (*Macaca mulatta*) (88-91), cynomolgus macaques (*Macaca fascicularis*) (90,92), African baboons (*Papio hamadryas*) (89), and African green monkeys (AGMs) (*Chlorocebus aethiops*) (93) have all been demonstrated to be permissive for SARS-CoV-2 infection and to exhibit symptoms also observed in COVID-19 patients such as fever, diarrhea and clinical manifestations of pneumonitis.

Recently, Lu et al. (90) compared SARS-CoV-2 infection in 3 different non-human primates, rhesus macaque, cynomolgus macaque, and common marmoset. Of the 3 species of NHPs, only rhesus and cynomolgus macaques were permissive to SARS-CoV-2 and manifested symptoms of infection. Moreover, both macaque species showed detectable viral RNA in different extrapulmonary organs, including the urinary and digestive tracts, as well as in hilar and mesenteric lymph nodes. However, rhesus macaques showed more severe lung lesions than those in cynomolgus macaques. Although SARS-CoV-2 specific Abs were

detectable as early as 4 dpi in the majority of the macaques used, adult and elderly rhesus macaques displayed higher virus-specific Ab titers than did young rhesus macaques. Rhesus and cynomolgus macaques also showed increased inflammatory cytokine expression, IL-1a, IL-8, IL-15, and MCP-1, including anti-inflammatory IL-10 that play a role in the underlying pathophysiology. In addition, transient lymphopenia was also observed, similar to what has been seen in humans (94,95), with the greatest decrease in cell number observed at 10–12 dpi.

A comprehensive study by Singh et al. (89), evaluated the fitness of the Indian rhesus macaque, African baboons, and common marmosets animal models for SARS-CoV-2 infection, and found that common marmosets suffered from a more mild form of infection. Compared to African baboons and Indian rhesus macaques, marmosets had accelerated clearance of viral RNA with only 1 of 6 infected marmosets positive at 6 dpi. Although African baboons and Indian rhesus macaques showed a similar degree of infection, African baboons, and especially older animals, develop a more severe lung pathology compared to Indian rhesus macaques. This is believed to be due to the persistence of SARS-CoV-2 in the lung compartment coupled with higher expression of several cytokines, chemokines, and inflammatory markers resulting in more severe inflammation in the lungs. Cytokine analysis of infected Indian rhesus macaques showed a significant elevation of IL-6, IFN $\alpha$ , IFN $\gamma$ , IL-8, perforin, IP-10, MIP1 $\alpha$ , and MIP1 $\beta$  in the broncho-alveolar lavage (BAL) fluid. In comparison, MIF, IL-6, C-reactive protein, and IP-10 were significantly increased in BAL fluid of African baboons. Moreover, IL1Ra, perforin IL-8, plasma IP-10, RANTES, and IFN $\alpha$  levels were also increased in BAL fluid and serum.

Similar to rhesus and cynomolgus macaques, AGMs recapitulate mild manifestations of human SARS-CoV-2 infection with a pronounced viral pneumonia. According to Woolsey et al. (93), a notable aspect of using AGMs is that they can develop virus-induced pneumonia, exhibited by pulmonary consolidation and hemorrhage along with increases in serum levels of IL-6, IFN- $\beta$ , IL-10, and other pro-inflammatory cytokines after infection. Moreover, AGMs seroconverted past 5 dpi with IgG titers as high as 1:6,400.

Age-related differences in disease manifestation following SARS-CoV-2 infection were observed in both the macaque NHP model (rhesus and cynomolgus) and in baboons (89,90,93). Singh et al. (89) showed a more conspicuous age-related effect of SARS-CoV-2 infection in African baboons compared to Indian rhesus macaques. Furthermore, older African baboons developed more severe infections of longer duration. Hence, this model could warrant further development for the study of SARS-CoV-2 in elderly COVID-19 patients with co-morbidities, such as diabetes and heart disease. This implies that age has an effect and may be a factor for the more severe disease manifestations seen in elderly COVID-19 patients. Thus, older macaques/baboons may be a suitable NHP animal model for the study of age-dependent pathogenesis of SARS-CoV-2. The NHP genera show varied levels of SARS-CoV-2 infection and exhibit different degrees of pathological manifestation suggesting NHP species-dependent pathogenesis. Moreover, difference in the mounting of protective Abs were also observed. In a study of rhesus macaques, Singh et al. (89) showed that old rhesus macaques had lower Ab titers than young rhesus macaques, even though other studies demonstrated the opposite (90).

Despite the similarity between NHP ACE2 and human ACE2, the NHP animal model does have several limitations. NHPs do not recapitulate severe human disease and no mortality was observed in this model. In addition, variability in the innate and adaptive immune

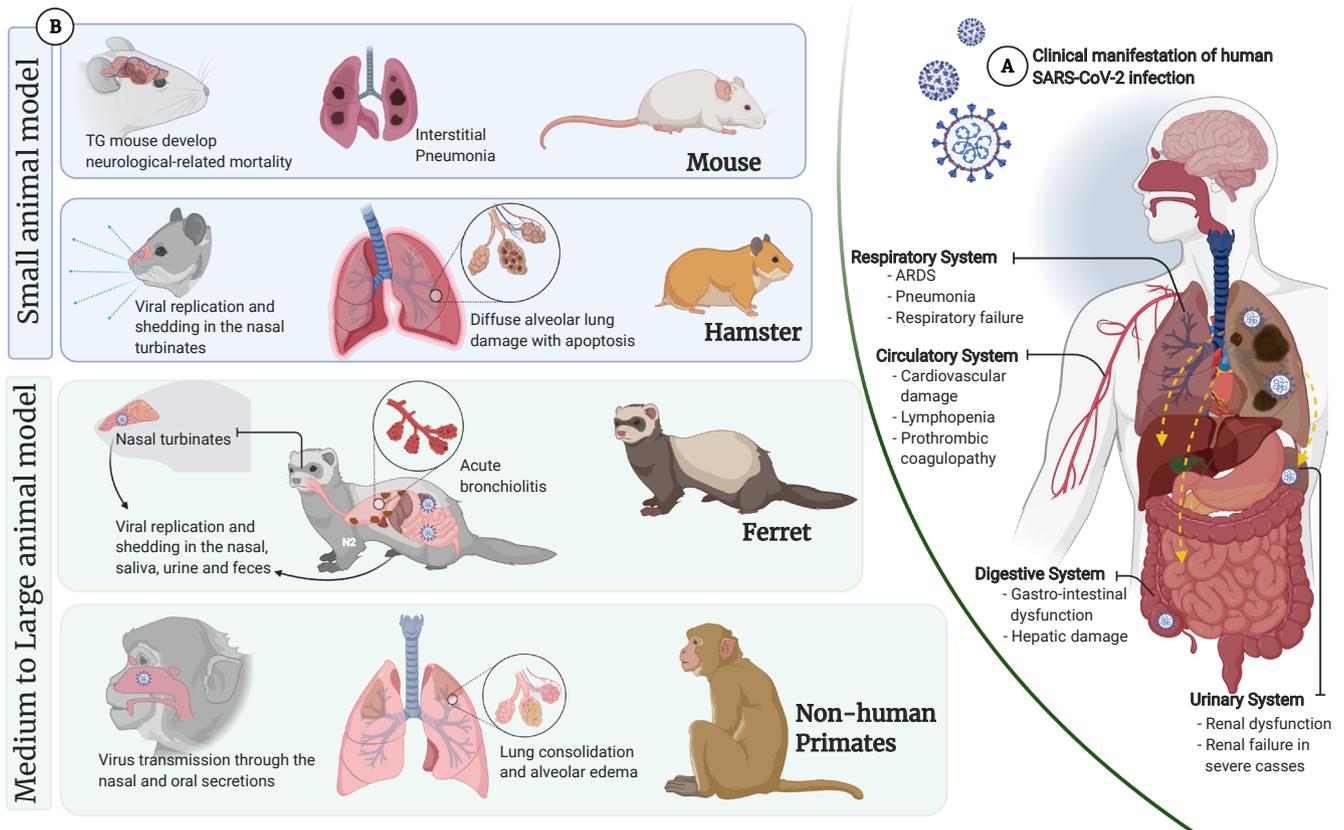
responses against SARS-CoV-2 infection has been reported for NHPs (90,96). It should be noted that there are several species and subspecies of NHPs, which may result in significant variation in the level of viral replication and clinical diseases. Additional disadvantages of using NHPs include significant expenses, limited numbers of animals, and the tight regulation of their use due to ethical reasons (97).

The use of animal models (Table 1) has allowed deducing experimental results into the context of human infection (Fig. 1) (98,99). Furthermore, the use of mammalian models of SARS-CoV-2 infection to evaluate both pathogenicity and transmissibility has resulted in a fuller understanding of the capacity of the virus to cause disease.

**Table 1.** General animal models of SARS-CoV-2

Variables	Description
<b>Mouse</b>	
Advantage	Cost-effective, ease of handling, and suitable for large scale studies (47).
Limitations	Wild-type mice not permissive to SARS-CoV-2 infection (47).
<b>Transgenic and transduced mice</b>	
Advantage	Permissive to SARS-CoV-2 infection (54,55). Virus replicates in the lungs causing lung inflammation. Bodyweight loss after infection (54,55).
Limitations	Limited availability in a timely manner. Neurological-related mortality (50,51,54). Possibility of antiviral immune response against the vector used. Variable hACE2 expression in the mouse lungs due to non-uniform transduction (55).
<b>Humanized animal model expressing hACE2</b>	
Advantage	Recapitulates the main human immunopathological events and generates human IgM and IgG SARS-CoV-2 RBD-specific Abs (59).
Limitations	
<b>Hamster</b>	
<b>Golden syrian hamster</b>	
Advantage	High similarity between the SARS-CoV-2 spike-contacting regions of human ACE2 and hamster ACE2 (62). Susceptible to SARS-CoV-2 infection (64). More consistent lung disease phenotype compared to other animal models against SARS-CoV-2 infections (64).
Limitations	Limited availability of immunological reagents (74). Lack of mortality.
<b>Roborovski dwarf hamsters</b>	
Advantage	Highly susceptible to SARS-CoV-2 infection (73). Developed a rapid onset of severe clinical disease mimicking the outcomes of severe COVID-19 cases.
Limitations	Real mortality was not observed.
<b>Ferret</b>	
Advantage	Histo-anatomical features of the ferret respiratory tract can reproduce condition similar to the human respiratory tract (78-81). SARS-CoV-2 replicate efficiently in the respiratory tract of ferrets without prior adaptation (78,79). Suitable for transmission study (78,79). Apt size allows repeated blood sampling at volumes (81).
Limitations	Inadequate availability of ferret-specific immunological reagents.
<b>NHP</b>	
Advantage	Phylogenetic proximity with humans. Permissive for SARS-CoV-2 infection. Similar symptoms observed in mild to moderate COVID-19. Develop virus-induced pneumonia. Suitable animal model for studying SARS-CoV-2 in elderly COVID-19 patients.
Limitations	Does not recapitulate severe human disease and lack of mortality. Variable disease outcome, clinical signs, and immune responses dependent on NHP species (90,96). Expensive, NHP studies are limited to a small number of animals and are highly regulated due to ethical reasons (97).

RBD, receptor-binding domain.



**Figure 1.** (A) Overview of human clinical manifestation of SARS-CoV-2 infection: showing common clinical features observed in hospitalized patients (98,99). Pneumonia, a major clinical feature in COVID-19 patients, complicated by ARDS leading to respiratory failure. Reports on human autopsies reported that the lung generally appeared congested and edematous, with different stages of diffuse alveolar damage characterized by fibrosis of the interstitium, desquamation of pneumocytes, hyaline membrane formation with inflammatory cell infiltration. In addition, direct assault to other organs by disseminated SARS-CoV-2 and immune pathogenesis were also observed such as cardiovascular complications, hypercoagulopathy, substantial reduction of lymphocytes, gastrointestinal dysfunction, and impaired liver function, and renal injury. (B) Overview of SARS-CoV-2 manifestations in different animal models. (created with BioRender.com). ARDS, acute respiratory distress syndrome; TG, transgenic.

## CONCLUSION

The emergence of the novel SARS-CoV-2 pathogen has resulted in the need for the development of an appropriate animal model to aid in the understanding of disease causation, transmission, and pathogenesis, as well as to elucidate the details of the host immune response. Perhaps the most perplexing aspect of SARS-CoV-2 infection is the wide range of disease severity in humans, which can vary from asymptomatic or mild presentation to severe and fatal disease. Developing animal models that can reflect such diverse clinical manifestations is remarkably important, albeit arduous. While many SARS-CoV-2 infection models have been developed and investigated thus far, including mice, hamsters, ferrets, and NHPs, a large variation in results has been obtained both between and within species.

While standard mouse strains are not susceptible to natural SARS-CoV-2 infection, several approaches to generate mouse strains susceptible to SARS-CoV-2 have been developed. Unfortunately, engineered mice exhibit uneven hACE2 transduction, and ectopic hACE2 expression results in changes in viral tissue tropism. In addition to the mouse model, hamsters have proven to be a useful animal model for SARS-CoV-2 infection due to their development of clinical signs of illness, including lung damage similar to that seen in

humans. While hamsters do offer several advantages, a caveat with this species is the scarcity of research reagents for immunological studies compared to those available for mice.

Several medium to large size animals are closer anatomically and physiologically to humans, having ACE2 receptors similar to human ACE2, and manifest many clinical similarities to human infection. With respect to ferrets, not only are they susceptible to SARS-CoV-2, they can spread the virus with high transmission rates via both direct contact and through airborne transmission. In addition, several studies have indicated that non-human primate animal models are both permissive to SARS-CoV-2 and are suitable for pathology studies. Moreover, a notable variation of infection was found with respect to the age of infected NHPs, with aged animals succumbing to infection and showing more severe symptoms and pathology. In addition, different NHP species exhibit heterogeneous responses to SARS-CoV-2 infection.

The use of an appropriate animal model can markedly accelerate understanding of the immunopathogenesis of novel pathogens such as SARS-CoV-2. Although numerous animal models are available, however, currently, no single existing animal model can really recapitulate the totality of human SARS-CoV-2 infection. Several possible reasons may explain why experimental animal models only reflect moderate lung pathology and disease manifestation. These include, route and method of virus inoculation, absence nor not been rigorously explored effects of co-morbidities and other risk factors associated with high-case fatality such as advancement in the age of the animal, obesity, diabetes, cardiovascular disease and immunocompromised state. Incorporation of these features may predispose existing animal models to reflect pathological states showing clinical manifestation similar to those observed in humans with severe COVID-19. Nevertheless, each animal model has advantages and disadvantages, and importantly, the value of different animal models is that they can be tailored, providing different options in order to achieve the scientific goals of the researcher.

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