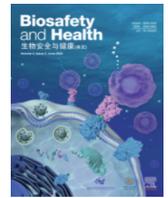




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Application of animal models to compare and contrast the virulence of current and future potential SARS-CoV-2 variants



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ABSTRACT

Since severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified during late 2019, the sustained spread of this pathogen within the human population has caused worldwide disruption with staggering infection rates and death tolls. Due to the accumulation of mutations in SARS-CoV-2, the virus has evolved into many variants, five of which have been listed as variants of concern (VOCs) by the World Health Organization (WHO). Multiple animal models of SARS-CoV-2 have been developed to evaluate vaccines and drugs and to assess the pathogenicity, transmissibility and antiviral measures of these VOCs. Here, we review the cutting-edge research based on mouse, hamster, ferret and non-human primate models for evaluating SARS-CoV-2 with a focus on the Omicron variant, and highlight the importance of updating vaccines in a timely manner in order to mitigate the negative effects of SARS-CoV-2 infections in the human population.

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1. Introduction

During December 2019, 41 cases of viral pneumonia of unknown origin were reported in China. The disease was named coronavirus disease 2019 (COVID-19) [1], and the causative agent was found to be severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. Initial clinical manifestations of COVID-19 include cough, fatigue, fever, breathing difficulties, as well as loss of smell and taste. A proportion of patients may develop more severe disease symptoms including pneumonia and acute respiratory distress syndrome (ARDS), acute kidney injury, coagulation disorders, acute myocardial injury, eventually leading to death [3,4]. The elderly and patients who have pre-existing medical conditions such as diabetes, chronic liver diseases, chronic lung diseases etc., have a higher risk of severe illness [5]. As of 20 March 2022, over 468 million COVID-19 cases have been confirmed, including over 6 million deaths, and the pandemic is still ongoing with over 1 million new infections globally per day [6].

SARS-CoV-2 belongs to the genus Betacoronavirus, lineage 2b, which also contains two other coronaviruses that are highly virulent

in humans, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). In addition, several Alphacoronaviruses (HCoV-229E, HCoV-NL63) and Betacoronaviruses (HCoV-OC43 and HCoV-HKU1) are also known to infect humans and cause mild disease, hence these are termed “seasonal coronaviruses” [7]. Since SARS-CoV, MERS-CoV and SARS-CoV-2 constitute the biggest threats to global health, the majority of current research efforts are focused on these pathogens, in which candidate vaccines, therapies and animal models have been developed against these viruses.

The SARS-CoV-2 virion consists of four structural proteins – the spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N), as well as encoding a ~ 30 kb-sized positive-sense, single-stranded RNA genome. The S protein recognizes and binds to several host cell receptors including angiotensin converting enzyme 2 (ACE2), and mediate membrane fusion, allowing the virion to enter the host cell. The S protein is composed of two subunits, S1 and S2. S1 contains the receptor binding domain (RBD), which can specifically recognize and bind to ACE2, while S2 mediates membrane fusion of virus and host cell [8].

There are at least four types of vaccines currently being deployed for SARS-CoV-2 immunizations: mRNA vaccines represented by Pfizer and Moderna, adenovirus vector vaccines represented by AstraZeneca, Johnson & Johnson and CanSino, recombinant subunit vaccines represented by Anhui-Zhifei, and inactivated vaccines represented by Sinovac and Sinopharm [9,10]. The target antigen for mRNA and adenovirus vector vaccines is the S protein, the recombinant subunit

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vaccine focuses on RBD, and the whole virion is the target for the inactivated vaccine [10,11].

Animal models play an indispensable role for developing vaccines and therapeutics against SARS-CoV-2, in addition to studying its viral pathogenesis and transmission. An ideal animal model should closely mimic and reproduce the disease symptoms and immune responses after SARS-CoV-2 infections in humans, as the efficacy of therapeutics and vaccines can then be interpreted with more confidence between different models.

2. Variants of concern

Previous research showed that the RNA genome of SARS-CoV-2 acquired approximately two nucleotide substitutions per month, which is lower than other RNA viruses, due to the proofreading mechanism of nonstructural gene *nsp14* [12]. Although most mutations are likely to have little or no observable effects on virulence, transmission and/or antiviral resistance, several mutations have been identified to substantially impact virus properties, which may enhance viral spread, or allow SARS-CoV-2 to evade the immune responses raised by vaccines or antibodies against earlier isolates. The SARS-CoV-2 sequences from patients are continually monitored, and any rapidly dominant mutations to the genome of SARS-CoV-2 which affect virus characteristics and causing intense transmission in multiple countries are flagged as variants of interest (VOIs), and if associated with increased virulence and resistance to available vaccines, variants of concern (VOCs) [13].

In April 2020, the D614G substitution on the SARS-CoV-2 S protein was first described, which enhanced the ability of binding to hACE2 [14,15]. In September 2020, the Alpha variant (Pango lineage B.1.1.7) was first detected in England and designated as a VOC [16]. Out of ten mutations in the S protein, the N501Y substitution was found to increase binding affinity to both human and murine ACE2 [17,18]. Almost simultaneously, the Beta VOC (Pango lineage B.1.351) with the same amino acid substitution (N501Y) was reported from South Africa. In addition, another two substitutions (K417N, E484K) located in its RBD were shown to reduce the antibody recognition and enhance binding affinity to hACE2 [19,20]. In January 2021, the Gamma VOC (Pango lineage P.1) with ten substitutions (L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I V1176, K417T, E484K, and N501Y) in the S protein was detected from four Brazilians travelling to Japan [21]. Similar with the Alpha and Beta VOCs, Gamma also had mutations on amino acids 417, 484 and 501, suggesting that these changes can greatly increase the transmissibility of the virus and are important adaptive mutations for viral spread in humans [18].

In 2021, the Delta and Omicron VOCs were reported and spread rapidly worldwide, replacing the previous VOCs in terms of prevalence. The Delta (Pango lineage B.1.617.2) was first detected in India at the end of 2020 [22]. In contrast with the previous three VOCs, Delta does not have N501Y but instead has two new mutations (L452R and T478K) on its RBD, which can also increase its affinity to hACE2 and avoid recognition by vaccine-induced antibodies [23]. In addition, P681R, a new substitution near the furin cleavage site, was shown to facilitate S1/S2 cleavage and enhances the fusion of the virus with the host cell [22].

Due to a series of mutations, the Delta variant showed particular resistance against neutralization by antibodies raised with previous VOCs. It showed on average 2.7-fold reduced neutralization to the plasma from convalescent individuals collected from the early stages of the pandemic, and some clinical samples from patients infected with Alpha, Beta and Gamma VOCs failed to neutralize the Delta VOC [24]. Antibodies induced by commonly-used vaccines are against the “prototype” SARS-CoV-2 from early isolates in Wuhan, such as ChAdOx1, BNT162b2 and BBIBP-CorV, which also displayed significantly reduced neutralizing activities to the Delta VOC [24–27]. However,

the effectiveness of these vaccines can be improved to a certain extent (BNT162b2: 88.0% neutralization and ChAdOx1: 67.0% neutralization) if two doses were administered [28].

On November 26, 2021, the Omicron (Pango lineage B.1.1.529) variant was designated a new VOC by the WHO, only two days after it had been assigned as a variant under monitoring (VOM). This newly emergent variant was first detected in South Africa and Botswana in early November 2021 [29], and spread rapidly around the world within a short period of time [30]. The Omicron VOC contains up to 45–52 amino acid changes across the whole genome, including 32 mutations in the S protein, which is substantially more than that recorded for previous variants [31]. It was found that 15 of 32 mutations are located on the RBD. Several mutations observed previously, such as T478K, N501Y, D614G and P681H are present on the S protein of the Omicron VOC. As a result, Omicron was found to be more resistant to neutralization by antibodies raised via vaccination, leading to more breakthrough infections [32].

As of March 2022, the Omicron VOC is dominant globally and is currently divided into three main lineages: BA.1, BA.2 and BA.3, as well as a sub-lineage of BA.1.1 [33]. These lineages share 21 mutations, in which BA.1 differs from BA.2 by 50 amino acids, including the amino acid 69–70 deletion in the S protein [33,34]. It has been reported that convalescent patients infected with BA.1 can still be infected with BA.2 [35]. Although infections with the BA.1 variant came out earlier than BA.2, the BA.2 is predicted to be more transmissible than BA.1 and other VOCs because of its high growth rate in the Calu-3 cell line and hamster animal model [35,36]. Due to the expected threat posed by BA.2, recent efforts have been focused on this emerging VOC.

3. Mouse models

Mice are the most widely used animal for biological research due to its small size and low cost [37]. Establishment of a SARS-CoV-2 mouse model was relatively rapid due to previous knowledge and experience accumulated in developing a mouse model for SARS-CoV, in which both viruses share the same receptor [38]. Wild-type mice are resistant to infection with clinical SARS-CoV and SARS-CoV-2 isolates to a certain degree [39,40]. Therefore, several mouse models have been developed to study mechanisms of pathogenicity and evaluation of candidate medical countermeasures. The strategies for generating mouse models include constructing transgenic mice expressing hACE2 in the lungs, using adenoviral vectors to transduce hACE2 into mouse lungs to render these animals transiently susceptible to infection, or developing mouse-adapted (MA) isolates that can replicate in mouse lungs and cause severe interstitial pneumonia [40–43].

Several strategies have been developed for studying SARS-CoV-2 in the first year of the pandemic. For transgenic mice, the hACE2 expression was controlled by an epithelial cell-specific HFH4/FOXJ1 promoter or K18. These transgenic mice can support SARS-CoV-2 replication and causes obvious clinical symptoms, such as weight loss and interstitial pneumonia [41,43]. However, due to the high price and time cost of developing, maintaining and validating transgenic mice for experimental use, a mouse model using the adenovirus serotype 5 vector expressing the hACE2 gene (Ad5-hACE2) was constructed and used to transduce wild-type mice 5 days-prior to infection, to render these mice temporarily susceptible to SARS-CoV-2. Robust viral growth and pneumonia was observed in these mice after virus challenge [40]. Based on continuously passaging of wild-type SARS-CoV-2 in the lungs of aged mice, MA SARS-CoV-2 has been isolated, in which infection results in interstitial pneumonia and inflammatory responses in both young and aged mice [42]. Interestingly, the N501Y mutation located at the RBD may be the key residue for SARS-CoV-2 to recognize the mouse ACE2 (mACE2) receptor to

facilitate entry. As mentioned earlier, the N501Y is also a common mutation observed in many VOCs, so there is some speculation that the VOCs can be potentially transmitted between infected humans and animals [44–46].

Mice were used extensively early in the pandemic to assess the virulence, pathogenicity and transmissibility of newly reported VOCs, as well as the effectiveness of specific vaccines and antiviral agents. The sera from mice immunized with the trimer S protein from the prototype SARS-CoV-2 showed a decreased ability to neutralize Beta, Gamma VOCs, as well as some other variants. The common characteristic is these VOCs all harbor the E484K mutation, which may be an adaptive mutation of the virus to escape the neutralizing antibodies induced from previous vaccines [47].

Several studies found that the capacity for utilizing TMPRSS2 and S1/S2 cleavage for the Omicron VOC was weaker than that for prototype and the Delta VOC, which led to significant reduction of viral replication in Calu-3 and Caco-2 cells [48,49]. The viral subgenomic RNA (sgRNA) level and lung lesions were attenuated in K18-hACE2 transgenic mice infected with the Omicron VOC. Compared to the prototype SARS-CoV-2, Alpha, Beta and Delta variants, Omicron is milder in terms of virulence, as evidenced by the slight weight loss and lowest mortality (Prototype: 80%, Alpha: 100%, Beta: 67%, Delta: 56% and Omicron: 43%) in mice [48]. Additionally, some inflammatory factors in nasal turbinates and lungs, such as IP-10 and IFN- γ , were at lower transcription levels compared to Omicron-infected mice [48]. Similarly, another study found that Omicron variant failed to cause weight loss in both young and aged mice. Compared to the D614G and Beta VOC, the viral burden, pulmonary function damage and immune responses were all attenuated for the Omicron variant in multiple strains of mice [50]. These results are consistent with the milder respiratory symptoms also observed in patients infected with the Omicron VOC, as opposed to other VOCs [51].

4. Hamster models

Using structural simulation and molecular dynamics simulation of the SARS-CoV-2 S protein and ACE2 receptor from different animal species, a study showed that the binding affinity between the S protein and ACE2 of the Syrian hamster (*Mesocricetus auratus*) is much stronger than those of ferrets, suggesting that hamsters may be permissive for SARS-CoV-2 infection [52]. Similar to mice, hamsters are widely used in the laboratory and tested for susceptibility to SARS-CoV-2 infection. It was shown that within one week after challenge at a dose of 8×10^4 TCID₅₀, the presence of viral antigens and inflammatory cell infiltration can be detected in the respiratory tract. In addition, hamsters can shed progeny virus in nasal wash and throat swab samples until 14 dpi and feces samples until 5 dpi, which in turn cause infections in other healthy, naive hamsters upon contact, thus serving as a potential animal model to study virus transmission [53,54]. Similar with transgenic mice, K18-hACE2 hamsters was also developed to study SARS-CoV-2. Most hamsters died at 5 dpi with 100 or 1,000 PFU infection of the SARS-CoV-2 WA1 prototype strain. Over 10% weight loss and approximately 10^6 PFU/g viral load in the lungs was observed at 5 dpi [55].

The virulence between different SARS-CoV-2 variants have been evaluated in hamster models. In these animals, the Alpha and Beta VOCs were able to replicate efficiently and the viral titer was determined to be 10^4 TCID₅₀/mg in lung tissues at 4 days post-infection (dpi). In addition, viral infection resulted in bronchopneumonia, similar to symptoms observed in COVID-19 patients. However, there were no significant differences in the levels of sgRNA and progeny virus titers, as well as pathology between the two VOCs [56]. The virulence of the Delta variant was also tested in hamster models. The results

showed that the viral sgRNA was continuously detected in the respiratory tract of hamsters infected with the Delta variant in 14 days [57]. The virulence of Delta VOC was also compared with B.1.1, the parent variant of Omicron VOC. The peak weight loss of the Delta VOC is 16% at 5 dpi, while B.1.1 is 13% at 6 dpi. For Delta-infected hamsters, the lung areas positive for the SARS-CoV-2 N protein was found to be diffused more rapidly compared to B.1.1, which explains why Delta VOC is more pathogenic [22]. The neutralizing antibodies produced by infection with the Delta VOC can effectively neutralize Beta VOC, B.1 and B.1.617.3 with an observed decrease of 2.5/1.8/1.8-fold, respectively [57].

Similar with results in mice, infection with the Omicron variant showed attenuated virulence in hamster models. Compared to D614G, the Omicron variant results in approximately 1000-fold lower viral RNA in lungs, and no infectious virus can be isolated at 4 dpi [49]. In contrast with D614G, B.1.1 and the Delta variant, the hamster infected with the Omicron variant did not show decreased body weights, while the uninfected control group gained body weight [50,58]. Additionally, no significant differences in viral load were found in the nasal turbinates among wild-type hamsters infected with different variants, but the Omicron variant had significantly fewer infections (12-fold lower of viral RNA level) in the lower respiratory tract compared to WA1/2020 D614G, and caused less instances of severe pneumonia [50]. The infection in K18-hACE2 transgenic hamsters is similar to those from other hamsters, with less observed weight loss, lower viral RNA levels (10^3 to 10^4 -fold) and mortality rates (25% versus 100%) compared to HP-095 D614G [50].

While BA.1 and BA.2 are both classified as Omicron, the high number of different mutations between these two variants result in a certain degree of difference in their pathology. Yamasoba et al. found that BA.2 has an approximate 100-fold higher replication capacity in primary human nasal epithelial cells and induced significantly stronger cell fusion in HEK293-ACE2/TMPRSS2 cells than BA.1, which means BA.2 is more fusogenic than BA.1. BA.2 infected-hamsters exhibited significant respiratory disorders, such as a decreased subcutaneous oxygen saturation (SpO₂) and increased lung enhanced pause (Penh), a marker of bronchoconstriction, with significant weight loss [50]. BA.1 infected hamsters, as previously described, were asymptomatic or had only mild symptoms. Viral RNA detection and immunohistochemistry (IHC) of pulmonary in hamsters also showed that BA.2 has a higher replication than BA.1. HE staining also showed that BA.2 infection led to more severe lung inflammation compared to BA.1 and B.1.1. In summary, BA.2 has higher virulence and transmissibility than BA.1 [36].

5. Ferret models

Ferrets have been frequently used as a model to study pathology in previous research into SARS-CoV and other human respiratory viruses [59], and thus were also investigated for its utility as a model for studying the infection and transmission of SARS-CoV-2 [60]. The results showed that ferrets infected with SARS-CoV-2 showed typical clinical symptoms such as cough, decreased activity, and increased body temperatures, but without significant weight loss. Viral shedding can be detected at up to 16 days after infection with a dose of 5×10^4 PFU [61]. Additionally, the ferret model was used to simulate human-to-human SARS-CoV-2 transmission. The exposure of direct and indirect contact animals to the challenged ferret were shown to be also infected at two days after exposure, producing the same symptoms of infection, and positive for viral RNA in nasal wash, saliva and fecal specimens [62].

This ferret model was then used to compare the competition and transmission ability of various SARS-CoV-2 variants. The prototype strain and the D614G variant were both shown to be able to infect fer-

rets, but the D614G variant was dominant in terms of progeny virus produced from these animals [15]. Additionally, healthy ferrets that came into contact with these infected animals subsequently became infected, and D614G was also found to be the dominant variant in these animals, which suggests that S^{D614G} provided a stronger transmission ability for SARS-CoV-2 [15]. Subsequent work showed that the Alpha variant was more transmissible in ferrets compared to the D614G variant, and transmitted ferrets can carry the similar viral RNA loads compared to donor ferrets [63]. These results closely reflected the replication and transmission tendency of SARS-CoV-2 in humans.

6. Non-human primate models

Non-human primates (NHP) are considered to be the closest animal model to humans in terms of genetics, receptor similarities, immune systems and infection symptoms. Therefore, NHPs are widely used in preclinical trials to test candidate countermeasures. In a study, four species of NHPs were shown to be susceptible to SARS-CoV-2, including rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), common marmosets (*Callithrix jacchus*), and African green monkeys (*Chlorocebus sabaues*) [64]. Rhesus macaques are considered to be the most suitable animal model for SARS-CoV-2 because they are most susceptible to SARS-CoV-2 infection and have the most severe symptoms [64]. In rhesus monkeys, SARS-CoV-2 infection resulted in fever, cough, interstitial pneumonia, which is very similar to COVID-19 patients. The virus shedding could be detected in the nose, throat, and rectum. Interestingly, SARS-CoV-2 infection induced the production of neutralizing antibodies in rhesus monkeys, which effectively protected these animals from secondary infection [65].

Published SARS-CoV-2 researches based on the NHP model are mainly focused on the evaluation of vaccine protection against VOCs. In one study, rhesus macaques were immunized with two doses of mRNA-1273 at a 4-week interval. Compared to the D614G variant, the neutralization against Beta, Gamma and Delta VOCs decreased by 4.1-fold, 3.3-fold and 6.9-fold, respectively, at two weeks after boost. The neutralizing antibody capacity against Delta VOC continuously declined with time. At week 48, 3 out of 8 vaccinated rhesus macaques lost detectable neutralizing antibodies against the Delta VOC [66]. Another published study showed that 3 doses of the SARS-CoV-2 inactivated vaccine VacKMS1 can provide a weakened but still effective protection for rhesus macaques against VOCs infection. The sera collected at 18 days after the third immunization showed a 2.7-fold, 5.2-fold, 4.3-fold and 31.6-fold decline of neutralizing antibody levels against Alpha, Beta, Delta and Omicron VOCs, respectively. The viral RNA level was found to be significantly lower than the unvaccinated group. Although the vaccinated group infected with Alpha, Beta and Delta VOCs showed typical interstitial pneumonia, such as inflammatory cell infiltration, thickened alveolar walls, hemorrhage and thrombosis, the histopathology changes were significantly improved compared to the unvaccinated group, which indicates the inactivated vaccine may still provide a certain degree of protection against SARS-CoV-2 VOCs [67].

7. Discussion

The COVID-19 pandemic, which began in late 2019, continues unabated in 2022, with over 1 million infections and thousands of deaths per day globally. A substantial number of vaccines and agents have been developed based on evaluation in animal models, which are summarized in Table 1. However, due to sustained transmission in humans and susceptible animals, SARS-CoV-2 is also undergoing constant evolution and breaking through the immunity stimulated by infection or vaccination from different variants.

Current research is focused on the Omicron VOCs. Based on studies from the mouse and hamster models, the Omicron variant was found to be of lower virulence but higher transmissibility [50,58]. This characteristic poses a great challenge for the controlling the pandemic, as high numbers of asymptomatic or mildly-symptomatic patients cannot be easily detected in time. Additionally, the Omicron variant is resistant to existing vaccines based on the prototype SARS-CoV-2, as even those who have received three doses of vaccines were susceptible to infection [68–70]. Recent research showed that, compared with the wild-type SARS-CoV-2, serum neutralization from individuals inoculated with two doses of BNT162b2 or mRNA 1273 was found to be over 23-fold and 42-fold lower, respectively, against the Omicron VOC at one month after vaccination [71]. In those individuals who have received three doses of these vaccines, the neutralization of Omicron in their serum was decreased 7.5 and 16.7-fold, respectively, at an average of 19 days after vaccination [72]. In another study, the vast majority of serum from individuals vaccinated with two doses of ChAdOx1 did not neutralize Omicron [73]. Since the level of neutralizing antibody against SARS-CoV-2 infection gradually decreases with a half-life of about 8 weeks, existing vaccines based on the prototype SARS-CoV-2 cannot be relied on indefinitely to provide strong cross-protection against emerging VOCs [74].

At present, the dominant Omicron variant is BA.1, but BA.2 is gradually outcompeting BA.1 as the new prevalent variant [75]. From 16 February to 17 March, 2022, 99.8% of the reported sequences were Omicron, in which BA.2 sequences accounts for 85.96% of cases [6]. Similar with BA.1, BA.2 was highly resistant to serum antibodies induced by prototype vaccines, such as mRNA-1273 and ChAdOx1. Moreover, BA.2 is also highly resistant to therapeutic monoclonal antibodies and serum from convalescent patients infected with other VOCs [36]. BA.2 has a certain degree of resistance to the serum of convalescent patients infected with BA.1, which has been further verified in hamster models [36]. It has been reported that some individuals who recovered from BA.1 were reinfected with BA.2. Fortunately, the symptoms were mild and did not result in hospitalization or death [35].

Compared to 2020, the severity and mortality caused by the SARS-CoV-2 infections have significantly declined, but on the other hand, the increased number of asymptomatic infections has brought new challenges towards containing the pandemic. An example is the Omicron VOC. Once Omicron emerged in November 2021, the variant spread rapidly and intensely, infecting more people than the peaks of Alpha, Beta, Gamma and Delta, despite the availability of vaccines against the prototype SARS-CoV-2 variant. Hong Kong, China, in particular, has recorded over 80 times more cases and over five thousand deaths during the Omicron wave compared to past waves with other VOCs [76]. These developments clearly indicate that if the ultimate goal of zero-COVID was to be achieved, it will be important to employ several newer strategies to decrease caseloads. In addition to diligent mass testing and quarantine measures, which have been crucial for lowering viral transmission and the chances for the pathogen to evolve in a new host, the development of specific vaccines against the Omicron variant to rapidly reduce the number of infections, hospitalizations and deaths (and timely updating of vaccines against future dominant variants, perhaps in a manner similar to how seasonal influenza vaccines are updated) will also be a crucial component to “flattening the pandemic curve” to relieve the stress placed on medical workers, laboratory workers and the general community.

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Table 1
Comparison of characteristics between different SARS-CoV-2 animal models.

Animal models		Presentation of disease	Applications	Advantages	Limitations	References
Mice	Transgenic mice	Weight loss and interstitial pneumonia	Pathology, vaccine evaluation, therapeutics	Convenient, cheap, causes systemic infection, permissive to infection for all VOCs	Time-consuming for construction	[41,43,50]
	Transduced mice	Weight loss, severe pneumonia, and robust virus replication in lungs	Pathology, vaccine evaluation, therapeutics	Convenient, economical, permissive for all VOCs	Only infection in lung, nonlethal model	[40]
	Mouse-adapted strain	The virus replicates effectively in the lungs, and causes severe lung damage in aged mice, but without significant weight loss.	Pathology, vaccine evaluation	Can infect wild-type mice directly, convenient and cheap	Incomplete symptoms, no mouse-adapted VOC variants	[42]
Syrian hamsters	Wild-type hamsters	Weight loss, interstitial pneumonia and intestinal inflammation	Pathology, transmission, vaccine evaluation, therapeutics	Easy to handle, cheap, have digestive symptoms and can transmit to healthy individuals, permissive for all VOCs	Not as convenient to manipulate as mice, virus cleared in 7 days and nonlethal	[53,54,56,57]
	Transgenic hamsters	Significant weight loss, severe and lethal pneumonia, central nervous system infection and heart injury	Pathology, transmission, vaccine evaluation, therapeutics	Hypersensitive to SARS-CoV-2 infection, can develop multiple severe symptoms	Have not been widely used, cannot represent mild and asymptomatic infections.	[50,55]
Ferrets	Ferrets	Cough, sneeze, fever, mild pneumonia	Pathology, transmission, vaccine evaluation, therapeutics	Useful model for transmission by direct contact and aerosol	Expensive and related reagents are difficult to acquire.	[15,59–63]
Non-human primates	Rhesus macaques and Cynomolgus macaques	Fever, cough, interstitial pneumonia, weight loss, inflammatory cell infiltration in the ileum, colon, etc.	Pathology, vaccine evaluation, therapeutics	High similarity to humans	NHPs are expensive and difficult to acquire. Infection symptoms are mild and resistant to a second infection.	[64–67]

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Ding Zhe: Writing – Original Draft. **Tong Chen:** Writing – Original Draft. **Jiaming Lan:** Funding Acquisition, Writing – Review & Editing. **Gary Wong:** Conceptualization, Funding Acquisition, Supervision, Writing - Review & Editing.

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