Transmissibility and pathogenicity of SARS-CoV-2 variants in animal models

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As of February 2022, SARS-CoV-2 is still one of the most serious public health threats due to its high mortality rate and rapid spread of novel variants. Since the first outbreak in 2019, general understanding of SARS-CoV-2 has been improved through basic and clinical studies; however, knowledge gaps still exist in our understanding of the emerging novel SARS-CoV-2 variants, which impacts the corresponding development of vaccines and therapeutics. Especially, accumulation of mutations in SARS-CoV-2 and rapid spread in populations with previous immunity has resulted in selection of variants that evade the host immune response. This phenomenon threatens to render current SARS-CoV-2 vaccines ineffective for controlling the pandemic. Proper animal models are essential for detailed investigations into the viral etiology, transmission and pathogenesis mechanisms, as well as evaluation of the efficacy of vaccine candidates against recent SARS-CoV-2 variants. Further, the choice of animal model for each research topic is important for researchers to gain better knowledge of recent SARS-CoV-2 variants. Here, we review the advantages and limitations of each animal model, including mice, hamsters, ferrets, and non-human primates, to elucidate variant SARS-CoV-2 etiology and transmission and to evaluate therapeutic and vaccine efficacy.

Keywords: SARS-CoV-2, animal models, variants, transmissibility, pathogenicity

Introduction

In December 2019, a previously unknown respiratory disease caused by a beta-coronavirus was reported in a cluster of patients with severe respiratory disease in Wuhan, China (Zhu et al., 2020). This novel coronavirus is closely related to the severe acute respiratory syndrome (SARS)-coronavirus, hence the name SARS-CoV-2 (International Committee on Taxonomy of Viruses, 2020). Since the outbreak of SARS-CoV-2, there have been more than 260 million confirmed cases with a fatality rate of 2% (as of December 2021). In contrast to SARS-CoV, SARS-CoV-2 has significantly strong transmissibility resulting in the first pandemic caused by a coronavirus, although with a relatively lower mortality rate than that of SARS-CoV (10%) or MERS-CoV (40%) (Abdelrahman et al., 2020; Abdelghany et al., 2021). Although rapid development of various vaccine formulas has resulted in vaccination of a number of people worldwide, SARS-CoV-2 variants have occurred globally, significantly impacting vaccine efficacy (Moore and Offit, 2021). These SARS-CoV-2 variants may cause mismatches with vaccine-induced antibodies due to the occurrence of mutations in the spike gene, which plays an important role in infection, and may result in breakthrough infections. Thus far, vaccines have been developed based on the early SARS-CoV-2 variants, and poor cross-protective efficacy between the vaccine strain and recent SARS-CoV-2 variants has been reported (Cromer et al., 2021; Martinez-Flores et al., 2021; Stamatatos et al., 2021; Zhou et al., 2021a). In addition, it was reported that a variant SARS-CoV-2 strain containing the Asn501 to Tyr (N501Y) substitution in the spike protein could proliferate in the BALB/c mouse model (Gu et al., 2020; Muruato et al., 2021), in which previous variants could not proliferate, suggesting this mutation changes the host specificity of SARS-CoV-2. Although N501Y alone could induce binding of the virus to the murine ACE2, additional mutations in the RBD are necessary to establish effective infections in mice. In addition to human infections, SARS-CoV-2 was first detected in minks April 23, 2020. Since then, outbreaks on mink farms have been reported in Europe and North America (Oreshkova et al., 2020; Fenollar et al., 2021; Munnink et al., 2021). At least 170 mutations have been identified by whole-genome sequencing of mink SARS-CoV-2 samples from 40 mink farms, and mink-specific mutations of SARS-CoV-2 (including a Y453F mutation in spike) have been found in humans (Mallapaty, 2020). Recently, infections of captive and wild animals such as white-tailed deer (Odocoileus virginianus) (Chandler et al., 2021) and Asiatic lions (Panthera leo persica) (Mishra et al., 2021) were also demonstrated suggesting an alteration of tropism of SARS-CoV-2 and the possibility of a natural reservoir of this deadly virus.
Experimental animal model
hACE2 transgenic/knock-in mice, transient somatic expression of hACE2 mice, aged Balb/c mice, aged Balb/c mice

Hassan

None reported yet.

factors that possess proofreading activity (Pachetti et al., 2020). Due to the RNA-dependent RNA polymerase (RdRp) and co-factors that possess proofreading activity (Pachetti et al., 2020). However, an extremely high mutation rate can be detrimental and cause viruses to go extinct. Since its emergence in late 2019, SARS-CoV-2 exhibited a period of relative evolutionary stasis lasting almost a year (Harvey et al., 2021). This is likely due to the RNA-dependent RNA polymerase (RdRp) and co-factors that possess proofreading activity (Pachetti et al., 2020). However, in February 2020, a D614G substitution in the RBD of the S protein, which is believed to increase infectivity of the SARS-CoV-2, was identified in Europe and strains carrying this mutation became dominant globally (Groves et al., 2021). Furthermore, since late 2020, SARS-CoV-2 has exhibited continuous convergent evolution, characterized by the generation of sets of mutations that impact the transmissibility and antigenicity of the virus (ECDC, 2021). The mutations occurring in the spike (S) protein may affect virus entry into target cells and the efficacy of antibody protection. Particularly, receptor-binding domain (RBD) mutations are of high significance since most of the current vaccines target this domain (Ju et al., 2020). Furthermore, mutations in the N-terminal domain (NTD) of the spike gene could impair the efficacy of neutralizing antibodies (McCallum et al., 2021). Emergent SARS-CoV-2 variants classified as variants of concern by the World Health Organization include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) (Table 1) (WHO, 2021b).

SARS-CoV-2 Variants of Concern (VOCs)
Genetic mutations are building blocks of evolution, and the novelty in the genetic make-up that occurs during evolution leads to the emergence of new variants (Baer, 2008). High mutation rates have always been associated with RNA viruses, and these mutations are correlated with traits that are considered beneficial for viruses, such as altered virulence and transmissibility (Sanjuán and Domingo-Calap, 2016). However, an extremely high mutation rate can be detrimental and cause viruses to go extinct. Since its emergence in late 2019, SARS-CoV-2 exhibited a period of relative evolutionary stasis lasting almost a year (Harvey et al., 2021). This is likely due to the RNA-dependent RNA polymerase (RdRp) and co-factors that possess proofreading activity (Pachetti et al., 2020). However, in February 2020, a D614G substitution in the RBD of the S protein, which is believed to increase infectivity of the SARS-CoV-2, was identified in Europe and strains carrying this mutation became dominant globally (Groves et al., 2021). Furthermore, since late 2020, SARS-CoV-2 has exhibited continuous convergent evolution, characterized by the generation of sets of mutations that impact the transmissibility and antigenicity of the virus (ECDC, 2021). The mutations occurring in the spike (S) protein may affect virus entry into target cells and the efficacy of antibody protection. Particularly, receptor-binding domain (RBD) mutations are of high significance since most of the current vaccines target this domain (Ju et al., 2020). Furthermore, mutations in the N-terminal domain (NTD) of the spike gene could impair the efficacy of neutralizing antibodies (McCallum et al., 2021). Emergent SARS-CoV-2 variants classified as variants of concern by the World Health Organization include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) (Table 1) (WHO, 2021b).

Table 1. Experimental animal models used for SARS-CoV-2 variant infection studies

| SARS-CoV-2 Variants of Concern (VOCs) | Experimental animal model | Reference |
|--------------------------------------|---------------------------|-----------|
| Alpha                                | hACE2 transgenic/knock-in mice, transient somatic expression of hACE2 mice, aged Balb/c mice | Abdelnabi et al. (2021) |
|                                       | Golden Syrian hamster, Ferret, NHP (African green monkey) | Mohandas et al. (2021a) |
|                                       |                           | Mok et al. (2021) |
|                                       |                           | Port et al. (2021) |
|                                       |                           | Rosenke et al. (2021) |
|                                       |                           | Zhang et al. (2021a) |
| Beta                                 | hACE2 transgenic/knock-in mice, transient somatic expression of hACE2 mice, aged Balb/c mice | Abdelnabi et al. (2021) |
|                                       | Golden Syrian hamster, Ferret, NHP (rhesus macaques) | Corbett et al. (2021) |
|                                       |                           | Hassan et al. (2021) |
|                                       |                           | Yu et al. (2021) |
|                                       |                           | Zhang et al. (2021a) |
| Gamma                                | hACE2 transgenic/knock-in mice, transient somatic expression of hACE2 mice, golden Syrian hamster | Zhang et al. (2021a) |
| Delta                                | hACE2 transgenic mice, golden Syrian hamster | Imai et al. (2021) |
|                                       |                           | Hassan et al. (2021) |
| Omicron                              | None reported yet.        | Mohandas et al. (2021b) |

ALPHA (B.1.1.7)
The Alpha (B.1.1.7) variant was the first major VOC identified in the United Kingdom in 2020 (CDC, 2021b). Recent studies suggest that the Alpha variant exhibits an increase in transmissibility of about 40–50% compared to the original SARS-CoV-2 strain (Davies et al., 2021; Volz et al., 2021; Washington et al., 2021). The increased fitness is attributed to the presence of mutations in the spike (S) protein, including N501Y, D614G, and P681H (Davies et al., 2021). N501Y in the RBD within the S protein enhances the binding affinity to the host ACE2 receptor, leading to increased viral establishment and propagation of infection (Davies et al., 2021). While P681H, located adjacent to the furin cleavage site, is a known important region for infection and transmission and is a key indicator for increased transmission (Hoffmann et al., 2020). Furthermore, Alpha also has two deletions (ΔH69/ΔV70 and Δ144) in the spike protein linked to immune escape in immunocompromised patients and enhancement of viral infectivity in vivo (Kemp et al., 2021; Meng et al., 2021). Although Alpha showed resistance to monoclonal antibodies (mAbs) against the NTD and RBD, it is less resistant to convalescent plasma and sera from vaccinated individuals (Graham et al., 2021). Thus, reinfection with the Alpha variant does not frequently occur compared to other strains (Graham et al., 2021). Notably, the Alpha variant has de-escalated since fall 2020 in Europe. Moreover, there has been a drastic reduction in the circulation of the Alpha variant following the emergence of Delta, and Alpha is susceptible to vaccine-induced immunity (ECDC, 2021).

BETA (B.1.351)
Beta (B.1.351) was first identified from samples collected in
the beginning of October 2020 in South Africa. This variant is characterized by 18 amino acid changes and a deletion (Δ242–244) in the S protein (Boehm et al., 2021). Compared to the original strain, the Beta variant exhibits vaccine escape along with increased risk of hospitalization and mortality (Funk et al., 2021; Karim and de Oliveira, 2021; Wibmer et al., 2021). A study by Pearson et al. (2021) revealed that Beta also appears to have an increased transmission rate compared to preexisting strains in South Africa. Owing to the N501Y mutation, Beta also exhibits an increase in transmissibility. In addition, E484K and K417N were found to be associated with increased ACE binding affinity, and the combination of the three mutations could further enhance binding affinity. Moreover, recent studies reported that the Beta variant is more resistant to neutralization by convalescent plasma and sera from vaccinated individuals due to the E484K substitution (Wang et al., 2021; Wibmer et al., 2021). This resistance of the Beta variant to neutralizing antibodies (NAb) formed after infection with previously circulating strains means that even recovered patients have a high risk of reinfection. Thus, Beta has been considered the most NAb-resistant variant seen thus far, but it is important to note that it still seems to be outcompeted by Delta (Ramesh et al., 2021). A growing body of evidence suggests that Omicron is resistant to therapeutic antibodies and efficiently evades neutralization by antibodies from convalescent patient plasma and fully vaccinated individuals (Hoffmann et al., 2021b).

**GAMMA (P.1)**

Gamma was first identified in Brazil in early December 2020, and was subsequently shown to be descended from the B.1.1.28 lineage (Faria et al., 2021). B.1.1.28 was one of the two SARS-CoV-2 lineages predominant during the first wave of COVID-19 in Brazil. However, genetic and phylogenetic analysis revealed that the Gamma variant is distinct from B.1.1.28, hence it was given a new lineage designation (Rambaut et al., 2020). Beginning in January 2021, Gamma was detected at an increasing rate throughout Brazil and consequently, Brazil became the COVID-19 epicenter with over 22 million cases and ~610,000 deaths (WHO, 2021a). Gamma contains 21 mutations, 10 located in the spike protein, of which, biologically important mutations located in the RBD include N501Y, E484K, and K417T (Dejnirattisai et al., 2021). Combined together these mutations have been shown to increase receptor binding affinity and virus transmissibility. Further, recent studies reported that the mutations in the Gamma variants are associated with increased transmissibility, viral load, and a propensity for immune evasion and reinfection (Sabino et al., 2021), as well as cases of breakthrough infection of the Gamma variant even in fully vaccinated individuals (Vignier et al., 2021).

**DELTA (B.1.617.2)**

In May 2021, Delta was designated by the WHO as a VOC (WHO, 2021b), and subsequently found to be more highly contagious, cause more severe disease, and evade immunity more efficiently than existing variants (Campbell et al., 2021; Hoffmann et al., 2021a; Planas et al., 2021; Zhang et al., 2021b). Furthermore, research also showed that Delta is as much as 50% more transmissible than Alpha (Twohig et al., 2021). Among the seven mutations in the Delta S protein, L452R and T478K are likely to be associated with vaccine escape and increased transmissibility (Mohammadi et al., 2021). Recent studies reported that the L452R may stabilize the interaction between the viral S protein and host ACE2, thereby increasing infectivity (Deng et al., 2021; Teng et al., 2021). The T478K is located at the interface of spike/ACE2 interactions, which may play a key role in virus infectivity (Di Giacomo et al., 2021). Further, Muecksch et al. (2021) demonstrated the role of the T478K/R mutation in immune evasion. Liu et al. (2021b) demonstrated that P681R, within the furin cleavage site of S, could enhance viral fitness over that of the Alpha variant. Moreover, Saito et al. (2021) reported that although the P618R mutation does not increase infectivity in vitro, the P618R mutation imparts higher pathogenicity in hamsters compared to the original SARS-CoV-2 strains. A recent report by the WHO showed that Delta has outcompeted other variants in terms of prevalence, including Alpha, Beta and Gamma, in most countries and is by far the world’s most dominant SARS-CoV-2 variant (WHO, 2021c).

In light of growing concern of SARS-CoV-2 variants, an additional mutation in the Delta variant was found to give rise to Delta plus (B.1.671.2.1), also known as the AY.1 variant (Kannan et al., 2021). According to the US Center for Disease Control (US-CDC), the Delta plus variant includes signature mutations such as V70F, A222V and W258L in the S protein (CDC, 2021a). Furthermore, Delta plus carries the K417N mutation in the S protein, which is also found in the Beta variant and may contribute to immune escape. In fact, the Cryo-EM structure shows that K417 interacts with the Y52 of a neutralizing monoclonal Fab-Spike complex, and thus, K417N should result in loss of interaction, reducing binding of the antibody with the S protein (Kannan et al., 2021). The Delta plus variant was first isolated in India on April 2021 and subsequently reported in 42 other countries (GVN, 2021), although there has been increasing cases of Delta plus, it still uncertain whether this variant is exhibits increased transmissibility, severe disease, and immune evasion.

**OMICRON (B.1.1.529)**

The Omicron variant was first reported in South Africa on November 24, 2021, and was declared a variant of concern on November 26, 2021 (WHO, 2021b). Epidemiological reports in South Africa revealed a steep increase in reported cases coinciding with the detection of Omicron. This variant has a large number of mutations, most of which (>30 amino acid mutations and deletions) are found in the S protein and include ΔH69/ΔV70, H655Y, N679K, P681H, and D614G with enhanced ACE2 binding, increased transmissibility, and altered recognition by antibodies (Benvenuto et al., 2020; Covariants, 2021; Gong et al., 2021; Zahradnik et al., 2021). Preliminary reports showed that the Omicron
variant is highly transmissible even in fully vaccinated individuals (Liu et al., 2021a). This report is in parallel with the accumulating evidence that Omicron is markedly resistant to both vaccine-elicited neutralizing antibodies and sera of convalescent patients (Hoffmann et al., 2021b). Moreover, neutralization capabilities of most of the therapeutic antibodies currently available for COVID-19 are abolished or severely impaired (Mannar et al., 2022). Intriguingly, a recent study revealed that Omicron exhibits lower replication fitness and attenuated pathogenicity in mice compared to earlier VOCs, which is speculated to be a potential trade-off for escaping neutralization (Shuai et al., 2022). Disease characteristics observed in mice include negligible bodyweight loss with more moderate lung pathology than observed in animals infected with other VOCs. Furthermore, the lower pathogenicity exhibited in mice infected with Omicron is congruous with the current reports of Omicron variant infection in humans, including data from South Africa and England that showed significantly reduced odds of hospitalization (UK Health Security Agency, 2021; Wolter et al., 2022). The reduction in the risk of severe disease might be attributed to the lower intrinsic virulence of the virus. Although these preliminary reports are encouraging, it is not safe to assume that Omicron epidemics will also have a milder health impact than previous. Furthermore, existing data regarding Omicron is prefatory since studies regarding transmissibility, virulence, and immunity towards this newly emerged VOC are still ongoing.

Natural Infection Cases

Bats are considered to be a natural reservoir for many viruses, including SARS-like coronaviruses (Calisher et al., 2006). Recent data suggest that SARS-CoV-2 has undergone little evolutionary change from the putative bat coronavirus from which it originated (MacLean et al., 2021). In addition, it was hypothesized that pangolins may have served as an intermediate host, which was believed to play a role in the evolutionary change of the virus (Liu et al., 2020). A study revealed that impounded smuggled pangolins in China in 2017–2018 harbored two different sub-lineages of SARS-CoV-2-related coronaviruses (Fig. 1) (Lam et al., 2020). Notably, the receptor-binding domain in the virus isolated from the pangolin showed 97.4% sequence similarity with the SARS-CoV-2 (Lam et al., 2020). Thus, the possibility exists that pangolins served as a potential intermediate host for the transmission of SARS-CoV-2 to humans.

Fig. 1. Potential and established routes of transmission of SARS-CoV-2 variants between human and animal species. This figure focuses on the SARS-CoV-2 variants and shows the putative origin of SARS-CoV-2 and transmission to various animal hosts and humans. Companion animals such as cats and dogs are susceptible to SARS-CoV-2 variants and humans can be a source of their infection, but the potential role of pets in transmitting the disease to humans is unknown. Studies reported that mink can be a source of infection to humans. In addition, several animals, such as lions, gorillas, white-tailed deers, hamsters, rhesus monkeys, ferrets, humanized mice were also susceptible to SARS-CoV-2. In this, humanized mice, hamsters, rhesus monkeys, and ferrets were used as infection and transmission animal models outlining the current understanding of these variants, and potentially to omicron variant of concern. The figure was created with BioRender.com.
as the SARS-CoV-2 vector to mediate the jump to humans. However, this is still somewhat controversial and many scientists feel another intermediate species may have been involved.

Naturally acquired SARS-CoV-2 infections have been demonstrated among pets as well as captive and wild animals (Fig. 1). SARS-CoV-2 human-to-animal transmission has been well-documented in companion animals, particularly dogs (*Canis familiaris*) and cats (*Felis catus*) (Hobs and Reid, 2021; Hosie et al., 2021a). SARS-CoV-2 infection in companion animals was first described in February 2020 in Hong Kong (Sit et al., 2020) followed by a report in Belgium in March 2020 (Garigliany et al., 2020). Since then, several infections in companion animals have been reported (Halfmann et al., 2020; Sailleau et al., 2020; Hamer et al., 2021b; Hosie et al., 2021b) including recent B.1.1.7 infections (Barroso-Arévalo et al., 2021; Hamer et al., 2021a). A longitudinal household transmission study in the USA revealed that companion animals were confirmed to be infected with the B.1.1.7 variant (Hamer et al., 2021a). In addition, B.1.1.7 infections in dogs were also reported in Spain, and although no clinical symptoms were displayed, a high viral load in the nasal and rectal swabs was demonstrated, suggesting active virus shedding (Barroso-Arévalo et al., 2021). Interestingly, a pre-print study by Ferasin et al. (2021) showed that companion animals in the UK positive with the B.1.1.7 variant exhibited acute onset of cardiac disease, characterized by severe myocarditis. Severe heart abnormalities are a well-recognized complication in COVID-19 patients; however, this was considered an atypical clinical manifestation in companion animals and were not previously reported (Butt et al., 2021; Farshidfar et al., 2021). In March 2021, Italy and Germany has reported its first case of SARS-CoV-2 VOC in domestic cats (Keller et al., 2021; Zoccola et al., 2021). The report showed that cats tested positive with the B.1.1.7 a few days after COVID-19 appeared in their owners. Furthermore, a domestic cat in Peru was also reported to be infected with the Lambda variant (C.37), although cats appeared to be asymptomatic. Compared to cats, canines are generally considered less susceptible to SARS-CoV-2 infection (Shi et al., 2020); however, the potential for viral shedding from companion animals raises the possibility that they may serve as a viral reservoir and thus, further research is needed to elucidate their role in the COVID-19 disease cycle.

Recently, SARS-CoV-2 infections among captive and wild animals have also been demonstrated (Fig. 1). A significant percentage (40%) of white-tailed deer (*Odocoileus virginianus*) has been found to have antibodies against SARS-CoV-2 (Chandler et al., 2021), a sign that this species has been infected for some time. Human-to-deer transmission is believed to have caused this infection of the deer population (Chandler et al., 2021). Given the susceptibility of white-tailed deer to SARS-CoV-2 (Palmer et al., 2021), it was speculated that deer-to-deer transmission might have also contributed to the spread of SARS-CoV-2 within this population in the USA. Meanwhile, captive animals, including gorillas in zoos in the USA, have tested positive for SARS-CoV-2 (USDA, 2021b). In this case, it is believed that an asymptomatic zookeeper passed the virus to the gorillas. Given that gorillas exhibit large genetic similarities with humans, including in the ACE2 gene (Dam et al., 2020; Melin et al., 2020), their susceptibility to SARS-CoV-2 infection is not surprising. In addition, cases of SARS-CoV-2 infection among several species of large cats have also been reported (USDA, 2020, 2021a; Fernández-Bellon et al., 2021). Interestingly, Asiatic lions (Panthera leo persica) were reported to be naturally infected with the B.1.617.2 variant at two different locations in India (Karakalan et al., 2021; Mishra et al., 2021). Notably, the genome sequence of SARS-CoV-2 isolated from the lions showed spike protein deletions (ΔE156 and ΔF157) and a mutation (R158G) not found in human-isolated SARS-CoV-2 sequences (Mishra et al., 2021). Further, these reported deletions and the mutation were not found in previously reported SARS-CoV-2 isolated from lions, thus excluding the possibility of host adaptation. However, further studies are warranted to determine if the mutations are associated with increased transmissibility or pathogenicity in a wider host range.

Although studies of cross-species infection of SARS-CoV-2 and variants are limited, the constant emergence of novel variants raises the possibility of a broader host range for this virus. Likewise, these incidents also highlight the possibility of establishment of viral reservoirs where virus mutation and adaptation to the new host allow opportunity for reverse zoonosis events, which should be monitored closely.

**Experimental Animal Models**

**Mouse model**

The mouse (*Mus musculus*) has been the primary animal model adopted for the study of infectious diseases due to several advantages, such as the convenience of genome manipulation, cost-effectiveness, rapid reproduction rate, and ease of handling (Sarkar and Heise, 2019). However, due to the incompatibility between SARS-CoV-2 and the mouse ACE2 receptor, wild-type mouse strains are less susceptible to SARS-CoV-2 (Wan et al., 2020). To circumvent this limitation, several strategies have been utilized with the aim to express human ACE2 (hACE2) in the respiratory tract of mice. These include the generation of transgenic mice (Bao et al., 2020; Jiang et al., 2020) and expression of hACE2 by transduction (Table 1) (Hassan et al., 2020; Israelow et al., 2020). Although hACE2 transgenic or hACE2-transduced mice support SARS-CoV-2 replication (Rathnasinghe et al., 2020), these animal models exhibit subtle differences in disease manifestation. hACE2 TG mice manifest more severe disease, greater weight loss, and virus dissemination to multiple organs compared to hACE2 transduced mice, wherein virus replication is restricted to the respiratory tract. Nonetheless, a huge advantage of the transduced-hACE2 mouse model is that it allows immediate studies using multiple mice compared to the time-consuming breeding of hACE2 TG mice. However, despite these advantages, these mouse models have several limitations. For example, hACE2 mice generated by viral-vector-mediated delivery systems or TG mice can exhibit consequential effects, due to the delivery system used or ectopic hACE2 expression, causing confusion over which effects are caused by SARS-CoV-2. Most studies of the pathogenesis and host immune response to SARS-CoV-2 have used the hACE2 mouse model, but disparities between human and murine...
immune systems bring the extrapolation of immune response from this animal model into debate. Thus, researchers developed a human immune system/humanized mouse model expressing hACE2 (MISTRG6-hACE2) (Sefik et al., 2021). Aside from the fact that MISTRG6-hACE2 can be readily infected with SARS-CoV-2, this humanized mouse model comprehensively recapitulates the human innate and adaptive immune response during infection with SARS-CoV-2 and hence, allows study of the anti-viral immune response and resulting immunopathology. Furthermore, this animal model could provide a platform for testing immunopathological therapeutic options.

Meanwhile, in addition to hACE2 mice, researchers also developed a mouse-adapted SARS-CoV-2 (MA SARS-CoV-2) strain both by serial in vivo passaging (Gu et al., 2020; Huang et al., 2021) and through reverse genetics (Dinnon et al., 2020). For the latter approach, a SARS-CoV-2 strain was remodeled to express an S binding interface that can utilize mouse ACE2 (mACE2) for host entry. Furthermore, results showed that mice infected with the MA SARS-CoV-2 strain generated by successive lung passages develop interstitial pneumonia (Gu et al., 2020). Deep sequencing of this MA SARS-CoV-2 strain revealed a particular mutation (N501Y) in the RBD of S, which might be associated with increased binding affinity to mACE2, leading to increased infectivity and virulence in mice (Gu et al., 2020). Interestingly, the N501Y mutation has also been found among the recent SARS-CoV-2 variants, B.1.1.7 (Alpha), B.1.351 (Beta), and P.1 (Gamma) (Zhang et al., 2021a). Moreover, N501Y-carrying variants have been shown to infect wild-type mice (Shuai et al., 2021), hence, this suggests breakage of the cross-species barrier and the capability to expand species tropism. Furthermore, the K417N mutation was also observed in the mouse-adapted strains of SARS-CoV-2 (Muruato et al., 2021). The presence of both of these mutations in the S protein has also been observed in human isolates of SARS-CoV-2, particularly the Beta, Gamma, and the Delta-plus variants. Interestingly, N501Y and K417N mutations are also found in the newly emergent Omicron variant (CoVariants, 2021). This convergent evolution in humans and in the mouse model provides significant insight into SARS-CoV-2 adaptation.

Mouse models are employed in virus studies to evaluate virulence, pathogenesis, and vaccine effectiveness (Hassan et al., 2021). However, adaptation studies of earlier SARS-CoV-2 isolates have helped reveal key factors driving the rapid evolution of this virus. Thus, comparison of the results of these mouse adaptation experiments with newly emerging variants may also provide valuable information regarding how SARS-CoV-2 adapts during human transmission.

Hamster model

The golden Syrian hamster (Mesocricetus auratus) has been utilized for studying SARS-CoV-2 pathology, transmission, and host immune responses (Sia et al., 2020). Among rodent animal models used for SARS-CoV-2 studies, hamsters are readily infected without the need for virus-host adaptation. Molecular docking studies revealed a high binding affinity of SARS-CoV-2 S protein to the hamster ACE2 receptor (Chan et al., 2020; Luan et al., 2020). Furthermore, studies showed that upon intranasal inoculation, SARS-CoV-2 replicates remarkably well in the upper and lower respiratory tracts of these animals, causing pneumonia with significant lung histological changes (Imai et al., 2020; Sia et al., 2020).

Following the success of the hamster animal model and given the emergence of new SARS-CoV-2 variants, hamsters have been used to characterize the replicative fitness of viral variants and the resulting disease manifestation (Table 1). Although, similar to infection studies using an earlier SARS-CoV-2 variant, studies using variant SARS-CoV-2 inocula reportedly result in transient mild disease with minimal or no weight loss (Imai et al., 2021; Mohandas et al., 2021a).

Previously, a D614G competition and transmission experiment in hamsters suggested that the D614G mutation increases transmissibility (Plante et al., 2021). Moreover, the addition of the N501Y mutation is predicted to increase affinity for human ACE2, hence resulting in increased risk of airborne transmission, which is an important trait of VOCs and partially explains the dominance of new variants containing both mutations. Notably, a study by Port et al. (2021) highlighted the efficiency of the Alpha variant in airborne transmission in Syrian hamsters at a 200 cm distance after an hour of exposure. Meanwhile, the study by Mohandas et al. (2021a) found no difference in transmission by different routes (contact, fomite, or short distance airborne transmission) between Alpha (B.1.1.7) and D614G in Syrian hamsters. An earlier study showed that SARS-CoV-2 is inefficiently transmitted through fomites (Sia et al., 2020); however, a fomite transmission study showed that Alpha is efficiently transmitted and infectious virus can be recovered from nasal washes. Thus, this suggest that VOCs exhibit enhanced transmissibility and can be spread through other measures.

Given the high susceptibility of hamsters to SARS-CoV-2 infection, Mok et al. (2021) investigated infection kinetics of low viral titer Alpha-variant (B.1.1.7) inoculum and compared it to HK-405 (GH-clade), HK-95 (G-clade), and HK-15 (a non-D614G strain) in the hamster model. Interestingly, the results showed that the Alpha variant can replicate efficiently resulting in significantly higher titers in the nasal epithelium compared to other strains. Further, another study also showed that the increased transmission of Alpha may not be due to the magnitude of shedding, but to the fact that a lower dose of Alpha variant may be sufficient for transmission (Port et al., 2021). Thus, this suggests the Alpha variant can initiate effective infection with fewer infectious particles. It should be noted that humans are exposed to varying doses of infectious particles during virus transmission, hence, some exposures may result in a failure to establish infection due to insufficient transmission of infectious virus titers.

Abdelnabi et al. (2021) used a hamster animal model to investigate infectivity and virulence of clinical SARS-CoV-2 isolates, particularly B.1.1.7 (Alpha) and B.1.351 (Beta). Results showed that Alpha and Beta variants could establish very efficient infections in the lower respiratory tract resulting in a pathology comparable to the earlier SARS-CoV-2 lineage. Furthermore, a comparative study of viral pathogenesis of Alpha and Beta in comparison to the prototypic Wuhan-Hu-1 isolate has recently been conducted in the hamster model. Results showed that hamsters infected with the three virus strains exhibited no discernable differences in gross pathology and viral burden in the lungs. A similar observation...
was also reported in other studies comparing variants of concern with the D614G mutation and the earlier SARS-CoV-2 variant (Imai et al., 2021; Mohandas et al., 2021a).

Recently, B.1.617 is becoming a dominant variant globally, and sublineage B.1.617.2 (Delta) has been a variant of concern. A comparison study in hamsters (Mohandas et al., 2021b) between B.1.617.2, B.1.617.3, and B.1 showed no significant differences, although higher sub-genomic viral RNA could be detected in Delta-infected hamsters for a prolonged period of time. Furthermore, the Delta variant possesses mutations in the S protein that affect neutralization; however, no marked reduction in neutralization was observed in hamsters.

**Ferret model**

The ferret (*Mustela putorius furo*) is a common laboratory animal model in studying human respiratory infections, as ferrets respiratory tracts have anatomical and physiological features that are similar to that of humans (Enkirch and von Messling, 2015). Experimental SARS-CoV-2 infections have shown the susceptibility of ferrets to this virus and their ability to transmit the virus both directly and indirectly to naïve ferrets (Table 1) (Kim et al., 2020; Richard et al., 2020). Intriguingly, ferrets inoculated experimentally with wild-type SARS-CoV-2 demonstrated spike protein mutations (N501T and Y453F), which have been associated with experimental adaptation (Everett et al., 2021). During the reverse zoonotic-outbreaks in April 2020, SARS-CoV-2 adapted to mink populations bearing mutations in the RBD of S protein (Y453 and N501T) that enhance the affinity of the virus for mink ACE2. In addition, virus spillover to farmworkers from an outbreak among mink farms in Denmark resulted in an estimated 4,000 mink-associated human cases from June to November 2020 (Larsen et al., 2021). Of particular concern, sequence analysis of the isolated virus revealed the Y453F mutation. Minks and ferrets are closely related, as both belonging to Mustelidae family. Mink and ferret ACE2 are almost identical, potentially explaining the susceptibility of mink to SARS-CoV-2 infection. Although the Y453F containing ferret-adapted SARS-CoV-2 strain showed enhanced binding affinity to both human and ferret ACE2 (Zhou et al., 2021b), this strain is more easily neutralized than the earlier SARS-CoV-2 strains. Hence, the Y453F mutation negatively impacts SARS-CoV-2 replication kinetics in humans, suggesting this mutation is a species-specific adaptive mutation.

While G354R in the ferret ACE2 protein may act as a host barrier which limits natural SARS-CoV-2 infection within this host, N501T appeared as a selective mutational adaptation resulting in increased transmissibility to and infectivity of ferrets and minks (Sawatzki et al., 2021). However, additional work is needed to determine whether N501T is a required adaptation for ferret transmission and how it affects transmission dynamics.

**Non-human primate model**

Previous studies investigated different species of non-human primates as possible models of SARS-CoV-2 infection (Chandrashekar et al., 2020; Lu et al., 2020; Munster et al., 2020; Rockx et al., 2020; Yu et al., 2020; Blair et al., 2021) and reported a heterogeneous spectrum of clinical manifestations of infection (Table 1). Comparative analysis using African green monkeys revealed that Alpha variants are more prevalent in the lower respiratory tissues causing more severe pulmonary lesions than the contemporary progenitor D614G variant (Rosenke et al., 2021). Further, the Alpha variant exhibited higher virus in nasal and oropharyngeal swabs suggestive of higher transmissibility than the D614G variant. Intriguingly, D614G replicated at a higher level in the gastrointestinal tract (GIT), suggesting a different organ tropism. Notably, during early to mid-2020, 15–20% of COVID-19 patients reportedly had gastrointestinal (GI) symptoms (Lui et al., 2021); however, the possibility of altered tropism between Alpha and D614G variants warrants further study. In addition, non-human primates provide several advantages as preclinical animal models for assessing the immunogenicity and protection of human vaccines. Although all animals have limitations for extrapolation to human species, rhesus macaques have been useful for assessing immunity against SARS-CoV-2 as these animals share immune properties with humans. Thus far, rhesus (Macaca mulatta) and cynomolgus macaques (Macaca fascicularis) have been most frequently utilized for COVID-19 drug and vaccine research (Munoz-Fontela et al., 2020; Salguero et al., 2021). Although one limitation of the macaque animal model is the mild to moderate clinical manifestations of SARS-CoV-2 infection, it is important to keep in mind that this does reflect the majority of human infections.

NHPs are proven valuable animal models have been a critical resource in preclinical investigation of therapeutics and vaccines developed for humans, including those against SARS-CoV-2. Thus, they have been essential in understanding SARS-CoV-2 cellular immune responses, specially in vulnerable populations, and for studies of post-acute sequelae of COVID-19. Thus far, multiple authorized vaccines tested in macaques showed strong protection against disease symptoms in the lower respiratory tract. However, there has been emergence of several variants of concern that exhibit greater transmissibility, pathogenicity, and resistance to antibodies and vaccine-mediated immunity. Ongoing assessments of authorized vaccines against SARS-CoV-2 variants have been performed. Recently, Corbett et al. showed that administration of mRNA-1273 vaccine can protect rhesus macaques from lung pathology after experimental infection with the Beta variant (Corbett et al., 2021). Compellingly, the two-dose regimen of mRNA-1273 vaccine not only provides protection against viral replication in the lower respiratory tract, but also reduces the amount of virus in the upper respiratory tract. In addition, broadly similar findings have been reported in a study with the Ad26.COV2.S vaccine against Beta variant infection of macaques (Yu et al., 2021). Macaques in both studies receiving either of the vaccines showed decreased disease symptoms, which was attributed to the reduction in viral load, particularly in the lower respiratory tract. However, it is challenging to compare studies of different vaccines, since too many variables are involved. Notwithstanding these differences, both vaccines significantly reduce viral loads in the lower and upper respiratory tracts of these animals. Results of these studies are promising, given that ten-fold higher neutralizing antibody titers are needed to control the Beta variant compared to Wuhan and D614G strains. Furthermore,
this comparison study highlights the need for authorized vaccine-induced titers to be high enough to fare with the loss in protective capabilities of neutralizing antibodies in the face of Beta or other variants of concern.

**Conclusion**

As was verified at the beginning of the pandemic, SARS-CoV-2 can infect different species of animals, and the emergence of new variants may expand this capacity, as emerging variants with multiple mutations or deletions could potentially be highly contagious to humans and other animals. Further, changes in the amino acids that mediate receptor binding can affect the ability of the virus to bind more efficiently to human and/or animal ACE2 receptors. Currently circulating variants include one or more such amino-acid changes in the RBD of the S protein conferring fitness advantages, increased infectivity, and reduced serum neutralization. In particular, the Alpha, Beta, and Gamma variants contain a particular set of mutations, D614G and N501Y. Moreover, Beta and Gamma both exhibit substitutions at the 417 site, replacing lysine with asparagine (Beta) or threonine (Gamma). On the other hand, Delta carries L452R and T478K changes in addition to D614G. Thus, the presence of common mutations among variants suggests co-evolutionary and convergent mutation processes.

Active monitoring of the emergence of SARS-CoV-2 variants has enabled different institutions to further support the public health response. Potential consequences of the emergence of novel variants include more rapid transmission, severe disease manifestation, evasion of detection by diagnostic tests, decreased susceptibility to therapeutics, and the ability to evade natural and/or vaccine-induced immunity. Hence, to investigate these properties among variants, animal studies are pivotal. Since the start of the pandemic, many studies using different animal models have been undertaken to elucidate differences in the mechanisms and variability of disease manifestation and severity, transmissibility and pathogenesis, and the host immune response to and evasion by novel emerging SARS-CoV-2 variants of concern. While new SARS-CoV-2 variants are detected weekly, most of are present but not yet prevalent worldwide. Although a growing body of evidence shows that circulating VOCs do not differ enough to require changes in COVID-19 guidelines to mitigate the spread of the virus, we must stay vigilant for such phenomena.

Traditionally, mouse models have been employed to study viruses and evaluate their virulence, host pathogenic mechanisms, and vaccine effectiveness. However, in this case adaptation studies of SARS-CoV-2 have been used to understand the key factors driving the rapid evolution of this virus. Thus, results of mouse adaptation experiments in parallel with the emergence of novel variants have provided valuable insight into how SARS-CoV-2 adapts during human-to-human transmission. Susceptible animal models, particularly golden Syrian hamsters and ferrets, are also of great significance. Several studies using hamster and ferret animal models have shed light on disease pathogenesis and hence, have been utilized for testing medical countermeasures. NHPs offer several advantages due to their close genetic proximity, physiology, and immune system similarities to humans. Despite limitations associated with the fact that disease manifestation does not recapitulate severe cases of human infection, NHPs provide distinct insight with significant implications for development of therapeutics and vaccines.

Nonetheless, no single animal model yet developed can fully recapitulate the wide range of disease manifestations exhibited during human infections. Furthermore, the limited lethality across SARS-CoV-2 animal models constitutes a critical shortcoming. Several key elements in understanding COVID-19 pathophysiology should be included to maximize the translational value of the animal models being employed. These factors include age (aged animals would correspond to the most affected patient demographic), presence of most frequent COVID-19-related comorbidities such as diabetes, obesity, and cardiovascular diseases, and presence of severe COVID-19 phenotypes. Other shortcomings are difficult to overcome when using animal models, thus over-reliance on a single animal species as a disease model may produce misleading findings until contraindicated in other species. Nevertheless, animal research plays a key role in the fight against COVID-19, and important points have been addressed through extensive research using currently available animal models.

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**Conflict of Interest**

The authors have no conflict of interest to report.

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