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REVIEW

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 (Coronaviridae Study Group of the 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.

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Thus, beside the existing animal models for SARS-CoV-2, the discovery of new animal models will further advance studies of the etiology of transmission, cross-reactivity, and aid in development of vaccines and therapeutics against variant viruses. Therefore, in this review, we discuss naturally susceptible animals and various experimental animal models for recent SARS-CoV-2 strains to generate a better and more diverse understanding of variant virus etiology and transmissibility.

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 cofactors 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).

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

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 Golden Syrian hamster, Ferret, NHP (African green monkey)	Abdelnabi *et al.* (2021) 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 Golden Syrian hamster, Ferret, NHP (rhesus macaques)	Abdelnabi *et al.* (2021) 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) Imai *et al.* (2021) Hassan *et al.* (2021)
Delta	hACE2 transgenic mice, golden Syrian hamster	Hassan *et al.* (2021) Mohandas *et al.* (2021b)
Omicron	None reported yet.	

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 comple, 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 increased 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 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

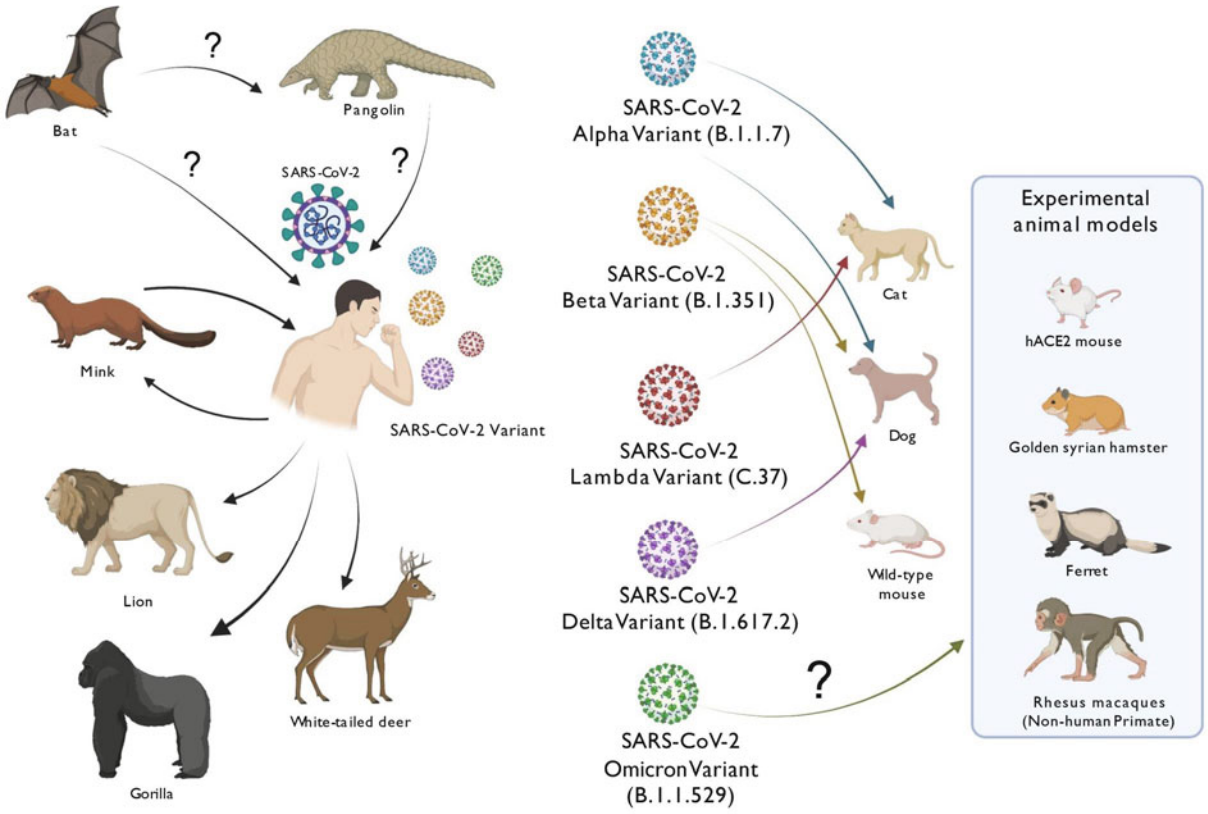

Fig. 1. Potential and established routes of transmission of SARS-CoV-2 variants between human and animal species. This figure focuses on the SARS-CoV-2 variants and shows the putative origin of SARS-CoV-2 and transmission to various animal hosts and humans. Companion animals such as cats and dogs are 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*) (Hobbs 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 (Damas *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 (Karikalan *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 broaden 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 fascicullariis*) 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 decrease 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.

References

Abdelghany, T.M., Ganash, M., Bakri, M.M., Qanash, H., Al-Rajhi, A.M.H., and Elhussieny, N.I. 2021. SARS-CoV-2, the other face to SARS-CoV and MERS-CoV: future predictions. *Biomed. J.* **44**, 86–93.

Abdelnabi, R., Boudewijns, R., Foo, C.S., Seldeslachts, L., Sanchez-Felipe, L., Zhang, X., Delang, L., Maes, P., Kaptein, S.J.F., Weynand, B., *et al.* 2021. Comparing infectivity and virulence of emerging SARS-CoV-2 variants in syrian hamsters. *EBioMedicine* **68**, 103403.

Abdelrahman, Z., Li, M.Y., and Wang, X.S. 2020. Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza a respiratory viruses. *Front. Immunol.* **11**, 552909.

Baer, C.F. 2008. Does mutation rate depend on itself? *PLoS Biol.* **6**, e52.

Bao, L.N., Deng, W., Huang, B., Gao, H., Liu, J., Ren, L., Wei, Q., Yu, P., Xu, Y., Qi, F., *et al.* 2020. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* **583**, 830–833.

Barroso-Arévalo, S., Rivera, B., Dominguez, L., and Sánchez-Vizcaíno, J.M. 2021. First detection of SARS-CoV-2 B.1.1.7 variant of concern in an asymptomatic dog in Spain. *Viruses* **13**, 1379.

Benvenuto, D., Angeletti, S., Giovanetti, M., Bianchi, M., Pascarella, S., Cauda, R., Ciccozzi, M., and Cassone, A. 2020. Evolutionary analysis of SARS-CoV-2: how mutation of non-structural protein 6 (NSP6) could affect viral autophagy. *J. Infect.* **81**, e24–e27.

Blair, R.V., Vaccari, M., Doyle-Meyers, L.A., Roy, C.J., Russell-Lod-

rigue, K., Fahlberg, M., Monjure, C.J., Beddingfield, B., Plante, K.S., Plante, J.A., *et al.* 2021. Acute respiratory distress in aged, SARS-CoV-2-infected african green monkeys but not rhesus macaques. *Am. J. Pathol.* **191**, 274–282.

Boehm, E., Kronig, I., Neher, R.A., Eckerle, I., Vetter, P., Kaiser, L., and Geneva Centre for Emerging Viral Diseases. 2021. Novel SARS-CoV-2 variants: the pandemics within the pandemic. *Clin. Microbiol. Infect.* **27**, 1109–1117.

Butt, N., Arshid, A., Ahmad, S.A., Khalid, N., and Kayani, W.T. 2021. Cardiovascular complications in COVID-19. *Am. J. Emerg. Med.* **45**, 540.

Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V., and Schountz, T. 2006. Bats: important reservoir hosts of emerging viruses. *Clin. Microbiol. Rev.* **19**, 531–545.

Campbell, F., Archer, B., Laurenson-Schafer, H., Jinnai, Y., Konings, F., Batra, N., Pavlin, B., Vandemaele, K., Van Kerkhove, M.D., Jombart, T., *et al.* 2021. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Euro Surveill.* **26**, 2100509.

Centers for Disease Control and Prevention, CDC. 2021a. SARS-CoV-2 Variant Classifications and Definitions. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html.

Centers for Disease Control and Prevention, CDC. 2021b. Science brief: Emerging SARS-CoV-2 Variants. https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-emerging-variants.html.

Chan, J.F.W., Zhang, A.N.J., Yuan, S., Poon, V.K.M., Chan, C.C.S., Lee, A.C.Y., Chan, W.M., Fan, Z., Tsoi, H.W., Wen, L., *et al.* 2020. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin. Infect. Dis.* **71**, 2428–2446.

Chandler, J.C., Bevins, S.N., Ellis, J.W., Linder, T.J., Tell, R.M., Jenkins-Moore, M., Root, J.J., Lenoch, J.B., Robbe-Austerman, S., DeLiberto, T.J., *et al.* 2021. SARS-CoV-2 exposure in wild white-tailed deer (*Odocoileus virginianus*). *Proc. Natl. Acad. Sci. USA* **118**, e2114828118.

Chandrashekar, A., Liu, J., Martinot, A.J., McMahan, K., Mercado, N.B., Peter, L., Tostanoski, L.H., Yu, J., Maliga, Z., Nekorchuk, M., *et al.* 2020. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science* **369**, 812–817.

Corbett, K.S., Werner, A.P., O'Connell, S., Gagne, M., Lai, L., Moliva, J.I., Flynn, B., Choi, A., Koch, M., Foulds, K.E., *et al.* 2021. mRNA-1273 protects against SARS-CoV-2 beta infection in nonhuman primates. *Nat. Immunol.* **22**, 1306–1315.

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. 2020. The species *Severe acute respiratory syndrome-related coronavirus*: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* **5**, 536–544.

Cromer, D., Steain, M., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Kent, S.J., Triccas, J.A., Khoury, D.S., and Davenport, M.P. 2021. Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* **3**, e52–e61.

Damas, J., Hughes, G.M., Keough, K.C., Painter, C.A., Persky, N.S., Corbo, M., Hiller, M., Koepfli, K.P., Pfenning, A.R., Zhao, H., *et al.* 2020. Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *Proc. Natl. Acad. Sci. USA* **117**, 22311–22322.

Davies, N.G., Abbott, S., Barnard, R.C., Jarvis, C.I., Kucharski, A.J., Munday, J.D., Pearson, C.A.B., Russell, T.W., Tully, D.C., Washburne, A.D., *et al.* 2021. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science* **372**, eabg3055.

Dejnirattisai, W., Zhou, D.M., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y.G., Duyvesteyn, H.M.E., Tuekprakhon, A., Nutalai, R., *et al.* 2021. Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell* **184**, 2939–2954.

Deng, X., Garcia-Knight, M.A., Khalid, M.M., Servellita, V., Wang, C., Morris, M.K., Sotomayor-González, A., Glasner, D.R., Reyes, K.R., Gliwa, A.S., *et al.* 2021. Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant. *Cell* **184**, 3426–3437.

Di Giacomo, S., Mercatelli, D., Rakhimov, A., and Giorgi, F.M. 2021. Preliminary report on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Spike mutation T478K. *J. Med. Virol.* **93**, 5638–5643.

Dinnon, K.H., Leist, S.R., Schäfer, A., Edwards, C.E., Martinez, D.R., Montgomery, S.A., West, A., Yount, B.L.Jr, Hou, Y.J., Adams, L.E., *et al.* 2020. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. *Nature* **586**, 560–566.

Enkirch, T. and von Messling, V. 2015. Ferret models of viral pathogenesis. *Virology* **479–480**, 259–270.

European Centre for Disase Prevention and Control, ECDC. 2021. Situation updates on covid-19: Variants of concern. https://www.ecdc.europa.eu/en/covid-19/variants-concern.

Everett, H.E., Lean, F.Z.X., Byrne, A.M.P., van Diemen, P.M., Rhodes, S., James, J., Mollett, B., Coward, V.J., Skinner, P., Warren, C.J., *et al.* 2021. Intranasal infection of ferrets with SARS-CoV-2 as a model for asymptomatic human infection. *Viruses* **13**, 113.

Faria, N.R., Mellan, T.A., Whittaker, C., Claro, I.M., Candido, D.D.S., Mishra, S., Crispim, M.A.E., Sales, F.C.S., Hawryluk, I., McCrone, J.T., *et al.* 2021. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* **372**, 815–821.

Farshidfar, F., Koleini, N., and Ardehali, H. 2021. Cardiovascular complications of COVID-19. *JCI Insight.* **6**, e148980.

Fenollar, F., Mediannikov, O., Maurin, M., Devaux, C., Colson, P., Levasseur, A., Fournier, P.E., and Raoult, D. 2021. Mink, SARS-CoV-2, and the human-animal interface. *Front. Microbiol.* **12**, 663815.

Ferasin, L., Fritz, M., Ferasin, H., Becquart, P., Corbet, S., Gouilh, M.A., Legros, V., and Leroy, E.M. 2021. Infection with SARS-CoV-2 variant B.1.1.7 detected in a group of dogs and cats with suspected myocarditis. *Vet. Rec.* **189**, e944.

Fernández-Bellon, H., Rodon, J., Fernández-Bastit, L., Almagro, V., Padilla-Solé, P., Lorca-Oró, C., Valle, R., Roca, N., Grazioli, S., Trogu, T., *et al.* 2021. Monitoring natural SARS-CoV-2 infection in lions (*Panthera leo*) at the Barcelona zoo: viral dynamics and host responses. *Viruses* **13**, 1683.

Funk, T., Pharris, A., Spiteri, G., Bundle, N., Melidou, A., Carr, M., Gonzalez, G., Garcia-Leon, A., Crispie, F., O'Connor, L., *et al.* 2021. Characteristics of SARS-CoV-2 variants of concern B.1.1.7, B.1.351 or P.1: data from seven EU/EEA countries, weeks 38/2020 to 10/2021. *Euro Surveill.* **26**, 2100348.

Garigliany, M., Van Laere, A.S., Clercx, C., Giet, D., Escriou, N., Huon, C., van der Werf, S., Eloit, M., and Desmecht, D. 2020. SARS-CoV-2 natural transmission from human to cat, Belgium, March 2020. *Emerg. Infect. Dis.* **26**, 3069–3071.

Global Virus Network, GVN. 2021. Delta (B.1.617.2). https://gvn.org/covid-19/delta-b-1-617-2/.

CoVariants. 2021. Variant: 21K (Omicron). https://covariants.org/variants/21K.Omicron.

Gong, S.Y., Chatterjee, D., Richard, J., Prévost, J., Tauzin, A., Gasser, R., Bo, Y.X., Vezina, D., Goyette, G., Gendron-Lepage, G., *et al.* 2021. Contribution of single mutations to selected SARS-CoV-2 emerging variants spike antigenicity. *Virology* **563**, 134–145.

Graham, M.S., Sudre, C.H., May, A., Antonelli, M., Murray, B., Varsaysky, T., Kläser, K., Canas, L.S., Molteni, E., Modat, M., *et al.* 2021. Changes in symptomatology, reinfection, and transmissibility associated with the SARS-CoV-2 variant B.1.1.7: An ecological study. *Lancet Public Health* **6**, e335–e345.

Groves, D.C., Rowland-Jones, S.L., and Angyal, A. 2021. The D614G mutations in the SARS-CoV-2 spike protein: implications for

viral infectivity, disease severity and vaccine design. *Biochem. Biophys. Res. Commun.* **538**, 104–107.

Gu, H.J., Chen, Q., Yang, G., He, L., Fan, H., Deng, Y.Q., Wang, Y.X., Teng, Y., Zhao, Z.P., Cui, Y.J., *et al.* 2020. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* **369**, 1603–1607.

Halfmann, P.J., Hatta, M., Chiba, S., Maemura, T., Fan, S., Takeda, M., Kinoshita, N., Hattori, S., Sakai-Tagawa, Y., Iwatsuki-Horimoto, K., *et al.* 2020. Transmission of SARS-CoV-2 in domestic cats. *N. Eng. J. Med.* **383**, 592–594.

Hamer, S.A., Ghai, R.R., Zecca, I.B., Auckland, L.D., Roundy, C.M., Davila, E., Busselman, R.E., Tang, W., Pauvolid-Correa, A., Killian, M.L., *et al.* 2021a. SARS-CoV-2 B.1.1.7 variant of concern detected in a pet dog and cat after exposure to a person with COVID-19, USA. *Transbound Emerg Dis.* doi: https://doi.org/10.1111/tbed.14122.

Hamer, S.A., Pauvolid-Correa, A., Zecca, I.B., Davila, E., Auckland, L.D., Roundy, C.M., Tang, W., Torchetti, M.K., Killian, M.L., Jenkins-Moore, M., *et al.* 2021b. SARS-CoV-2 infections and viral isolations among serially tested cats and dogs in households with infected owners in texas, USA. *Viruses* **13**, 938.

Harvey, W.T., Carabelli, A.M., Jackson, B., Gupta, R.K., Thomson, E.C., Harrison, E.M., Ludden, C., Reeve, R., Rambaut, A., Peacock, S.J., *et al.* 2021. SARS-CoV-2 variants, spike mutations and immune escape. *Nat. Rev. Microbiol.* **19**, 409–424.

Hassan, A.O., Case, J.B., Winkler, E.S., Thackray, L.B., Kafai, N.M., Bailey, A.L., McCune, B.T., Fox, J.M., Chen, R.E., Alsoussi, W.B., *et al.* 2020. A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. *Cell* **182**, 744–753.

Hassan, A.O., Shrihari, S., Gorman, M.J., Ying, B., Yaun, D., Raju, S., Chen, R.E., Dmitriev, I.P., Kashentseva, E., Adams, L.J., *et al.* 2021. An intranasal vaccine durably protects against SARS-CoV-2 variants in mice. *Cell Rep.* **36**, 109452.

Hobbs, E.C. and Reid, T.J. 2021. Animals and SARS-CoV-2: Species susceptibility and viral transmission in experimental and natural conditions, and the potential implications for community transmission. *Transbound. Emerg. Dis.* **68**, 1850–1867.

Hoffmann, M., Hofmann-Winkler, H., Krüger, N., Kempf, A., Nehlmeier, I., Graichen, L., Arora, P., Sidarovich, A., Moldenhauer, A.S., Winkler, M.S., *et al.* 2021a. SARS-CoV-2 variant B.1.617 is resistant to bamlanivimab and evades antibodies induced by infection and vaccination. *Cell Rep.* **36**, 109415.

Hoffmann, M., Kleine-Weber, H., and Pöhlmann, S. 2020. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol. Cell* **78**, 779–784.

Hoffmann, M., Krüger, N., Schulz, S., Cossmann, A., Rocha, C., Kempf, A., Nehlmeier, I., Graichen, L., Moldenhauer, A.S., Winkler, M.S., *et al.* 2021b. The Omicron variant is highly resistant against antibody-mediated neutralization–implications for control of the COVID-19 pandemic. *Cell* **185**, 447–456.

Hosie, M.J., Epifano, I., Herder, V., Orton, R.J., Stevenson, A., Johnson, N., MacDonald, E., Dunbar, D., McDonald, M., Howie, F., *et al.* 2021a. Detection of SARS-CoV-2 in respiratory samples from cats in the UK associated with human-to-cat transmission. *Vet. Rec.* **188**, e247.

Hosie, M.J., Hofmann-Lehmann, R., Hartmann, K., Egberink, H., Truyen, U., Addie, D.D., Belák, S., Boucraut-Baralon, C., Frymus, T., Lloret, A., *et al.* 2021b. Anthropogenic infection of cats during the 2020 COVID-19 pandemic. *Viruses* **13**, 185.

Huang, K., Zhang, Y., Hui, X., Zhao, Y., Gong, W., Wang, T., Zhang, S., Yang, Y., Deng, F., Zhang, Q., *et al.* 2021. Q493K and Q498H substitutions in Spike promote adaptation of SARS-CoV-2 in mice. *eBioMedicine* **67**, 103381.

Imai, M., Halfmann, P.J., Yamayoshi, S., Iwatsuki-Horimoto, K., Chiba, S., Watanabe, T., Nakajima, N., Ito, M., Kuroda, M., Kiso, M., *et al.* 2021. Characterization of a new SARS-CoV-2 variant that emerged in Brazil. *Proc. Natl. Acad. Sci. USA* **118**, e2106535118.

Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P.J., Nakajima, N., Watanabe, T., Ujie, M., Takahashi, K., Ito, M., *et al.* 2020. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl. Acad. Sci. USA* **117**, 16587–16595.

Israelow, B., Song, E., Mao, T., Lu, P., Meir, A., Liu, F., Alfajaro, M.M., Wei, J., Dong, H., Homer, R., *et al.* 2020. Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. *J. Exp. Med.* **217**, e20201241.

Jiang, R.D., Liu, M.Q., Chen, Y., Shan, C., Zhou, Y.W., Shen, X.R., Li, Q., Zhang, L., Zhu, Y., Si, H.R., *et al.* 2020. Pathogenesis of SARS-CoV-2 in transgenic mice expressing human angiotensin-converting enzyme 2. *Cell* **182**, 50–58.

Ju, B., Zhang, Q., Ge, J., Wang, R., Sun, J., Ge, X., Yu, J., Shan, S., Zhou, B., Song, S., *et al.* 2020. Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* **584**, 115–119.

Kannan, S.R., Spratt, A.N., Cohen, A.R., Naqvi, S.H., Chand, H.S., Quinn, T.P., Lorson, C.L., Byrareddy, S.N., and Singh, K. 2021. Evolutionary analysis of the delta and delta plus variants of the SARS-CoV-2 viruses. *J. Autoimmun.* **124**, 102715.

Karikalan, M., Chander, V., Mahajan, S., Deol, P., Agrawal, R.K., Nandi, S., Rai, S.K., Mathur, A., Pawde, A., Singh, K.P., *et al.* 2021. Natural infection of delta mutant of SARS-CoV-2 in Asiatic lions of India. *Transbound. Emerg. Dis.* doi: https://doi.org/10.1111/tbed.14290.

Karim, S.A.S. and de Oliveira, T. 2021. New SARS-CoV-2 variants - clinical, public health, and vaccine implications. *N. Eng. J. Med.* **384**, 1866–1868.

Keller, M., Hagag, I.T., Balzer, J., Beyer, K., Kersebohm, J.C., Sadeghi, B., Wernike, K., Höper, D., Wylezich, C., Beer, M., *et al.* 2021. Detection of SARS-CoV-2 variant B.1.1.7 in a cat in Germany. *Res. Vet. Sci.* **140**, 229–232.

Kemp, S.A., Collier, D.A., Datir, R.P., Ferreira, I.A.T.M., Gayed, S., Jahun, A., Hosmillo, M., Rees-Spear, C., Mlcochova, P., Lumb, I.U., *et al.* 2021. SARS-CoV-2 evolution during treatment of chronic infection. *Nature* **592**, 277–282.

Kim, Y.I., Kim, S.G., Kim, S.M., Kim, E.H., Park, S.J., Yu, K.M., Chang, J.H., Kim, E.J., Lee, S., Casel, M.A.B., *et al.* 2020. Infection and rapid transmission of SARS-CoV-2 in ferrets. *Cell Host Microbe* **27**, 704–709.

Lam, T.T.Y., Jia, N., Zhang, Y.W., Shum, M.H.H., Jiang, J.F., Zhu, H.C., Tong, Y.G., Shi, Y.X., Ni, X.B., Liao, Y.S., *et al.* 2020. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *Nature* **583**, 282–285.

Larsen, H.D., Fonager, J., Lomholt, F.K., Dalby, T., Benedetti, G., Kristensen, B., Urth, T.R., Rasmussen, M., Lassauniere, R., Rasmussen, T.B., *et al.* 2021. Preliminary report of an outbreak of SARS-CoV-2 in mink and mink farmers associated with community spread, Denmark, June to November 2020. *Euro Surveill.* **26**, 2100009.

Liu, L., Iketani, S., Guo, Y., Chan, J.F.W., Wang, M., Liu, L., Luo, Y., Chu, H., Huang, Y., Nair, M. S., *et al.* 2021a. Striking antibody evasion manifested by the omicron variant of SARS-CoV-2. *Nature.* doi: https://doi.org/10.1038/s41586-021-04388-0.

Liu, P., Jiang, J.Z., Wan, X.F., Hua, Y., Li, L., Zhou, J., Wang, X., Hou, F., Chen, J., Zou, J., *et al.* 2020. Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)? *PLoS Pathog.* **16**, e1008421.

Liu, Y., Liu, J., Johnson, B.A., Xia, H., Ku, Z., Schindewolf, C., Widen, S.G., An, Z., Weaver, S.C., Menachery, V.D., *et al.* 2021b. Delta spike P681R mutation enhances SARS-CoV-2 fitness over Alpha variant. *bioRxiv.* doi: https://doi.org/10.1101/2021.08.12.456173.

Lu, S., Zhao, Y., Yu, W., Yang, Y., Gao, J., Wang, J., Kuang, D., Yang, M., Yang, J., Ma, C., *et al.* 2020. Comparison of nonhuman primates identified the suitable model for COVID-19. *Sig. Transduct. Target.Ther.* **5**, 157.

Luan, J., Lu, Y., Jin, X., and Zhang, L. 2020. Spike protein recogni-

tion of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochem. Biophys. Res. Commun.* **526**, 165–169.

Lui, K., Wilson, M.P., and Low, G. 2021. Abdominal imaging findings in patients with SARS-CoV-2 infection: a scoping review. *Abdom. Radiol.* **46**, 1249–1255.

MacLean, O.A., Lytras, S., Weaver, S., Singer, J.B., Boni, M.F., Lemey, P., Pond, S.K.L., and Robertson, D.L. 2021. Natural selection in the evolution of SARS-CoV-2 in bats created a generalist virus and highly capable human pathogen. *PLoS Biol.* **19**, e3001115.

Mallapaty, S. 2020. Covid mink analysis shows mutations are not dangerous - yet. *Nature* **587**, 340–341. doi: https://doi.org/10.1038/d41586-020-03218-z.

Mannar, D., Saville, J.W., Zhu, X., Srivastava, S.S., Berezuk, A.M., Tuttle, K.S., Marquez, A.C., Sekirov, I., and Subramaniam, S. 2022. SARS-CoV-2 Omicron variant: antibody evasion and cryo-EM structure of spike protein–ACE2 complex. *Science*, **375**, 760–764.

Martinez-Flores, D., Zepeda-Cervantes, J., Cruz-Reséndiz, A., Aguirre-Sampieri, S., Sampieri, A., and Vaca, L. 2021. SARS-CoV-2 vaccines based on the spike glycoprotein and implications of new viral variants. *Front. Immunol.* **12**, 701501.

McCallum, M., De Marco, A., Lempp, F., Tortorici, M.A., Pinto, D., Walls, A.C., Beltramello, M., Chen, A., Liu, Z.M., Zatta, F., *et al.* 2021. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell* **184**, 2332–2347.

Melin, A.D., Janiak, M.C., Marrone, F., Arora, P.S., and Higham, J.P. 2020. Comparative ACE2 variation and primate COVID-19 risk. *Commun. Biol.* **3**, 641.

Meng, B., Kemp, S.A., Papa, G., Datir, R., Ferreira, I.A.T.M., Marelli, S., Harvey, W.T., Lytras, S., Mohamed, A., Gallo, G., *et al.* 2021. Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. *Cell Rep.* **35**, 109292.

Mishra, A., Kumar, N., Bhatia, S., Aasdev, A., Kanniappan, S., Sekhar, A.T., Gopinadhan, A., Silambarasan, R., Sreekumar, C., Dubey, C.K., *et al.* 2021. SARS-CoV-2 Delta variant among Asiatic lions, India. *Emerg. Infect. Dis.* **27**, 2723–2725.

Mohammadi, M., Shayestehpour, M., and Mirzaei, H. 2021. The impact of spike mutated variants of SARS-CoV2 [Alpha, Beta, Gamma, Delta, and Lambda] on the efficacy of subunit recombinant vaccines. *Braz. J. Infect. Dis.* **25**, 101606.

Mohandas, S., Yadav, P.D., Nyayanit, D., Shete, A., Sarkale, P., Hundekar, S., Kumar, S., and Lole, K. 2021a. Comparison of SARS-CoV-2 variants of concern 202012/01 (U.K. Variant) and D614G variant transmission by different routes in Syrian hamsters. *Vector Borne Zoonotic Dis.* **21**, 638–641.

Mohandas, S., Yadav, P.D., Shete, A., Nyayanit, D., Sapkal, G., Lole, K., and Gupta, N. 2021b. SARS-CoV-2 Delta variant pathogenesis and host response in Syrian hamsters. *Viruses* **13**, 1773.

Mok, B.W.Y., Liu, H.L., Deng, S., Liu, J., Zhang, A., Lau, S., Liu, S., Tam, R.C.Y., Cremin, C.J., Ng, T.T.L., *et al.* 2021. Low dose inocula of SARS-CoV-2 Alpha variant transmits more efficiently than earlier variants in hamsters. *Commun. Biol.* **4**, 1102.

Moore, J.P. and Offit, P.A. 2021. SARS-CoV-2 vaccines and the growing threat of viral variants. *JAMA* **325**, 821–822.

Muecksch, F., Weisblum, Y., Barnes, C.O., Schmidt, F., Schaefer-Babajew, D., Lorenzi, J.C.C., Flyak, A.I., DeLaitsch, A.T., Huey-Tubman, K.E., Hou, S., *et al.* 2021. Development of potency, breadth and resilience to viral escape mutations in SARS-CoV-2 neutralizing antibodies. *bioRxiv*. doi: https://doi.org/10.1101/2021.03.07.434227.

Munnink, B.B.O., Sikkema, R.S., Nieuwenhuijse, D.F., Molenaar, R.J., Munger, E., Molenkamp, R., van der Spek, A., Tolsma, P., Rietveld, A., Brouwer, M., *et al.* 2021. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* **371**, 172–177.

Munoz-Fontela, C., Dowling, W.E., Funnell, S.G.P., Gsell, P.S., Riveros-Balta, A.X., Albrecht, R.A., Andersen, H., Baric, R.S., Carroll, M.W., Cavaleri, M., *et al.* 2020. Animal models for COVID-19. *Nature* **586**, 509–515.

Munster, V.J., Feldmann, F., Williamson, B.N., van Doremalen, N., Perez-Perez, L., Schulz, J., Meade-White, K., Okumura, A., Callison, J., Brumbaugh, B., *et al.* 2020. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. *Nature* **585**, 268–272.

Muruato, A., Vu, M.N., Johnson, B.A., Davis-Gardner, M.E., Vanderheiden, A., Lokugamage, K., Schindewolf, C., Crocquet-Valdes, P.A., Langsjoen, R.M., Plante, J.A., *et al.* 2021. Mouse-adapted SARS-CoV-2 protects animals from lethal SARS-CoV challenge. *PLoS Biol.* **19**, e3001284.

Oreshkova, N., Molenaar, R.J., Vreman, S., Harders, F., Munnink, B.B.O., Hakze-van Der Honing, R.W., Gerhards, N., Tolsma, P., Bouwstra, R., Sikkema, R.S., *et al.* 2020. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Euro Surveill.* **25**, 2001005.

Pachetti, M., Marini, B., Benedetti, F., Giudici, F., Mauro, E., Storici, P., Masciovecchio, C., Angeletti, S., Ciccozzi, M., Gallo, R.C., *et al.* 2020. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *J. Transl. Med.* **18**, 179.

Palmer, M.V., Martins, M., Falkenberg, S., Buckley, A., Caserta, L.C., Mitchell, P.K., Cassmann, E.D., Rollins, A., Zylich, N.C., Renshaw, R.W., *et al.* 2021. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to SARS-CoV-2. *J. Virol.* **95**, e00083-21.

Pearson, C.A.B., Russell, T.W., Davies, N., Kucharski, A.J., CMMID COVID-19 working group, Edmunds, W.J., and Eggo, R.M. 2021. Estimates of severity and transmissibility of novel SARS-CoV-2 variant 501y.V2 in south africa. *CMMID Repository*. https://cmmid.github.io/topics/covid19/sa-novel-variant.html.

Planas, D., Veyer, D., Baidaliuk, A., Staropoli, I., Guivel-Benhassine, F., Rajah, M.M., Planchais, C., Porrot, F., Robillard, N., Puech, J., *et al.* 2021. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature* **596**, 276–280.

Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G., Zhang, X., Muruato, A.E., Zou, J., Fontes-Garfias, C.R., *et al.* 2021. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* **592**, 116–121.

Port, J.R., Yinda, C.K., Avanzato, V.A., Schulz, J.E., Holbrook, M.G., van Doremalen, N., Shaia, C., Fischer, R.J., and Munster, V.J. 2021. Increased aerosol transmission for B.1.1.7 (Alpha variant) over lineage A variant of SARS-CoV-2. *bioRxiv*. doi: https://doi.org/10.1101/2021.07.26.453518.

Rambaut, A., Holmes, E.C., O'Toole, Á., Hill, V., McCrone, J.T., Ruis, C., du Plessis, L., and Pybus, O.G. 2020. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat. Microbiol.* **5**, 1403–1407.

Ramesh, S., Govindarajulu, M., Parise, R.S., Neel, L., Shankar, T., Patel, S., Lowery, P., Smith, F., Dhanasekaran, M., and Moore, T. 2021. Emerging SARS-CoV-2 variants: a review of its mutations, its implications and vaccine efficacy. *Vaccines.* **9**, 1195.

Rathnasinghe, R., Strohmeier, S., Amanat, F., Gillespie, V.L., Krammer, F., Garcia-Sastre, A., Coughlan, L., Schotsaert, M., and Uccellini, M.B. 2020. Comparison of transgenic and adenovirus hACE2 mouse models for SARS-CoV-2 infection. *Emerg. Microbes Infect.* **9**, 2433–2445.

Richard, M., Kok, A., de Meulder, D., Bestebroer, T.M., Lamers, M.M., Okba, N.M.A., van Vlissingen, M.F., Rockx, B., Haagmans, B.L., Koopmans, M.P.G., *et al.* 2020. SARS-CoV-2 is transmitted via contact and via the air between ferrets. *Nat. Commun.* **11**, 3496.

Rockx, B., Kuiken, T., Herfst, S., Bestebroer, T., Lamers, M.M., Munnink, B.B.O., de Meulder, D., van Amerongen, G., van den Brand, J., Okba, N.M.A., *et al.* 2020. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science* **368**, 1012–1015.

Rosenke, K., Feldmann, F., Okumura, A., Hansen, F., Tang-Huau,

T.L., Meade-White, K., Kaza, B., Callison, J., Lewis, M.C., Smith, B.J., *et al.* 2021. UK B.1.1.7 (Alpha) variant exhibits increased respiratory replication and shedding in nonhuman primates. *Emerg. Microbes Infect.* **10**, 2173–2182.

Sabino, E.C., Buss, L.F., Carvalho, M.P.S., Prete, C.A., Crispim, M.A.E., Fraiji, N.A., Pereira, R.H.M., Parag, K.V., Peixoto, P.D., Kraemer, M.U.G., *et al.* 2021. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. *Lancet* **397**, 452–455.

Sailleau, C., Dumarest, M., Vanhomwegen, J., Delaplace, M., Caro, V., Kwasiborski, A., Hourdel, V., Chevaillier, P., Barbarino, A., Comtet, L., *et al.* 2020. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. *Transbound. Emerg. Dis.* **67**, 2324–2328.

Saito, A., Irie, T., Suzuki, R., Maemura, T., Nasser, H., Uriu, K., Kosugi, Y., Shirakawa, K., Sadamasu, K., Kimura, I., *et al.* 2021. SARS-CoV-2 spike P681R mutation, a hallmark of the Delta variant, enhances viral fusogenicity and pathogenicity. *bioRxiv*. doi: https://doi.org/10.1101/2021.06.17.448820.

Salguero, F.J., White, A.D., Slack, G.S., Fotheringham, S.A., Bewley, K.R., Gooch, K.E., Longet, S., Humphries, H.E., Watson, R.J., Hunter, L., *et al.* 2021. Comparison of rhesus and cynomolgus macaques as an infection model for COVID-19. *Nat. Commun.* **12**, 1260.

Sanjuán, R. and Domingo-Calap, P. 2016. Mechanisms of viral mutation. *Cell. Mol. Life Sci.* **73**, 4433–4448.

Sarkar, S. and Heise, M.T. 2019. Mouse models as resources for studying infectious diseases. *Clin. Ther.* **41**, 1912–1922.

Sawatzki, K., Hill, N.J., Puryear, W.B., Foss, A.D., Stone, J.J., and Runstadler, J.A. 2021. Host barriers to SARS-CoV-2 demonstrated by ferrets in a high-exposure domestic setting. *Proc. Natl. Acad. Sci. USA* **118**, e2025601118.

Sefik, E., Israelow, B., Mirza, H., Zhao, J., Qu, R.H., Kaffe, E., Song, E.R., Halene, S., Meffre, E., Kluger, Y., *et al.* 2021. A humanized mouse model of chronic covid-19. *Nat. Biotechnol.* doi: https://doi.org/10.1038/s41587-021-01155-4.

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Liu, R., He, X., Shuai, L., Sun, Z., Zhao, Y., *et al.* 2020. Susceptibility of ferrets, cats, dogs, and different domestic animals to SARS-coronavirus-2. **368**, 1016–1020.

Shuai, H., Chan, J.F.W., Hu, B., Chai, Y., Yuen, T.T.T., Yin, F., Huang, X., Yoon, C., Hu, J.C., and Liu, H. 2022. Attenuated replication and pathogenicity of SARS-CoV-2 B. 1.1. 529 omicron. *Nature* doi: https://doi.org/10.1038/s41586-022-04442-5.

Shuai, H., Chan, J.F.W., Yuen, T.T.T., Yoon, C., Hu, J.C., Wen, L., Hu, B.J., Yang, D., Wang, Y.X., Hou, Y.X., *et al.* 2021. Emerging SARS-CoV-2 variants expand species tropism to murines. *eBioMedicine* **73**, 103643.

Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L., Kaewpreedee, P., Perera, R.A.P.M., Poon, L.L.M., Nicholls, J.M., *et al.* 2020. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* **583**, 834–838.

Sit, T.H.C., Brackman, C.J., Ip, S.M., Tam, K.W.S., Law, P.Y.T., To, E.M.W., Yu, V.Y.T., Sims, L.D., Tsang, D.N.C., Chu, D.K.W., *et al.* 2020. Infection of dogs with SARS-CoV-2. *Nature* **586**, 776–778.

Stamatatos, L., Czartoski, J., Wan, Y.H., Homad, L.J., Rubin, V., Glantz, H., Neradilek, M., Seydoux, E., Jennewein, M.F., MacCamy, A.J., *et al.* 2021. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. *Science* **372**, 1413–1418.

Teng, S.L., Sobitan, A., Rhoades, R., Liu, D., and Tang, Q. 2021. Systemic effects of missense mutations on SARS-CoV-2 spike glycoprotein stability and receptor-binding affinity. *Brief Bioinform.* **22**, 1239–1253.

Twohig, K.A., Nyberg, T., Zaidi, A., Thelwall, S., Sinnathamby, M.A., Aliabadi, S., Seaman, S.R., Harris, R.J., Hope, R., Lopez-Bernal, J., *et al.* 2021. Hospital admission and emergency care attendance risk for SARS-CoV-2 Delta (B.1.617.2) compared with Alpha (B.1.1.7) variants of concern: a cohort study. *Lancet Infect. Dis.* **22**, 35–42.

UK Health Security Agency. 2021. SARS-CoV-2 variants of concern and variants under investigation in England. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1045619/Technical-Briefing-31-Dec-2021-Omicron_severity_update.pdf.

US Department of Agriculture, USDA. 2021a. Confirmation of COVID-19 in a cougar at a wild animal exhibitor in Texas. https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2021/sa-02/sars-cov-2-texas-cougar.

US Department of Agriculture, USDA. 2020. Confirmation of COVID-19 in a snow leopard at a Kentucky zoo. https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2020/sa-12/ky-snow-leopard-covid.

US Department of Agriculture, USDA. 2021b. Confirmation of COVID-19 in gorillas at a California zoo. https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2021/sa-01/ca-gorillas-sars-cov-2.

Vignier, N., Bérot, V., Bonnave, N., Peugny, S., Ballet, M., Jacoud, E., Michaud, C., Gaillet, M., Djossou, F., Blanchet, D., *et al.* 2021. Breakthrough infections of SARS-CoV-2 Gamma variant in fully vaccinated gold miners, French Guiana, 2021. *Emerg. Infect. Dis.* **27**, 2673–2676.

Volz, E., Mishra, S., Chand, M., Barrett, J.C., Johnson, R., Geidelberg, L., Hinsley, W.R., Laydon, D.J., Dabrera, G., Toole, A.O., *et al.* 2021. Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. *Nature* **593**, 266–269.

Wan, Y.S., Shang, J., Graham, R., Baric, R.S., and Li, F. 2020. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J. Virol.* **94**, e00127-20.

Wang, P., Nair, M.S., Liu, L., Iketani, S., Luo, Y., Guo, Y.C., Wang, M., Yu, J., Zhang, B.S., Kwong, P.D., *et al.* 2021. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* **593**, 130–135.

Washington, N.L., Gangavarapu, K., Zeller, M., Bolze, A., Cirulli, E.T., Barrett, K.M.S., Larsen, B.B., Anderson, C., White, S., Cassens, T., *et al.* 2021. Emergence and rapid transmission of SARS-CoV-2 B.1.1.7 in the United States. *Cell* **184**, 2587–2594.

Wibmer, C.K., Ayres, F., Hermanus, T., Madzivhandila, M., Kgagudi, P., Oosthuysen, B., Lambson, B.E., de Oliveira, T., Vermeulen, M., van der Berg, K., *et al.* 2021. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat. Med.* **27**, 622–625.

Wolter, N., Jassat, W., Walaza, S., Welch, R., Moultrie, H., Groome, M., Amoako, D.G., Everatt, J., Bhiman, J.N., Scheepers, C., *et al.* 2022. Early assessment of the clinical severity of the SARS-CoV-2 Omicron variant in South Africa: a data linkage study. *Lancet* **399**, 437–446.

World Health Organization, WHO. 2021a. Brazil: who coronavirus disease (COVID-19) dashboard. https://covid19.who.int/region/amro/country/br.

World Health Organization, WHO. 2021b. Tracking SARS-CoV-2 variants. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.

World Health Organization, WHO. 2021c. Weekly epidemiological update on COVID-19 - 9 November 2021.

Yu, J.Y., Tostanoski, L.H., Mercado, N.B., McMahan, K., Liu, J.Y., Jacob-Dolan, C., Chandrashekar, A., Atyeo, C., Martinez, D.R., Anioke, T., *et al.* 2021. Protective efficacy of Ad26.COV2.S against SARS-CoV-2 B.1.351 in macaques. *Nature* **596**, 423–427.

Yu, J.Y., Tostanoski, L.H., Peter, L., Mercado, N.B., McMahan, K., Mahrokhian, S.H., Nkolola, J.P., Liu, J.Y., Li, Z.F., Chandrashekar, A., *et al.* 2020. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science* **369**, 806–811.

Zahradnik, J., Marciano, S., Shemesh, M., Zoler, E., Harari, D., Chiaravalli, J., Meyer, B., Rudich, Y., Li, C.N., Marton, I., *et al.*

2021. SARS-CoV-2 variant prediction and antiviral drug design are enabled by RBD *in vitro* evolution. *Nat. Microbiol.* **6**, 1188–1198.

Zhang, L., Cui, Z.M., Li, Q.Q., Wang, B., Yu, Y.L., Wu, J.J., Nie, J.H., Ding, R.X., Wang, H.X., Zhang, Y., *et al.* 2021a. Ten emerging SARS-CoV-2 spike variants exhibit variable infectivity, animal tropism, and antibody neutralization. *Commun. Biol.* **4**, 1196.

Zhang, M., Xiao, J., Deng, A., Zhang, Y., Zhuang, Y., Hu, T., Li, J., Tu, H., Li, B., Zhou, Y., *et al.* 2021b. Transmission dynamics of an outbreak of the COVID-19 Delta variant B.1.617.2-Guangdong province, China, May-June 2021. *China CDC Wkly.* **3**, 584–586.

Zhou, D., Dejnirattisai, W., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Tuekprakhon, A., Nutalai, R., *et al.* 2021a. Evidence of escape of SARS-CoV-2 variant b.1.351 from natural and vaccine-induced sera. *Cell* **184**, 2348–2361.

Zhou, J., Peacock, T.P., Brown, J.C., Goldhill, D.H., Elrefaey, A.M.E., Penrice-Randal, R., Cowton, V.M., Lorenzo, G.D., Furnon, W., Harvey, W.T., *et al.* 2021b. Mutations that adapt SARS-CoV-2 to mustelid hosts do not increase fitness in the human airway. *bioRxiv*. doi: https://doi.org/10.1101/2021.08.20.456972.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., *et al.* 2020. A novel coronavirus from patients with pneumonia in China, 2019. *N. Eng. J. Med.* **382**, 727–733.

Zoccola, R., Beltramo, C., Magris, G., Peletto, S., Acutis, P., Bozzetta, E., Radovic, S., Zappulla, F., Porzio, A.M., Gennero, M.S., *et al.* 2021. First detection of an italian human-to-cat outbreak of SARS-CoV-2 Alpha variant-lineage B.1.1.7. *One Health* **13**, 100295.