Open Access

Check for updates

Review Article

Journal of Veterinary and Animal Sciences

ISSN (Print): 0971-0701, (Online): 2582-0605

https://doi.org/10.51966/jvas.2024.55.3.479-492

Animal Models in COVID-19 Research: Insights and Outcomes

Chithra C. Sreenivasan[*]

Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville, Louisville, KY 40222, USA

Citation: Chithra C.S., 2024. Animal Models in COVID-19 Research: Insights and Outcomes. *J. Vet. Anim. Sci.* **55** (3):479-492

Received: 30.07.2024 Accepted: 13.08.2024 Published: 30.09.2024

Abstract

Animal models have been critical in understanding the pathogenesis, transmission, and therapeutic interventions for COVID-19, caused by SARS-CoV-2. The primary challenges include species-specific differences in viral receptor expression, particularly the ACE2 receptor, and immune system responses, which can affect how closely these models mimic human COVID-19 pathology. Additionally, ethical concerns around the use of animals, especially non-human primates, and logistical issues such as high costs, housing requirements, and limited availability of certain models, further complicate research efforts. Transgenic mice expressing human ACE2 (hACE2) are widely used because of their ease of handling and cost-effectiveness. Other animal models include non-human primates, hamsters, cats, and ferrets, which were used to study viral transmission and host-pathogen interactions. The rapid dynamics of the SARS-CoV-2 evolution is one of the challenges and the complementary use of multiple models provides a more comprehensive understanding of the disease. Nonetheless, these models have been essential for rapid advancements in COVID-19 vaccine and therapeutic development, offering insights that continue to guide public health strategies. The review discusses the various animal models used in SARS-CoV-2 research, the outcomes, and the challenges and questions to be addressed

Keywords: *Animal models, SARS-CoV-2, COVID-19*

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in Wuhan, China, in late December 2019. The virus continues to impact humans globally with evolving variants, fluctuating case numbers, and diverse clinical manifestations. Since the pandemic began in late 2019, the virus has mutated into several variants of concern, causing different waves of infections worldwide. As of March 10, 2023, the devastating pandemic impacted 187 countries, with 770 million confirmed cases of COVID-19 and 6 million deaths (source: https://coronavirus.jhu.edu/map.html, accessed September 26, 2024).

Coronaviruses are positive-sense, single-stranded RNA viruses with 30 kb genome size encoding 13-15 (12 functional) open reading frames (ORFs) with 4 structural proteins, 8 accessory proteins, and 16 non-structural proteins affecting humans and multiple mammalian hosts (Jahirul *et al.*, 2023). Taxonomically, these viruses belong to the order, *Nidovirales*; family, *Coronaviridae*; subfamily *orthocoronavirinae* which are divided into four genera—alpha, beta,

---

[*]*Corresponding author : chithra.sreenivasan@louisville.edu, Ph. 502-852-1521*

gamma, and delta affecting a wide variety of domesticated and wild species of birds/animals. SARS-CoV-2 is a novel betaCoV, belonging to the same subgenus as severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV).

The spike protein of the ancestral strain Wuhan type 01 (WA-01) SARS CoV-2 mutated over time, leading to the emergence of multiple variants. Some of the key variants such as Alpha (B.1.1.7), Gamma (P.1), Delta (B.1.617.2), and the subsequent Omicron (B.1.1.529) variants were more transmissible than the ancestral strain, leading to case surges globally. An efficacy study with existing monoclonal antibody (mAb) products in hamsters showed absence or minimal neutralization ability against Delta and the mAb against Delta did not show any cross-reactivity with Omicron variants (Cong *et al.*, 2024). Omicron is highly transmissible; yet causes less severe disease than Delta, especially in vaccinated individuals. Omicron's immune escape properties allow it to infect people with previous immunity, leading to breakthrough infections. Omicron subvariants currently in circulation as of late 2023 and 2024, are XBB.1.5 (Kraken) and BA.2.75 (Centaurus) and these variants are far more highly transmissible and demonstrate immune escape properties than earlier Omicron strains. Such rapid evolution of viruses needs effective animal models to study the pathobiology of the variants and to develop therapeutic strategies.

SARS-CoV-2 virus spike protein uses angiotensin-converting enzyme 2 (ACE2) receptor on cells for virus entry and the spike protein is primed by transmembrane serine protease 2 (TMPRSS2) (Hoffmann *et al*., 2020). Multisystemic cellular tropism of SARS-CoV-2 enables the virus to infect multiple lineages of cells that express ACE2, TMPRSS2, or both. Localisation of viral RNA was demonstrated in epithelial, endothelial, and mesenchymal cells in almost all organs (Wong *et al*., 2021). Analyses of formalin-fixed paraffin-embedded (FFPE) human tissues/organs from fatal COVID-19 cases demonstrated that viral RNA was also present in tonsils, salivary glands, thyroid, adrenal, testicles, prostate, ovaries, lymph nodes, intestines, skin, and skeletal muscle in addition to the respiratory and vital organs such as lung, trachea, kidney, heart, or liver (Wong *et al*., 2021).

Homology modeling of the SARS-CoV-2 spike protein with ACE2 from old-world monkeys, orangutans, baboons, mustelids, civets, various species of horseshoe bats, pigs, ferrets, dogs, cats, pangolins, Malayan fruit bats, horses, cows, rabbits, red foxes, sheep, Chinese hamsters, other hamster species, marmosets, naked mole-rats, and ground squirrels demonstrated that these species could bind to the SARS-CoV-2 spike protein (Niu *et al*., 2021). Further, *in vitro* assays using pseudo typed viruses have confirmed these findings. Some species that are not susceptible include camels, raccoons, Greater horseshoe bats, rats, mice, platypuses, African bush elephants, European hedgehogs, mongooses, kangaroo rats, and guinea pigs (Luan *et al*., 2020).

COVID-19 is a multifaceted disease and the clinical manifestations shown by the patients showed a variable range of outcomes affecting the respiratory, olfactory, gustatory, neurological, and locomotor functions. Some of the caveats in animal model development are the reproducibility of the clinical and pathological correlates of the disease found in humans and the development of animal models with comorbidities that can mimic human conditions. The review aims to discuss the animal models that can closely replicate the pathophysiological, and immunological correlates of SARS-CoV-2. It is extremely challenging to find all the favourable outcomes in one animal model. So far, most animal models support viral replication in the upper and lower respiratory tract, facilitating transmission, and seroconversion.

Transgenic Mice models

The ACE2 receptor in standard laboratory mice strains (*Mus musculus*) does not naturally allow efficient binding of SARS-CoV-2 and hence failed to replicate and cause the disease. To address this issue, transgenic mice or modified viruses were developed to increase the suitability and efficiency of the animal model. Transgenic Mice expressing human ACE2 (hACE2) receptors are widely used to study SARS-CoV-2 as these mice support efficient viral replication and develop clinicopathological aspects of COVID-19, including lung inflammation and immune responses. Compared to normal mice, transgenic mice expressing human ACE2 allowed SARS-CoV-2 replication to reproduce interstitial pneumonia with weight loss until 10 dpi upon the intranasal challenge of 10^5 $TCID_{50}$ of SARS-CoV-2 (Bao *et al*., 2020b).

Transgenic K18-hACE2 mice model: Different transgenic mice models were employed with hACE2 driven under different promoters such as CMV early enhancer/chicken β actin (CAG), keratin 18 (K18), etc. to express the human ACE2, the key receptor of SARS-CoV-2 for virus entry. Compared to the CAG promoter, K18 mice worked better as hACE2 expressed under the K18 promoter restricts the ACE2 expression only on epithelial cells. A comparative pathogenesis study between K18-hACE2 and CAG-ACE2 mice demonstrated significant weight loss in K18-hACE2 mice resulting in fatal infections and showed a high viral load in the lungs, cerebrum, and cerebellum (Seo *et al*., 2022). Hence, K18-hACE2 mice are more susceptible to SARS-CoV-2 and the height of infection was observed on days 4-7 dpi, and viral load decreases from 7 to 14 dpi leading to virus clearance.

The K18-hACE2 mice express higher levels of hACE2 in multiple organ systems such as lungs, kidneys, liver, brain, and small and large intestines and this animal model can recapitulate the fatal CVID-19 outcomes. This model supported efficient infection and replication in the respiratory tract, causing severe lung infection mirroring the disease progression in humans, thus making it a valuable model for studying COVID-19 pathogenesis and testing vaccines and treatments (Myeni *et al*., 2024). A spatiotemporal dynamics study of SARS-CoV-2 until 14 dpi revealed that clinical deterioration and death in K18-hACE2 mice were linked with viral neuroinvasion and neuronal injury of brain and spinal neurons (Carossino *et al*., 2021). At 4 dpi, SARS-CoV-2 uses olfactory neuroepithelium as the main entry portal for neuroinvasion and localizes to the olfactory bulb supporting axonal transport. In K18-hACE2 mice, ACE2 was not detected in neurons, hence its expression in nasal passages and neuroepithelium determines the level of neuroinvasion. This makes it a good model to study the neuropathogenesis of SARS-CoV-2, especially when neurological consequences are widely reported for long COVID (Carossino *et al*., 2021).

K18-hACE2 mice have also been used to study the variants of concern Alpha, and Delta and found that Delta caused severe lung inflammatory changes with increased type I and II interferon responses (Lee *et al*., 2022). K18-hACE2 mice were also used to study co-infection models involving a sequential infection of influenza and followed by SARS-CoV-2 exacerbated encephalitis in mice along with severe pulmonary lesions, prolonged innate immune response, but reduced SARS-CoV-2 RNA synthesis (Clark *et al*., 2024).

Premature aging-related complications were analysed using the Hutchinson-Gilford progeria syndrome (HGPS) mouse model, with humanized ACE2 receptors to study premature aging and found that SARS-CoV-2 infection caused mild innate interferon responses and virus defense compared to young mice. Aged hACE2 mice demonstrated pathological phenotypes induced by SARS-CoV-2 infection (Haoyu *et al*., 2024).

AC70 line of human ACE2 transgenic (AC70 hACE2 Tg) mice demonstrated COVID-19-associated coagulopathy characterized by acute leukopenia, lymphopenia, and induction of circulating neutrophil extracellular traps (NETs), activation of platelet/endothelial markers (Drelich *et al*., 2024). The survival rate of aged (85-112 weeks) mice is less than young (12-15 weeks) mice (Subramaniam *et al*., 2024). A knock-in mouse model developed by CRISPR -Cas9 technology mACE2 F83Y, H353K mouse carrying a mouse-human hybrid form of ACE2 under the endogenous mouse promoter did not show clinical symptoms, while the Rosa26 conditional model (Rosa26^{hACE2}) where human ACE2 was expressed in cell and tissue-specific fashion allowed significant weight loss, clinical scores, viral load, and shedding (Song *et al*., 2024).

Transgenic hACE2/hTMPRSS2 knock-in mouse model: Another transgenic model, hACE2/hTMPRSS2 knock-in mouse model can support SARS-CoV-2 better than just the hACE2 model. This model can reproduce the multisystemic outcomes affecting the pulmonary, cardiovascular, locomotion, and behavioral responses. The male mice showed reduced locomotor responses supporting the sex-based differences. However, other pulmonary functions such as oxygen saturation, and heart rate variability changes were not observed in either sex (Liu *et al*., 2024). Also, this model was able to reproduce the same clinical signs on reinfection after 6 months showing that this model is suitable for studying mild COVID-19 (Liu *et al*., 2024).

Other transgenic mice models

Another humanized mouse model called human immune system (HIS)-DRAGA mice (HLA-A2.HLA-DR4.Rag1KO.IL-2RgKO.NOD) was developed after infusing Human Leukocyte Antigen (HLA)-matched, human hematopoietic stem cells from umbilical cord blood. This surrogate in vivo model has a functional human immune system, and lung epithelial and circulatory endothelial cells of this model express human ACE2 receptors. This model can recapitulate the long sequelae of COVID-19 as they could sustain infection for 25 days (Ghosh Roy *et al*., 2024).

Adenoviral Delivery of hACE2: Another approach involves adenovirus delivery of the human ACE2 gene into wild-type mice. This transient expression of hACE2 promotes the susceptibility of mice to SARS-CoV-2 infection, instead of using a full transgenic line (Glazkova *et al*., 2022). This approach provides flexibility in studying the pathobiological correlates in different genetic backgrounds and can be used to explore therapeutics and prophylactics.

Mouse-Adapted SARS-CoV-2 Strains: An alternative to tackle the refractory nature of mice towards SARS-CoV-2 is to use mouse-adapted SARS-CoV-2 strains. Serial passaging of SARS-CoV-2 in mice enables selection of viral variants that can bind to the mouse ACE2 receptor. These mouse-adapted strains can cause disease in non-transgenic mice, allowing for broader research applications in various mouse strains (Ellsworth *et al*., 2024) .

Immunocompromised Mice: Severe combined immune deficient (SCID) mice lacking key components of the immune system, have been used to study the role of immune responses in SARS-CoV-2 infection and to test potential therapeutic strategies (Abdelnabi *et al*., 2022). These models can provide insights into how the virus interacts with different elements of the immune system and can be used to evaluate the effectiveness of antiviral drugs or monoclonal antibodies.

Potential treatments, including antiviral drugs, monoclonal antibodies, and other therapeutics, are tested in mouse models to assess their efficacy and safety before advancing to human trials (Dhanushkodi *et al*., 2024; Ko *et al*., 2023; Tatham *et al*., 2024). A vaccine study in C57BL/6 mice to investigate the efficacy of two spike subunit proteins with selected dominant substitution variants fused with transmembrane protein vaccines in the young and aged mice caused a decline in antibody titer after 6 months, however, a third booster significantly increased the humoral responses in the aged mice (Cui *et al*., 2024). Another study with single-dose murine CMV (MCMV) vector expressing spike protein elicited the humoral response that lasted 5 months in K18-hACE2 mice and protected against Beta and Omicron variants (Metzdorf *et al*., 2024).

Non-human primates

Nonhuman primates (NHP) are the closest surrogates for humans, because of the close genetic and physiological similarities to humans. Hence, NHP studies on the virus pathogenesis, host-pathogen interactions, prophylactics, and therapeutics will offer high translational value. NHPs such as *rhesus macaques and cynomolgus monkeys,* exhibit mild to moderate COVID-19 symptoms and will recapitulate the human disease. Various routes such as conjunctival, intranasal, intra-tracheal, oral, and intragastric routes were employed for establishing infection in NHP (Deng *et al*., 2020; Munster *et al*., 2020). However, their high cost, ethical concerns, and limitations in modeling severe disease progression present challenges.

Rhesus macaques (*Macaca mulatta)* were widely used as an experimental model to study the SARS-CoV-2 based on the high sequence identity of the 23 critical amino acid residues between the human and macaque ACE2 responsible for the receptor binding domain (RBD) interaction which facilitates effective binding of the SARS-CoV-2. Common lab animals–rat and mouse ACE2 possess three key substitutions D30N, Y83F, and K353H, which disrupt the hydrogen bond limiting its receptor activity and virus entry, as demonstrated by the gain/loss of functions (Zhao *et al*., 2020a). Sequence analysis of ACE2 performed to determine the infection risk of NHP in comparison to its orthologs in ferrets, bats, cats, dogs, and pangolins found that all apes including gorillas, bonobos, chimpanzees, other African and Asian monkeys share a homologous receptor binding site like humans, while some species as in tarsiers, lemurs, lorisoids and monkeys of the Americas show some difference in the key amino acid residues which affect their binding affinity to SARS-CoV-2 (Melin *et al*., 2020). Lu *et al*. (2020) compared three species of monkeys, *Macaca mulatta, Macaca fascicularis* (Old World monkeys), and *Callithrix jacchus* (new world monkey) for studying the SARS-CoV-2 pathogenesis and all three species demonstrated virus shedding as detected in the nasal, throat, anal swabs and blood. The least susceptible species was *Callithrix* sp. and *M. mulatta* was the most (Lu *et al*., 2020).

Rhesus macaques: Munster *et al*.(2020) reported clinical disease for 8-16 days with mild to moderate interstitial pneumonia in infected rhesus macaques, with no virus isolated from blood/urogenital swab throughout the study. When the COVID-19 started, viral transmission through tears was considered as a potential portal for virus transmission. Deng *et al*.(2020) used conjunctival, intratracheal, and intragastric routes in rhesus macaques and demonstrated that the conjunctival route caused higher viral load only in the nasolacrimal ducts, and associated tissues with mild pneumonia and no other clinical signs compared to intratracheal inoculation. While SARS-CoV-2 viral load was detected in nasal and oropharyngeal swabs by both conjunctival and intratracheal groups, viral shedding through feces was only seen in intratracheally infected animal (Deng *et al*., 2020; Xia *et al*., 2020). Although RT-PCR of conjunctival swabs from COVID-19 human patients tested negative, the viral load in the nasolacrimal duct and conjunctival swab of NHP (only at 1 dpi) indicated that conjunctiva could act as virus entry portal, channeling the virus through the nasolacrimal duct to the inferior nasal meatus of the nasal cavity and hence, recommends proper personal eye protection (Deng *et al*., 2020). Intragastric inoculation did not cause any clinical signs or viral load, similar to the intragastric infection of SARS-CoV in cynomolgus monkeys (Deng *et al*., 2020; Nagata *et al*., 2007).

Intratracheal inoculation of 7×10^6 50% tissue-culture infectious doses (TCID50) of SARS-CoV-2 into rhesus macaques by ChaoShan (2020) did not cause any bodyweight and temperature changes in macaques, but chest X-ray revealed patchy ground-glass opacity in both sides of the lungs which intensified with the infection. Evidence of viral shedding in the nasal (until 3 dpi) and oropharyngeal (until 9 dpi) and anal swabs (until 11 dpi) was noticed. SARS-CoV-2 was also isolated from the tissues (trachea, bronchus, and lungs). Histopathological changes were observed in the inferior lobes of both sides of the lungs with peribronchial inflammatory changes, thickened alveolar walls, edema, pulmonary hyaline-membrane formation, and haemorrhage in the interalveolar septa (ChaoShan, 2020). Another study in rhesus macaques was conducted to test the COVID-19 relapse and found that neither active viral replication in tissues nor viral shedding occurred after re-infection with the SARS-CoV-2 virus which indicated that virus-specific IgG antibodies protected the macaques from re-infection with a homologous strain (Bao *et al*., 2020a).

These studies also screened for the extra-pulmonary tissue tropism of SARS-CoV-2 in macaques and demonstrated viral replication in the bladder, kidney, liver heart, skeletal muscle, thoracic spinal cord, rectum, colon, spleen, duodenum in addition to lungs, pulmonary lymph nodes, trachea, soft palate, tonsils, cervical cord and lymph nodes, nasal turbinate, and mucosa (Bao *et al*., 2020a; Deng *et al*., 2020; Lu *et al*., 2020; Munster *et al*., 2020). Taken together, the rhesus monkeys did not recapitulate the disease severity as in humans, nevertheless, they are susceptible to SARS-CoV-2 and supported viral replication in the upper and lower respiratory tract, and less frequently in the digestive tract.

The protective efficacy of a recombinant adenovirus vaccine containing fragments of S, N, and orf8 genes tested in rhesus macaques offered significant protection against live SARS-CoV challenge (Chen *et al*., 2020). Vaccine efficacy studies using spike stem-based broadly neutralizing antibodies in rhesus macaques also showed positive outcomes in terms of reduced viral load and decreased inflammatory cytokines and macrophages in the lower respiratory tract (Edwards *et al*., 2024). Studies were conducted in macaques to test the efficacy of the FDA-approved antiviral drug remdesivir, an RNA-dependent RNA polymerase inhibitor for COVID-19 patients (Pruijssers *et al*., 2020). In macaques, administration of remdesivir in the early phase of the disease (12 hours post-infection and then daily once, intravenously for 6 days) reduced the viral titers in bronchoalveolar lavage at 12 h post-remdesivir administration and also prevented severe pneumonia at 7 dpi (Williamson *et al*., 2020). African green monkeys (*Chlorocebus sabaeus*) upon SARS-CoV-2 infection caused dysregulated glucose metabolic profile, characterized by an increase in CCL25, hyperglycemia, dysfunctional pancreatic β-cells, impaired glucose clearance, increased gluconeogenesis or glycogenolysis, and insulin resistance (Palmer *et al*., 2024) which makes it a good model to study the disease with metabolic comorbidities.

A comparative study between MERS-CoV and SARS-CoV-2 involving both young adults and aged cynomolgus monkeys, age comparable to the real-time susceptible human population by intratracheal and intranasal routes revealed no overt clinical signs including weight loss after SARS-CoV-2 infection (Rockx *et al*., 2020), however, there were prolonged viral shedding, early peak, and viral tropism confined to the respiratory tract tissues except for the ileum (Rockx *et al*., 2020). For MERS-CoV, the marmoset model exhibited more severe pneumonia, alterations in blood chemistry, liver, and kidney functions (Falzarano *et al*., 2014). However, aerosol exposure of mean presented dose 8.7 × 10^4 $TCID_{50}$ of SARS-CoV-2 VIC01 in marmosets did not induce any clinical signs, except for the early weight loss and changes in respiratory activity. Immunohistochemical analyses showed that Marmoset respiratory tissues lacked ACE2 but expressed TMPRRS2. The presence of vRNA was observed in the lung but active virus replication was absent. However, innate immune responses were pronounced with activation of macrophages, circulating monocytes, neutrophils and reduction in circulating T cells (Ireland *et al*., 2022).

Ferrets

Ferrets are the closest human surrogates and are widely used for studying respiratory viruses. Ferrets are susceptible hosts for SARS-CoV-2 and have been used to study the pathogenesis and transmission of SARS-CoV-2 (Kim *et al*., 2020; Richard *et al*., 2020). Ferrets inoculated with SARS-CoV-2, SARS-CoV-2/F13/environment/2020/Wuhan (F13-E), which originated from an environmental sample from Huanan Seafood Market in Wuhan, and SARS-CoV-2/CTan/human/2020/Wuhan (CTan-H), of human origin, demonstrated vRNA and virus shedding of both strains, in nasal washes and upper respiratory tract (URT) tissues mainly (nasal turbinate, soft palate, and tonsils) at 4 dpi. No extrapulmonary tissues tested positive for the virus (Shi *et al*., 2020). Nasal swabs tested positive for both high RNA copy numbers and infectious virions, while vRNA without infectious virions was detected in few rectal swabs. Despite severe inflammatory changes in the alveolar space and septum in the ferret lungs, fatalities due to respiratory distress were absent (Shi *et al*., 2020). Intranasal inoculation of $10^{5.5}$ $TCID_{50}$ of the virus in 12-24-month-old male and female ferrets did not show obvious clinical signs except for a moderate increase in body temperature. However, viral shedding in the nasal wash, saliva, urine, and feces was observed in the directly inoculated animals and facilitated contact and aerosol transmission. Despite the presence of vRNA in nasal turbinate, trachea, lungs, kidney, and intestine, the viral titers were low especially in the lung (Kim *et al*., 2020).

Ferrets on intranasal/oral/ocular inoculation caused fever, weight loss and viral shedding via throat, nares, and rectum for 7-10 days post-infection (Reed *et al*., 2024). Ferret ACE2 was predicted to have a high affinity to SARS-CoV-2 spike protein, however, virus lung tropism in ferrets is very limited (low viral titer) causing only acute bronchiolitis without any interstitial pneumonia or diffuse alveolar damage (DAD), a hallmark lung lesion in COVID-19 patients (Kim *et al*., 2020). But this model certainly has advantages for studying the transmission of SARS-CoV-2, and to test the efficacy of prophylactic, therapeutics, or repurposing drugs. Ferrets have been used to test the efficacy of human polyclonal anti-SARS-CoV-2 IgG developed from hyper-immunized transchromosomic bovines (SAB-185) and found that low doses of IgG did not cause antibody-dependent enhancement (ADE) of disease (Reed *et al*., 2024).

Cats

Since COVID-19 affected small and big cats, cats were also tested to study its efficacy for model development. Subadult (6-9 months) and juvenile cats inoculated with 10^5 PFU of CTan-H intranasally did not cause any clinical signs, however, vRNA and infectious virions were present in nasal turbinate, tonsils, trachea, lungs, and small intestine on 3 dpi. Lungs did not show any vRNA on 6 dpi, even though vRNA and infectious virions were present in the URT tissues (Shi *et al*., 2020). In the same study, the sub-adult cats were also tested for aerosol transmission and reported that sentinel animals showed fecal shedding on 3 dpi and seroconverted. On the contrary, juvenile cats were more susceptible to SARS-CoV-2 infection and facilitated aerosol transmission. Taken together, there could be age-dependent variations in the incubation period, pathology, virus shedding, and persistence in cats (Shi *et al*., 2020).

Dogs

Natural infection of SARS-CoV-2 in dogs was reported from several parts of the world such as Italy, Hong Kong, and the USA (Goumenou *et al*., 2020). Five-month-old beagles were intranasally inoculated with 10^5 PFU of CTan-H and the direct inoculated animals demonstrated vRNA only in rectal swabs on 2 dpi, but failed to detect virus/vRNA in all the tissues, showing that dogs have a low susceptibility (Shi *et al*., 2020).

Although dogs can contract SARS-CoV-2, they are not a preferred model for studying the virus due to their mild disease presentation and limited role in transmission. However, their role as companion animals has made them important for understanding the potential risks of reverse zoonotic transmission from humans to pets. While their use in modeling human COVID-19 is limited, dogs remain important in broader epidemiological studies, providing insights for public health guidelines and pet management during the pandemic.

Rabbits

The receptor-mediated virus entry studies using pseudotyped viruses with ACE2 receptor orthologs suggested that rabbits could be a good animal model in addition to ferrets, monkeys, and cats (Zhao *et al*., 2020a). An intranasal application of 10^4, 10^5 and 10^6 $TCID_{50}$ SARS-CoV-2, in specific pathogen-free three-month-old female New Zealand White rabbits (*Oryctolagus cuniculus*), seronegative for SARS-CoV-2 demonstrated virions in nasal, throat and rectal swabs and seroconversion in 10^5 $TCID_{50}$ and 10^6 $TCID_{50}$ groups. Viral RNA was not detected in the lungs, however, mild to moderate inflammatory changes were observed (Mykytyn *et al*., 2021). Rabbit model was effectively used for developing monoclonal antibodies against different SARS-CoV-2 lineages (Guo *et al*., 2023).

Hamsters

Hamsters, particularly Golden Syrian hamsters (*Mesocricetus auratus),* are highly susceptible to SARS-CoV-2 and develop clinical features, including lung pathology, that closely resemble human COVID-19 (Blaurock *et al*., 2022). Roborovski (*Phodopus roborovskii*) hamsters, in particular, exhibit more severe disease and can be modeled to study critical cases seen in elderly and immunocompromised individuals.

Golden Syrian hamsters were used to study the pathogenesis and transmissibility of SARS-CoV-2, and they can facilitate contact and airborne transmission. Hamsters exhibited wide range of disease phenotypes such as weight loss, high viral load, diffuse alveolar damage, and bronchiole/airway inflammation, extrapulmonary lesions such as inflammatory infiltrations in the intestine, myocardial degenerations, apoptotic evidence in lymph nodes and spleen, and seroconversion at 14 dpi. Hamsters are a good model to study SARS-CoV-2 as the symptoms are moderate and cause self-limiting disease. Like ferrets, golden Syrian hamsters have shown extrapulmonary involvement after infection (Chan *et al*., 2020).

Golden Syrian hamsters can support prototypic SARS-CoV-2 and the variants of concern. Both Alpha and Delta strains caused moderate to severe lung pathology and replicated in tissues. However, neuropathological lesions were mild (Feng *et al*., 2022). SARS-CoV-2 can infect testicular cells in hamsters (Campos *et al*., 2021). Six-week-old Syrian hamsters were used for the pathogenicity studies against different SARS-CoV-2 variants and tested the antibody therapeutics against different variants. This study showed weight loss against all variants emerged before Omicron. The Omicron variants caused only secondary disease outcomes such as lung pathological correlates and viral load without weight loss (Cong *et al*., 2024). SARS-CoV-2 infection of hamsters with pre-existing liver conditions exacerbated liver lesions characterized by hepatitis with increased vascular lesions such as portal vein endotheliitis, and hepatocellular degeneration, in addition to the pulmonary lesions (Souza *et al*., 2024). Syrian golden hamsters support transmission studies by contact, aerosol and fomite routes of SARS-CoV-2 variants of concern (Mohandas *et al*., 2021).

Roborovski hamsters are also susceptible to SARS-CoV-2 infection, and they exhibit more severe clinical signs with acute respiratory distress and fatality (Trimpert *et al*., 2020). The fast aging of Roborovski hamsters makes them a suitable model for studying SARS-CoV-2 in elderly subjects. Just like K18-hACE2 mice, these hamsters show severe lung and neuropathology outcomes. SARS-CoV-2 in Roborovski hamsters induced severe acute diffuse alveolar damage and hyaline microthrombi in the lungs, hallmark changes found in human COVID-19 patients, a pathological feature not reproduced in any other animal models (Trimpert *et al*., 2020). Further, Roborovski hamsters are small and have shorter lifespan than Syrian hamsters and are suitable for studying age-related comorbidities.

Tree shrews

Tree shrews (*Tupaia belangeris)* became popular lately as experimental animals, as they are recently domesticated from the wild. Tree shrews of both sexes in three different age groups (young, adult, and old) inoculated with SARS-CoV-2 demonstrated an increase in temperature, especially in females. Main histopathological changes included mild inflammation in the lungs and extrapulmonary tissues such as liver, spleen, kidney, small intestine, and pancreas were affected randomly in all three age groups (Zhao *et al*., 2020b). Rats and guinea pigs ACE2 do not fall under the susceptible species of SARS-CoV-2 and hence have not been used for model development.

Sex differences in the pathobiology of SARS-CoV-2

Emerging evidence indicates that the male population is bearing the brunt of COVID-19 than the females worldwide (Wenham *et al*., 2020) which emphasizes the need to investigate the sex-dependent disease phenotype. Studies show that estrogen has an important role in alleviating the disease outcomes of SARS-CoV-2 as treatment of ovariectomized mice with estrogen receptor antagonists decreased the survival rate suggestive of the protective effect of estrogen (Channappanavar *et al*., 2017; Suba, 2020). Although normal mice were not used for any experimental infection of SARS-CoV-2, it was demonstrated that mucin 4 (Muc4)$^{-/-}$ female SARS-CoV-2 infected mice (42%) suffered ≥20% weight loss and had to be euthanized by 4 dpi, compared to infected Muc4$^{-/-}$ male and wild type (WT) mice (Plante *et al*., 2020). The sex-based body weight changes did not reflect in the viral load. Notably, the lung pathology was minimal in female Muc4$^{-/-}$ mice compared to the WT, whereas the male WT and Muc4$^{-/-}$ mice have similar lung pathology, indicating that the regulatory role of Muc4 is sex-dependent (Plante *et al*., 2020). Sex-based disease outcomes are also noticed in Syrian hamster models, in which they experienced severe clinical symptoms, lung pathology, slow recovery, and low antibody responses compared to female animals (Dhakal *et al*., 2021). Sex, age, and species-based differences were also noticed in rhesus macaques in terms of immunopathological aspects of the disease (Lu *et al*., 2020; Speranza *et al*., 2022).

Conclusions and Future Directions

An overview of the animal models currently in use, which support different phenotypic aspects of the COVID-19 disease is summarized in Fig. 1. Each model presents distinct advantages and challenges, thus impacting the relevance of findings and translational value for human studies.

Among the models, transgenic mice and hamster models are better in terms of rapid breeding, cost-effectiveness, housing requirements, ease of handling, and availability of well-characterized biological reagents. Despite their advantages in genetic manipulation and cost-effectiveness, mice generally exhibit mild disease, limiting their usefulness in modeling severe COVID-19. Additionally, differences in immune response between mice/Hamster and humans complicate the direct translation of findings. A comparison study of the microbiome in hamsters and mice demonstrated differences in the dominant microbial species. For hamsters, Lactobacillaceae is more abundant in forestomach and ileum, while Muribaculaceae dominates in the murine forestomach and ileum. Muribaculaceae were dominant in murine cecum and colon, while in hamsters, Lachnospiraceae and Erysipelotrichaceae were the dominant bacterial communities. This difference in microbiome plays an important role in the pathogenesis and further characterization, so these things have to be considered while considering the suitability of a model (Böswald *et al*., 2024). Ferrets are particularly useful for modeling respiratory transmission, while cats can develop subclinical infections, mirroring asymptomatic human cases. Both are valuable in studying zoonotic transmission but have limitations in replicating severe human pathology. In rhesus macaques, the clinical signs reported include mild to moderate respiratory disease, occasionally with initial body weight loss, temperature changes, and anorexia. However, the respiratory distress in macaques was not so pronounced to cause lethal pneumonia, not to mention the extrapulmonary involvement. A potentially serious condition emerged in children of all ages in the UK and the USA, possibly linked to COVID-19. Unlike adults, a ‘multi-system inflammatory syndrome’ characterized by cytokine storm, low blood pressure, fluid retention in lungs and other organs was manifested in children (Mahase, 2020). However, young adult macaques of 4-5 years old equivalent to 12.8-16 years of human age failed to replicate such inflammatory syndromes. Transgenic NHP models are an option, (Niu *et al*., 2010; Park and Silva, 2019) but the cost and ethical constraints limit the application.

Another major issue encountered in COVID-19 patients across demographics was the neurological sequelae of COVID-19 which include insomnia, anxiety, weariness, cognitive impairments, encephalopathy, and encephalomyelitis (Brola and Wilski, 2022) and the severity depends on the sensory and central nervous system (CNS) involvement. The affected individuals manifested stroke-like symptoms observed in young/middle-aged people, ageusia (loss of taste), and anosmia (loss of smell) were reported in 85.6% and 88.0% respectively (Lechien *et al*., 2020). Previous studies have shown that the neurological sequelae in acute SARS-CoV-2 infection and long-term COVID cognitive disorders are due to the rupture of the blood-brain barrier (BBB) in acute infection and subsequent systemic inflammation in the brain (Greene *et al*., 2024). A comprehensive analysis of the inflammatory, coagulative, and BBB functions in samples from the affected cohort with a clinical history of mild to severe clinical manifestations revealed moderate adaptive immune response affecting pro-inflammatory cytokines and dysregulated coagulative functions affecting

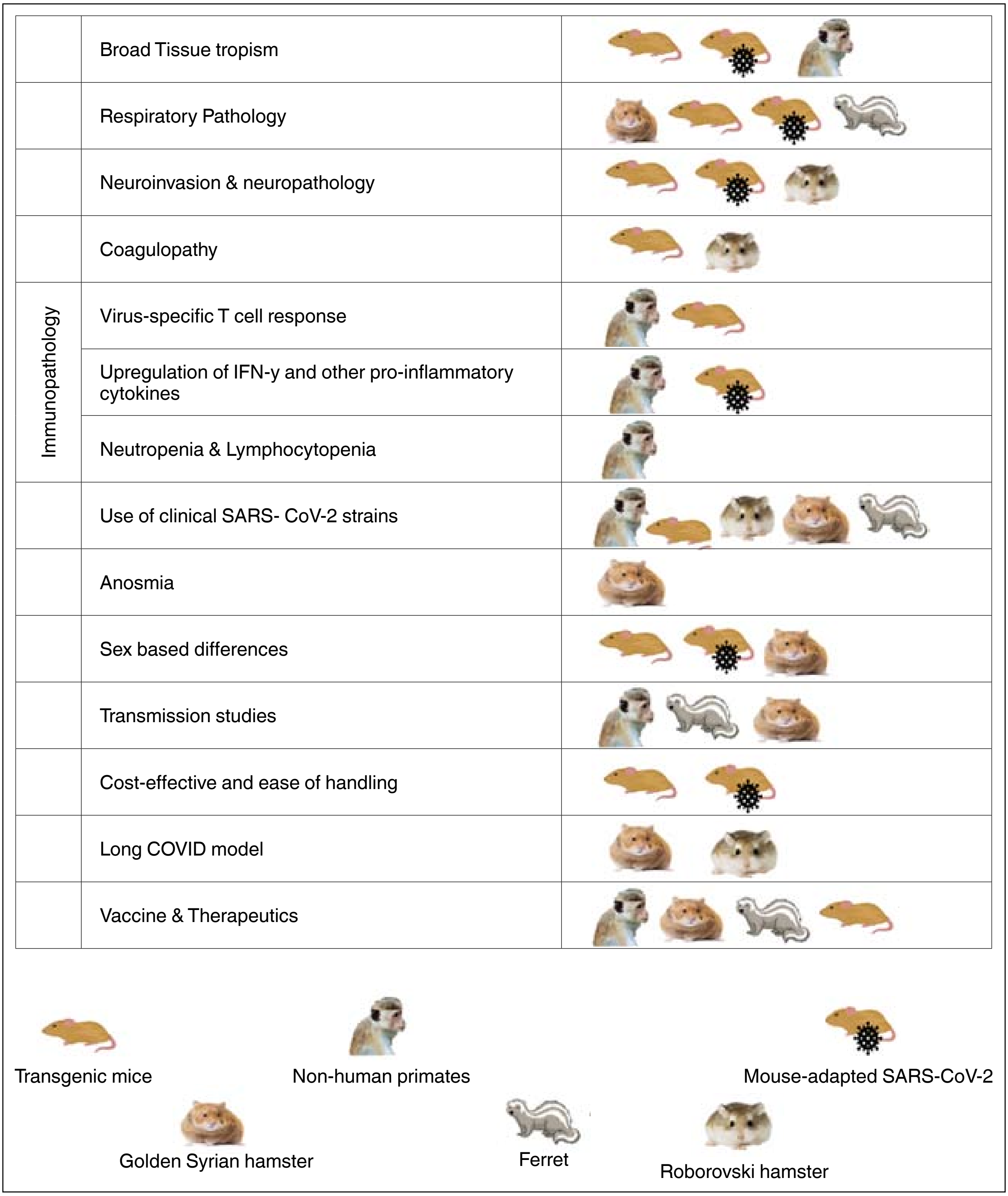

Fig. 1. Animal models and their applications for different disease phenotypes of COVID-19

thrombosis and endothelial cell activation (Greene *et al.*, 2024). SARS-CoV-2 impairs the glutamatergic receptors and affects the binding of glutamate, the most abundant excitatory neurotransmitter in the brain, to these receptors and subsequently affects the downstream pathway (Wang *et al.*, 2024).

Long COVID, also known as post-acute sequelae of SARS-CoV-2 infection (PASC), refers to symptoms that continue for weeks, months, or even years after the initial acute phase of a COVID-19 infection. These symptoms can vary widely, affecting multiple organ systems, and these may occur even in people with mild or asymptomatic COVID-19 cases. More than 200 symptoms have been identified with long-term COVID-19, the most common include lethargy, memory problems called brain fog, headache, dizziness, impaired gustatory and olfactory functions, sleep disorders, shortness of breath, cough, blood clots, fibromyalgia, irregular cardiac rate, digestive, circulatory, and metabolic diseases.

Currently, the practical feasibility of small animal models to replicate the neurological aspects of long COVID syndrome is limited. While ageusia and anosmia are highly subjective and difficult to test in animals, a model to study the CNS and sensory involvement would add great value. The inhalation method of infection without anesthesia seems to be a better route to reproduce the milder pathological lesions, and it was found that the brains of K18-hACE2 mice showed mild lesions after the virus clearance at 14 days post-infection giving its advantage in studying the long COVID. Inhalation of nebulized aerosols developed pathological lesions in the CNS and respiratory system better than the intranasal route of inoculation. The inhalation method caused milder lung pathology, focal lesions mimicking the chest CT pattern in humans, and pulmonary fibrosis as in recuperating lungs. Further, the inhalation method showed delayed brain involvement through the trigeminal nerve and olfactory bulb infection compared to the intranasal method in the long COVID mouse model (Jeon *et al*., 2024). Aerosol exposure of K18-hACE2 transgenic mice to SARS-CoV-2 caused fibrin deposition in lungs, immune cell infiltration, and transcriptional profile was comparable to the human patients, which underscores the suitability of this model to study the long COVID and therapeutic applications (Fumagalli *et al*., 2022).

Another factor that is linked to the grave prognosis of COVID-19 conditions is the underlying diseases or co-morbidities causing complications irrespective of the demographics. Transgenic or genetically modified susceptible animals prompting some underlying medical conditions such as myocarditis, diabetes, kidney failures, immune compromised conditions, and age-related neurodegenerative disorders like Parkinson's, and Alzheimer's diseases have to be considered, which would be a reasonable model choice for preliminary studies aimed at studying the pathogenic aspects of this multifaceted disease involving multiple organ systems.

Since the last few decades, coronaviruses have been a serious threat to human and animal health globally. Good animal models are instrumental in studying the pathobiological aspects of any viruses and COVID-19 certainly demands the development of new animal models to cater to our needs as the disease manifestations vary from asymptomatic to mild, moderate, and severe. Genetically engineered animals will be a great initiative and add great perspective for understanding the pathobiology of SARS-CoV-2.

Conflicts of Interest

The author declares no conflict of interest.

References

Abdelnabi, R., Foo Caroline, S., Kaptein Suzanne, J.F., Boudewijns, R., Vangeel, L., De Jonghe, S., Jochmans, D., Weynand, B. and Neyts, J. 2022. A SCID Mouse Model To Evaluate the Efficacy of Antivirals against SARS-CoV-2 Infection. *J. Virol.* **96**: e00758-00722.

Bao, L., Deng, W., Gao, H., Xiao, C., Liu, J., Xue, J., Lv, Q., Liu, J., Yu, P., Xu, Y., Qi, F., Qu, Y., Li, F., Xiang, Z., Yu, H., Gong, S., Liu, M., Wang, G., Wang, S., Song, Z., Zhao, W., Han, Y., Zhao, L., Liu, X., Wei, Q. and Qin, C. 2020a. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. *bioRxiv*, 2020.2003.2013.990226.

Bao, L., Deng, W., Huang, B., Gao, H., Ren, L., Wei, Q., Yu, P., Xu, Y., Liu, J., Qi, F., Qu, Y., Wang, W., Li, F., Lv, Q., Xue, J., Gong, S., Liu, M., Wang, G., Wang, S., Zhao, L., Liu, P., Zhao, L., Ye, F., Wang, H., Zhou, W., Zhu, N., Zhen, W., Yu, H., Zhang, X., Song, Z., Guo, L., Chen, L., Wang, C., Wang, Y., Wang, X., Xiao, Y., Sun, Q., Liu, H., Zhu, F., Ma, C., Yan, L., Yang, M., Han, J., Xu, W., Tan, W., Peng, X., Jin, Q., Wu, G. and Qin, C. 2020b. The Pathogenicity of 2019 Novel Coronavirus in hACE2 Transgenic Mice. *bioRxiv,* 2020.2002.2007.939389.

Blaurock, C., Breithaupt, A., Weber, S., Wylezich, C., Keller, M., Mohl, B.-P., Görlich, D., Groschup, M.H., Sadeghi, B., Höper, D., Mettenleiter, T.C. and Balkema-Buschmann, A. 2022. Compellingly high SARS-CoV-2 susceptibility of Golden Syrian hamsters suggests multiple zoonotic infections of pet hamsters during the COVID-19 pandemic. *Sci. Rep.* **12**: 15069.

Böswald, L.F., Popper, B., Matzek, D., Neuhaus, K. and Wenderlein, J. 2024. Characterization of the gastrointestinal microbiome of the Syrian hamster (Mesocricetus auratus) and comparison to data from mice. FEBS Open Bio. doi:10.1002/2211-5463.13869

Brola, W. and Wilski, M. 2022. Neurological consequences of COVID-19. *Pharmacol Rep* **74:** 1208-1222.

Campos, R.K., Camargos, V.N., Azar, S.R., Haines, C.A., Eyzaguirre, E.J. and Rossi, S.L. 2021. SARS-CoV-2 Infects Hamster Testes. *Microorganisms* **9.**1318

Carossino, M., Montanaro, P., O'Connell, A., Kenney, D., Gertje, H., Grosz, K., Ericsson, M., Huber, B.R., Subramaniam, S., Kirkland, T.A., Walker, J.R., Francis, K.P., Klose, A.D., Paragas, N., Kurnick, S., Bosmann, M., Saeed, M., Balasuriya, U., Douam, F. and Crossland, N. 2021. Fatal neuroinvasion and SARS-CoV-2 tropism in K18-hACE2 mice is partially independent on hACE2 expression. *bioRxiv*.2021-01

Chan, J.F.-W., Zhang, A.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., Liang, R., Cao, J., Chen, Y., Tang, K., Luo, C., Cai, J.-P., Kok, K.-H., Chu, H., Chan, K.-H., Sridhar, S., Chen, Z., Chen, H., To, K.K.-W. and Yuen, K.-Y. 2020. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clinic. Infect. Dis*.**71(**9):2428-2446.

Channappanavar, R., Fett, C., Mack, M., Ten Eyck, P.P., Meyerholz, D.K., Perlman, S., 2017. Sex-Based Differences in Susceptibility to Severe Acute Respiratory Syndrome Coronavirus Infection. *J. Immunol.* **198:** 4046-4053.

Chao Shan, Y.-F.Y., Xing-Lou Yang, Yi-Wu Zhou, Jia Wu, Ge Gao, Yun Peng, Lian Yang, Xue Hu, Jin Xiong, Ren-Di Jiang, Hua-Jun Zhang, Xiao-Xiao Gao, Cheng Peng, Juan Min, Ying Chen, Hao-Rui Si, Peng Zhou, Yan-Yi Wang, Hong-Ping Wei, Wei Pang, Zheng-Fei Hu, Long-Bao Lv, Yong-Tang Zheng, Zheng-Li Shi and Zhi-Ming Yuan. 2020. Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in the rhesus macaques. *Cell Res*. **30**:670-677.

Chen, Y., Wei, Q., Li, R., Gao, H., Zhu, H., Deng, W., Bao, L., Tong, W., Cong, Z., Jiang, H. and Qin, C. 2020. Protection of Rhesus Macaque from SARS-Coronavirus challenge by recombinant adenovirus vaccine. *bioRxiv*, 2020.2002.2017.951939.

Clark, J.J., Penrice-Randal, R., Sharma, P., Dong, X., Pennington, S.H., Marriott, A.E., Colombo, S., Davidson, A., Kavanagh Williamson, M., Matthews, D.A., Turtle, L., Prince, T., Hughes, G.L., Patterson, E.I., Shawli, G., Mega, D.F., Subramaniam, K., Sharp, J., Turner, J.D., Biagini, G.A., Owen, A., Kipar, A., Hiscox, J.A. and Stewart, J.P. 2024. Sequential Infection with Influenza A Virus Followed by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Leads to More Severe Disease and Encephalitis in a Mouse Model of COVID-19. *Viruses*, **16:** 863.

Cong, Y., Dixit, S., Perry, D.L., Huzella, L.M., Kollins, E., Byrum, R., Anthony, S.M., Drawbaugh, D., Lembirik, S., Postnikova, E., Eaton, B., Murphy, M., Kocher, G., Hadley, K., Marketon, A.E., Bernbaum, R.M., Hischak, A.M.W., Hart, R., Vaughan, N., Wada, J., Qin, J., St Claire, M.C., Schmaljohn, C.S. and Holbrook, M.R. 2024. Characterization of therapeutic antibody efficacy against multiple SARS-CoV-2 variants in the hamster model. *Antiviral Res*. **230**: 105987.

Cui, L., Wang, J., Orlando, F., Giacconi, R., Malavolta, M., Bartozzi, B., Galeazzi, R., Giorgini, G., Pesce, L., Cardarelli, F., Quagliarini, E., Renzi, S., Xiao, S., Pozzi, D., Provinciali, M., Caracciolo, G., Marchini, C. and Amici, A. 2024. Enhancing Immune Responses against SARS-CoV-2 Variants in Aged Mice with INDUK: A Chimeric DNA Vaccine Encoding the Spike S1-TM Subunits. *ACS Omega* **9**: 34624-34635.

Deng, W., Bao, L., Gao, H., Xiang, Z., Qu, Y., Song, Z., Gong, S., Liu, J., Liu, J., Yu, P., Qi, F., Xu, Y., Li, F., Xiao, C., Lv, Q., Xue, J., Wei, Q., Liu, M., Wang, G., Wang, S., Yu, H., Liu, X., Zhao, W., Han, Y. and Qin, C. 2020. Ocular conjunctival inoculation of SARS-CoV-2 can cause mild COVID-19 in Rhesus macaques. *bioRxiv*, 2020.2003.2013.990036.

Dhakal, S., Ruiz-Bedoya, C.A., Zhou, R., Creisher, P.S., Villano, J.S., Littlefield, K., Ruelas Castillo, J., Marinho, P., Jedlicka, A.E., Ordonez, A.A., Bahr, M., Majewska, N., Betenbaugh, M.J., Flavahan, K., Mueller, A.R.L., Looney, M.M., Quijada, D., Mota, F., Beck, S.E., Brockhurst, J., Braxton, A.M., Castell, N., Stover, M., D'Alessio, F.R., Metcalf Pate, K.A., Karakousis, P.C., Mankowski, J.L., Pekosz, A., Jain, S.K. and Klein, S.L. 2021. Sex Differences in Lung Imaging and SARS-CoV-2 Antibody Responses in a COVID-19 Golden Syrian Hamster Model. *mBio* **12**: e0097421.

Dhanushkodi, N.R., Prakash, S., Quadiri, A., Zayou, L., Srivastava, R., Shaik, A.M., Suzer, B., Ibraim, I.C., Landucci, G., Tifrea, D.F., Singer, M., Jamal, L., Edwards, R.A., Vahed, H., Brown, L. and BenMohamed, L. 2024. Antiviral and Anti-Inflammatory Therapeutic Effect of RAGE-Ig Protein against Multiple SARS-CoV-2 Variants of Concern Demonstrated in K18-hACE2 Mouse and Syrian Golden Hamster Models. *J. Immunol.* **212:** 576-585.

Drelich, A.K., Rayavara, K., Hsu, J., Saenkham-Huntsinger, P., Judy, B.M., Tat, V., Ksiazek, T.G., Peng, B.H. and Tseng, C.K. 2024. Characterization of Unique Pathological Features of COVID-Associated Coagulopathy: Studies with AC70 hACE2 Transgenic Mice Highly Permissive to SARS-CoV-2 Infection. *PLoS Pathog.* **20:** e1011777.

Edwards, C.T., Karunakaran, K.A., Garcia, E., Beutler, N., Gagne, M., Golden, N., Aoued, H., Pellegrini, K.L., Burnett, M.R., Honeycutt, C.C., Lapp, S.A., Ton, T., Lin, M.C., Metz, A., Bombin, A., Goff, K., Scheuermann, S.E., Wilkes, A., Wood, J.S., Ehnert, S., Weissman, S., Curran, E.H., Roy, M., Dessasau, E., Paiardini, M., Upadhyay, A.A., Moore, I., Maness, N.J., Douek, D.C., Piantadosi, A., Andrabi, R., Rogers, T.R., Burton, D.R. and Bosinger, S.E. 2024. Passive infusion of an S2-Stem broadly neutralizing antibody protects against SARS-CoV-2 infection and lower airway inflammation in rhesus macaques. *bioRxiv.*2024-07.

Ellsworth, C.R., Wang, C., Katz, A.R., Chen, Z., Islamuddin, M., Yang, H., Scheuermann, S.E., Goff, K.A., Maness, N.J., Blair, R.V., Kolls, J.K. and Qin, X. 2024. Natural Killer Cells Do Not Attenuate a Mouse-Adapted SARS-CoV-2-Induced Disease in Rag2(-/-) Mice. *Viruses*. **16:** 611.

Falzarano, D., de Wit, E., Feldmann, F., Rasmussen, A.L., Okumura, A., Peng, X., Thomas, M.J., van Doremalen, N., Haddock, E., Nagy, L., LaCasse, R., Liu, T., Zhu, J., McLellan, J.S., Scott, D.P., Katze, M.G., Feldmann, H. and Munster, V.J. 2014. Infection with MERS-CoV causes lethal pneumonia in the common marmoset. *PLoS Pathog*. **10:** e1004250.

Feng, X.L., Yu, D., Zhang, M., Li, X., Zou, Q.C., Ma, W., Han, J.B., Xu, L., Yang, C., Qu, W., Deng, Z.H., Long, J., Long, Y., Li, M., Yao, Y.G., Dong, X.Q., Zeng, J. and Li, M.H. 2022. Characteristics of replication and pathogenicity of SARS-CoV-2 Alpha and Delta isolates. *Virol Sin*. **37:** 804-812.

Fumagalli, V., Ravà, M., Marotta, D., Di Lucia, P., Laura, C., Sala, E., Grillo, M., Bono, E., Giustini, L., Perucchini, C., Mainetti, M., Sessa, A., Garcia-Manteiga, J.M., Donnici, L., Manganaro, L., Delbue, S., Broccoli, V., De Francesco, R., D'Adamo, P., Kuka, M., Guidotti, L.G. and Iannacone, M. 2022. Administration of aerosolized SARS-CoV-2 to K18-hACE2 mice uncouples respiratory infection from fatal neuroinvasion. *Sci. Immunol.* **7**: eabl9929.

Ghosh Roy, S., Karim, A.F., Brumeanu, T.D. and Casares, S.A. 2024. Reconstitution of human microglia and resident T cells in the brain of humanized DRAGA mice. *Front Cell Infect. Microbiol*. **14**: 1367566.

Glazkova, D.V., Bogoslovskaya, E.V., Urusov, F.A., Kartashova, N.P., Glubokova, E.A., Gracheva, A.V., Faizuloev, E.B., Trunova, G.V., Khokhlova, V.A., Bezborodova, O.A., Pankratov, A.A., Leneva, I.A. and Shipulin, G.A. 2022. [Generation of SARS-CoV-2 Mouse Model by Transient Expression of the Human ACE2 Gene Mediated by Intranasal Administration of AAV-hACE2]. *Mol Biol (Mosk)* **56**: 774-782.

Goumenou, M., Spandidos, D.A. and Tsatsakis, A. 2020. [Editorial] Possibility of transmission through dogs being a contributing factor to the extreme Covid-19 outbreak in North Italy. *Mol. Med. Rep*. **21**: 2293-2295.

Greene, C., Connolly, R., Brennan, D., Laffan, A., O'Keeffe, E., Zaporojan, L., O'Callaghan, J., Thomson, B., Connolly, E., Argue, R., Meaney, J.F.M., Martin-Loeches, I., Long, A., Cheallaigh, C.N., Conlon, N., Doherty, C.P. and Campbell, M.2024. Blood-brain barrier disruption and sustained systemic inflammation in individuals with long COVID-associated cognitive impairment. *Nat. Neurosci.* **27**: 421-432.

Guo, H., Yang, Y., Zhao, T., Lu, Y., Gao, Y., Li, T., Xiao, H., Chu, X., Zheng, L., Li, W., Cheng, H., Huang, H., Liu, Y., Lou, Y., Nguyen, H.C., Wu, C., Chen, Y., Yang, H. and Ji, X. 2023. Mechanism of a rabbit monoclonal antibody broadly neutralizing SARS-CoV-2 variants. *Commun.Biol*. **6:** 364.

Haoyu, W., Meiqin, L., Jiaoyang, S., Guangliang, H., Haofeng, L., Pan, C., Xiongzhi, Q., Kaixin, W., Mingli, H., Xuejie, Y., Lämmermann, I., Grillari, J., Zhengli, S., Jiekai, C. and Guangming, W. 2024. Premature aging effects on COVID-19 pathogenesis: new insights from mouse models. *Sci. Rep.* **14**: 19703.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., Müller, M.A., Drosten, C., Pöhlmann, S., 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* , **181**: 271-280.e278.

Ireland, R.E., Davies, C.D., Keyser, E., Findlay, J.S.F., Eastaugh, L., Laws, T.R., Salguero, F.J., Hunter, L. and Nelson, M. 2022. Histopathological and Immunological Findings in the Common Marmoset Following Exposure to Aerosolized SARS-CoV-2. *Viruses* **14**: 1580.

Jahirul Islam, M., Nawal Islam, N., Siddik Alom, M., Kabir, M. and Halim, M.A. 2023. A review on structural, non-structural, and accessory proteins of SARS-CoV-2: Highlighting drug target sites. *Immunobiology* **228**: 152302.

Jeon, D., Kim, S.H., Kim, J., Jeong, H., Uhm, C., Oh, H., Cho, K., Cho, Y., Park, I.H., Oh, J., Kim, J.J., Hwang, J.Y., Lee, H.J., Lee, H.Y., Seo, J.Y., Shin, J.S., Seong, J.K. and Nam, K.T. 2024. Discovery of a new long COVID mouse model via systemic histopathological comparison of SARS-CoV-2 intranasal and inhalation infection. *Biochem. Biophys. Acta Mol. Basis Dis* .**1870:** 167347.

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., Um, J., Song, M.S., Jeong, H.W., Lai, V.D., Kim, Y., Chin, B.S., Park, J.S., Chung, K.H., Foo, S.S., Poo, H., Mo, I.P., Lee, O.J., Webby, R.J., Jung, J.U. and Choi, Y.K. 2020. Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. *Cell host Microbe*, **27**(5):704-709.

Ko, M., Lee, J.Y., Shin, Y.S., Jeon, S., Myung, S., Cho, J.E., Jang, M.S., Song, J.H., Kim, H.R., Park, H.G., Park, C.M. and Kim, S. 2023. Novel SARS-CoV-2 entry inhibitors, 2-anilinoquinazolin-4(3H)-one derivatives, show potency as SARS-CoV-2 antivirals in a human ACE2 transgenic mouse model. *J. Med. Virol*. **95**: e28863.

Lechien, J.R., Chiesa-Estomba, C.M., De Siati, D.R., Horoi, M., Le Bon, S.D., Rodriguez, A., Dequanter, D., Blecic, S., El Afia, F., Distinguin, L., Chekkoury-Idrissi, Y., Hans, S., Delgado, I.L., Calvo-Henriquez, C., Lavigne, P., Falanga, C., Barillari, M.R., Cammaroto, G., Khalife, M., Leich, P., Souchay, C., Rossi, C., Journe, F., Hsieh, J., Edjlali, M., Carlier, R., Ris, L., Lovato, A., De Filippis, C., Coppee, F., Fakhry, N., Ayad, T. and Saussez, S. 2020. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur. Arch. Otorhinolaryngol*. **277**(8):251-2261.

Lee, K.S., Wong, T.Y., Russ, B.P., Horspool, A.M., Miller, O.A., Rader, N.A., Givi, J.P., Winters, M.T., Wong, Z.Y.A., Cyphert, H.A., Denvir, J., Stoilov, P., Barbier, M., Roan, N.R., Amin, M.S., Martinez, I., Bevere, J.R. and Damron, F.H. 2022. SARS-CoV-2 Delta variant induces enhanced pathology and inflammatory responses in K18-hACE2 mice. *PLoS One*, **17:** e0273430.

Liu, H., Brostoff, T., Ramirez, A., Wong, T., Rowland, D.J., Heffner, M., Flores, A., Willis, B., Evans, J.J., Lanoue, L., Lloyd, K.C.K. and Coffey, L.L. 2024. Establishment and characterization of an hACE2/hTMPRSS2 knock-in mouse model to study SARS-CoV-2. *Front. Immunol*. **15**: 1428711.

Lu, S., Zhao, Y., Yu, W., Yang, Y., Gao, J., Wang, J., Kuang, D., Yang, M., Yang, J., Ma, C., Xu, J., Qian, X., Li, H., Zhao, S., Li, J., Wang, H., Long, H., Zhou, J., Luo, F., Ding, K., Wu, D., Zhang, Y., Dong, Y., Liu, Y., Zheng, Y., Lin, X., Jiao, L., Zheng, H., Dai, Q., Sun, Q., Hu, Y., Ke, C., Liu, H. and Peng, X. 2020. Comparison of SARS-CoV-2 infections among 3 species of non-human primates. *bioRxiv*, 2020.2004.2008.031807.

Luan, J., Jin, X., Lu, Y. and Zhang, L. 2020. SARS-CoV-2 spike protein favors ACE2 from Bovidae and Cricetidae. *J.Med.Virol.* **92**(9):1649-1656.

Mahase, E. 2020. Covid-19: concerns grow over inflammatory syndrome emerging in children. *BMJ.* **369**:m1710.

Melin, A.D., Janiak, M.C., Marrone, F., Arora, P.S. and Higham, J.P. 2020. Comparative ACE2 variation and primate COVID-19 risk. *bioRxiv*, 2020.2004.2009.034967.

Metzdorf, K., Jacobsen, H., Kim, Y., Teixeira Alves, L.G., Kulkarni, U., Brdovčak, M.C., Materljan, J., Eschke, K., Chaudhry, M.Z., Hoffmann, M., Bertoglio, F., Ruschig, M., Hust, M., Šustić, M., Krmpotić, A., Jonjić, S., Widera, M., Ciesek, S., Pöhlmann, S., Landthaler, M. and Čičin-Šain, L. 2024. A single-dose MCMV-based vaccine elicits long-lasting immune protection in mice against distinct SARS-CoV-2 variants. *Front. Immunol.* **15**: 1383086.

Mohandas, S., Yadav, P.D., Nyayanit, D., Shete, A., Sarkale, P., Hundekar, S., Kumar, S. and Lole, K. 2021. 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.

Munster, V.J., Feldmann, F., Williamson, B.N., van Doremalen, N., Pérez-Pérez, L., Schulz, J., Meade-White, K., Okumura, A., Callison, J., Brumbaugh, B., Avanzato, V.A., Rosenke, R., Hanley, P.W., Saturday, G., Scott, D., Fischer, E.R. and de Wit, E. 2020. Respiratory disease and virus shedding in rhesus macaques inoculated with SARS-CoV-2. *bioRxiv,* 2020.2003.2021.001628.

Myeni, S.K., Leijs, A.A., Bredenbeek, P.J., Morales, S.T., Linger, M.E., Fougeroux, C., van Zanen-Gerhardt, S., Zander, S.A.L., Sander, A.F. and Kikkert, M. 2024. Protection of K18-hACE2 Mice against SARS-CoV-2 Challenge by a Capsid Virus-like Particle-Based Vaccine. *Vaccines* (Basel) 12(7):766.

Mykytyn, A.Z., Lamers, M.M., Okba, N.M.A., Breugem, T.I., Schipper, D., van den Doel, P.B., van Run, P., van Amerongen, G., de Waal, L., Koopmans, M.P.G., Stittelaar, K.J., van den Brand, J.M.A. and Haagmans, B.L. 2021. Susceptibility of rabbits to SARS-CoV-2. *Emerg. Microbes Infect.***10** : 1-7.

Nagata, N., Iwata, N., Hasegawa, H., Sato, Y., Morikawa, S., Saijo, M., Itamura, S., Saito, T., Ami, Y., Odagiri, T., Tashiro, M. and Sata, T. 2007. Pathology and virus dispersion in cynomolgus monkeys experimentally infected with severe acute respiratory syndrome coronavirus via different inoculation routes. *Int. J. Exp. Pathol*. **88**: 403-414.

Niu, S., Wang, J., Bai, B., Wu, L., Zheng, A., Chen, Q., Du, P., Han, P., Zhang, Y., Jia, Y., Qiao, C., Qi, J., Tian, W.x., Wang, H.W., Wang, Q. and Gao, G.F. 2021. Molecular basis of cross-species ACE2 interactions with SARS-CoV-2-like viruses of pangolin origin. T*he EMBO J.* **40**: e107786.

Niu, Y., Yu, Y., Bernat, A., Yang, S., He, X., Guo, X., Chen, D., Chen, Y., Ji, S., Si, W., Lv, Y., Tan, T., Wei, Q., Wang, H., Shi, L., Guan, J., Zhu, X., Afanassieff, M., Savatier, P., Zhang, K., Zhou, Q. and Ji, W. 2010. Transgenic rhesus monkeys produced by gene transfer into early-cleavage-stage embryos using a simian immunodeficiency virus-based vector. *Proc. Nat. Acad. Sci.USA*.**107**: 17663-17667.

Palmer, C.S., Perdios, C., Abdel-Mohsen, M., Mudd, J., Datta, P.K., Maness, N.J., Lehmicke, G., Golden, N., Hellmers, L., Coyne, C., Moore Green, K., Midkiff, C., Williams, K., Tiburcio, R., Fahlberg, M., Boykin, K., Kenway, C., Russell-Lodrigue, K., Birnbaum, A., Bohm, R., Blair, R., Dufour, J.P., Fischer, T., Saied, A.A. and Rappaport, J. 2024. Non-human primate model of long-COVID identifies immune associates of hyperglycemia. *Nat. Commun.* **15**: 6664.

Park, J.E. and Silva, A.C. 2019. Generation of genetically engineered non-human primate models of brain function and neurological disorders. *Am. J.Primatol.* **81:** e22931.

Plante, J.A., Plante, K.S., Gralinski, L.E., Beall, A., Ferris, M.T., Bottomly, D., Green, R., McWeeney, S.K., Heise, M.T., Baric, R.S. and Menachery, V.D. 2020. Mucin 4 Protects Female Mice from Coronavirus Pathogenesis. *bioRxiv,* 2020.2002.2019.957118.

Pruijssers, A.J., George, A.S., Schäfer, A., Leist, S.R., Gralinksi, L.E., Dinnon, K.H., Yount, B.L., Agostini, M.L., Stevens, L.J., Chappell, J.D., Lu, X., Hughes, T.M., Gully, K., Martinez, D.R., Brown, A.J., Graham, R.L., Perry, J.K., Du Pont, V., Pitts, J., Ma, B., Babusis, D., Murakami, E., Feng, J.Y., Bilello, J.P., Porter, D.P., Cihlar, T., Baric, R.S., Denison, M.R. and Sheahan, T.P. 2020. Remdesivir potently inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. *Cell Reports*, **32**(3): 107940

Reed, D.S., McElroy, A.K., Barbeau, D.J., McMillen, C.M., Tilston-Lunel, N.L., Nambulli, S., Cottle, E., Gilliland, T.C., Rannulu, H., Lundy, J., Olsen, E.L., O'Malley, K.J., Xia, M., Hartman, A.L., Luke, T.C., Egland, K., Bausch, C., Wu, H., Sullivan, E.J., Klimstra, W.B. and Duprex, W.P. 2024. No evidence for enhanced disease with human polyclonal SARS-CoV-2 antibody in the ferret model. *PLoS One*, **19**: e0290909.

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., Fouchier, R.A.M. and Herfst, S. 2020. SARS-CoV-2 is transmitted via contact and via the air between ferrets. *bioRxiv*, 2020.2004.2016.044503.

Rockx, B., Kuiken, T., Herfst, S., Bestebroer, T., Lamers, M.M., de Meulder, D., van Amerongen, G., van den Brand, J., Okba, N.M.A., Schipper, D., van Run, P., Leijten, L., Verschoor, E., Verstrepen, B., Langermans, J., Drosten, C., van Vlissingen, M.F., Fouchier, R., de Swart, R., Koopmans, M. and Haagmans, B.L. 2020. Comparative Pathogenesis Of COVID-19, MERS And SARS In A Non-Human Primate Model. *bioRxiv*, 2020.2003.2017.995639.

Seo, S.M., Son, J.H., Lee, J.H., Kim, N.W., Yoo, E.S., Kang, A.R., Jang, J.Y., On, D.I., Noh, H.A., Yun, J.W., Park, J.W., Choi, K.S., Lee, H.Y., Shin, J.S., Seo, J.Y., Nam, K.T., Lee, H., Seong, J.K. and Choi, Y.K. 2022. Development of transgenic models susceptible and resistant to SARS-CoV-2 infection in FVB background mice. *PLoS One*, **17:** e0272019.

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., Liu, R., He, X., Shuai, L., Sun, Z., Zhao, Y., Liu, P., Liang, L., Cui, P., Wang, J., Zhang, X., Guan, Y., Tan, W., Wu, G., Chen, H. and Bu, Z. 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. **368** :.1016-1020.

Song, I.W., Washington, M., Leynes, C., Hsu, J., Rayavara, K., Bae, Y., Haelterman, N., Chen, Y., Jiang, M.M., Drelich, A., Tat, V., Lanza, D.G., Lorenzo, I., Heaney, J.D., Tseng, C.K., Lee, B. and Marom, R. 2024. Generation of a humanized mAce2 and a conditional hACE2 mouse models permissive to SARS-COV-2 infection. *Mamm Genome* **35**:113-121.

Souza, A.J.S., Souza Filho, A.F., Zimpel, C.K., Ayupe, M.C., Araújo, M.V., Machado, R.R.G., Salles, E., Salgado, C.L., Tavares, M.S., Silva-Pereira, T.T., Souza, P.C., Durigon, E.L., Heinemann, M.B., Brandão, P.E., Fonseca, D.M.D., Guimarães, A.M.S. and Sá, L.R.M. 2024. Hepatic endotheliitis in Golden Syrian hamsters (Mesocricetus auratus) experimentally infected with SARS-CoV-2. *Re.v Inst. Med. Trop. Sao Paulo.* **66**:e44.

Speranza, E., Purushotham, J.N., Port, J.R., Schwarz, B., Flagg, M., Williamson, B.N., Feldmann, F., Singh, M., Pérez-Pérez, L., Sturdevant, G.L., Roberts, L.M., Carmody, A., Schulz, J.E., van Doremalen, N., Okumura, A., Lovaglio, J., Hanley, P.W., Shaia, C., Germain, R.N., Best, S.M., Munster, V.J., Bosio, C.M. and de Wit, E. 2022. Age-related differences in immune dynamics during SARS-CoV-2 infection in rhesus macaques. *Life Sci. Allian.***5**: e202101314.

Suba, Z. 2020. Prevention and therapy of COVID-19 via exogenous estrogen treatment for both male and female patients. Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, *Societecanadiennedessciencespharmaceutiques,* **23**:75-85.

Subramaniam, S., Kenney, D., Jayaraman, A., O'Connell, A.K., Walachowski, S., Montanaro, P., Reinhardt, C., Colucci, G., Crossland, N.A., Douam, F. and Bosmann, M. 2024. Aging is associated with an insufficient early inflammatory response of lung endothelial cells in SARS-CoV-2 infection. *Front. Immunol*. **15**: 1397990.

Tatham, L., Kipar, A., Sharp, J., Kijak, E., Herriott, J., Neary, M., Box, H., Gallardo Toledo, E., Valentijn, A., Cox, H., Pertinez, H., Curley, P., Arshad, U., Rajoli, R.K.R., Rannard, S., Stewart, J.P. and Owen, A. 2024. Ronapreve (REGN-CoV; casirivimab and imdevimab) reduces the viral burden and alters the pulmonary response to the SARS-CoV-2 Delta variant (B.1.617.2) in K18-hACE2 mice using an experimental design reflective of a treatment use case. *Microbiol Spectr*. **12**: e0391623.

Trimpert, J., Vladimirova, D., Dietert, K., Abdelgawad, A., Kunec, D., Dökel, S., Voss, A., Gruber, A.D., Bertzbach, L.D. and Osterrieder, N. 2020. The Roborovski Dwarf Hamster Is A Highly Susceptible Model for a Rapid and Fatal Course of SARS-CoV-2 Infection. *Cell Reports*, **33**: 108488-108488.

Wang, Q., Peng, W., Yang, Y., Wu, Y., Han, R., Ding, T., Zhang, X., Liu, J., Yang, J. and Liu, J. 2024. Proteome and ubiquitinome analyses of the brain cortex in K18-hACE2 mice infected with SARS-CoV-2. i.*Science*, **27:** 110602.

Wenham, C., Smith, J. and Morgan, R.2020. COVID-19: the gendered impacts of the outbreak. *The Lancet* , **395**: 846-848.

Williamson, B.N., Feldmann, F., Schwarz, B., Meade-White, K., Porter, D.P., Schulz, J., Doremalen, N.v., Leighton, I., Yinda, C.K., Pérez-Pérez, L., Okumura, A., Lovaglio, J., Hanley, P.W., Saturday, G., Bosio, C.M., Anzick, S., Barbian, K., Cihlar, T., Martens, C., Scott, D.P., Munster, V.J. and Wit, E.d. 2020. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. *bioRxiv*, 2020.2004.2015.043166.

Wong, D.W.L., Klinkhammer, B.M., Djudjaj, S., Villwock, S., Timm, M.C., Buhl, E.M., Wucherpfennig, S., Cacchi, C., Braunschweig, T., Knüchel-Clarke, R., Jonigk, D., Werlein, C., Bülow, R.D., Dahl, E., von Stillfried, S. and Boor, P. 2021. Multisystemic Cellular Tropism of SARS-CoV-2 in Autopsies of COVID-19 Patients. *Cells,* **10**: 1900.

Xia, J., Tong, J., Liu, M., Shen, Y. and Guo, D. 2020. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J. Med. Virol*. **92**:589-94.

Zhao, X., Chen, D., Szabla, R., Zheng, M., Li, G., Du, P., Zheng, S., Li, X., Song, C., Li, R., Guo, J.-T., Junop, M., Zeng, H. and Lin, H. 2020a. Broad and differential animal ACE2 receptor usage by SARS-CoV-2. *bioRxiv*, 2020.2004.2019.048710.

Zhao, Y., Wang, J., Kuang, D., Xu, J., Yang, M., Ma, C., Zhao, S., Li, J., Long, H., Ding, K., Gao, J., Liu, J., Wang, H., Li, H., Yang, Y., Yu, W., Yang, J., Zheng, Y., Wu, D., Lu, S., Liu, H. and Peng, X. 2020b. Susceptibility of tree shrew to SARS-CoV-2 infection. *Sci. Rep.* **10**(1): 16007. ■