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Preclinical Animal Models for COVID-19 Research-Making a Wise Choice

Preeti Vishwakarma[1], Ritika Khatri[1], Gazala Siddiqui[1], Naveen Yadav[1], Zaigham Abbas Rizvi[2], Amit Awasthi[2], Sweety Samal[1*]

[1]*Infection and Immunology centre, Translational Health Science and Technology Institute, NCR-Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad-121001, Haryana, India*

[2]*Immuno-biology Lab, Infection and Immunology centre, Translational Health Science and Technology Institute, NCR-Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad, Haryana, India*

Corresponding Author:** ***Sweety Samal, Ph.D*, Translational Health Science and Technology Institute (THSTI), NCR Biotech Science Cluster, 3rd Milestone, Faridabad–Gurgaon Expressway, Faridabad, Haryana, India.* ***Email:*** *sweety.samal@thsti.res.in*

Abstract: *The COVID-19 pandemic caused by a novel coronavirus, SARS-CoV2 has emerged as one of the global health threats causing unprecedented socio-economical loss with a rapid rise of the cases and high mortality rate. The scientific community and industry are racing for rapid development of COVID-19 preventive or therapeutic interventions such as vaccines, antiviral drugs, or antibodies. One of the major challenges for the development of these countermeasures is the availability and selection of an appropriate animal model that will mimic the disease symptoms as seen in humans. As of December, 2020, there are more than 100 candidates in vaccine and therapeutics in different stages of development. These candidates need to be tested in animal models before entering into human clinical trials. Hence it is the utmost essential to understand the SARS-CoV2 virus dynamics and host responses in animals to choose an appropriate model that will be able to measure both quantitative and qualitative immune responses. In this review, we will focus on the availability of animal models currently used for other important Coronavirus SARS-CoV and discuss herein the unique advantages or challenges of each animal model for SARS-CoV2 infection. While choosing an analog non-human model it is important to understand the intricate complex host-pathogen interactions and interpret results accordingly. The preclinical experiments of COVID-19 in various animals are very early in stage and more studies are in need with unique approaches so that outcomes can universally fit for validation of products or elucidating COVID-19 disease and pathogenesis.*

Keywords: *COVID-19; SARS-CoV; SARS-CoV2; animal model; pathophysiology; clinical symptoms*

1. INTRODUCTION

COVID-19 disease caused by SARS-CoV2 was first reported in Wuhan, Hubei province, China in December 2019 and within a few months the virus has widespread globally affecting 210 countries and causing severe pandemic [1]. In January 2020, the SARS-CoV2 has been isolated and found to be closely associated with the betacoronavirus family [1]. The sequence of SARS CoV-2 has been found to be similar to bat SARS-CoV-like coronaviruses [2], followed by pangolin coronaviruses, these data explain the strong likelihood of bats and pangolin as a primary animal reservoir from where the virus might have crossed the species barrier to infect and cause disease in humans [3]. The data from hospitalized patients have shown that the clinical manifestations of COVID-19 range from no symptoms (asymptomatic), symptoms associated with viral pneumonia most commonly seen as fever, sore throat, cough, fatigue and myalgia, and severe pneumonia (tachypnoea) that can lead to death [4]. Reports from around the world indicate that illness associated with COVID-19 disease also varies in demographic groups between male, female, children, and old people [4]. The severity of disease and mortality rate is higher in aged people (> 60 years), immunocompromised patients, and people with underlying other disease conditions.

As the pandemic is grappling around the globe, there is an urgent need for effective vaccine for mounting long-lasting immunity against SARS-CoV2. Other than vaccines, antiviral drugs and neutralizing antibodies are potential therapeutic approaches to combat the COVID-19. Though there are multiple academics and companies are

in the race to develop the interventions particularly vaccines, these candidates need to be efficiently evaluated in the preclinical animal models before moving into clinical trials. For testing vaccine candidates and therapeutic interventions pre-clinically, the most critical requirement is to have an animal model that closely mimics wide spectra of disease symptoms as seen in humans. Therefore, the development of an appropriate animal model for COVID-19 is one of the high priority research areas. Given the similarity to SARS, the animals that supported the replication of SARS virus could be of potential animal models for SARS-CoV2 as well. Nonetheless, it is absolutely important to test and select animal models as per experimental requirement such as infection model, transmission model and challenge model for evaluation of the vaccine or antiviral drugs. In addition, animal models could be valuable experimental tools that can also enable us to understand the pathophysiology of the COVID-19 disease and provide the mechanistic insight of anti-SARS-CoV2 immune responses.

In this review, we summarize currently available animal models for SARS and the challenges often associated with animal model development for Coronaviruses. We further discuss the influence of species and experimental goals on COVID-19 animal model development, extrapolating recently published reports on COVID-19 animal models and summarizing the strengths and limitations of animal models for COVID-19.

2. COVID-19 Disease Clinical Symptoms and Pathophysiology

Before selecting an appropriate animal model for SARS-CoV2 research and development, it is absolutely essential to understand clinical parameters and pathophysiology of infection. Since the SARS-CoV2 infection begins in human as a pandemic, human data become a valuable tool for selecting animal models for various coronavirus research. SARS-CoV2 belongs to Coronaviridae family which consists of four genus alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus. So far, six human Corona viruses (HCoVs) have been currently identified of which HCoV-229E and HCoV-NL63 viruses belongs to the genera alphacoronavirus, and SARS-CoV, MERS-CoV, HCoV-OC43, and HCoV-HKU1 belongs to the genus betacoronavirus. Because of the homology in the genetic structure and organization of the SARS-CoV2 to SARS-CoV, it has been categorized to betacoronavirus [5]. The Coronaviruses exhibit a wide range of disease signs and symptoms in epizootic animals ranging from respiratory, enteric, and neurological tropism. In humans, the disease is represented as mild, influenza-like illnesses to more severe acute respiratory syndrome (SARD), which is characterized by alveolar inflammation and diffuse alveolar damage, pneumonia, and hypoxic conditions in the lungs that may lead to respiratory failure and subsequent multiple organ failure and death [6]. If we talk about the pathology of SARS-COV2 not much is known. Broadly, the cellular pathology of SARS-COV2 can be divided into three phases. The first stage is asymptomatic phase in which virus enters the host through inhalation and replicates in the nasal epithelium [7,8]. Although ACE2 expression is low in the airways, the virus is still detected in nasal swabs [9] and it stays there for 1 – 2 days. The virus propagates locally and induces the first line of defense i.e the innate immune response. In the second stage, the virus migrates into the upper respiratory tract that leads to a stronger innate response . At this stage, virus could be detected both in the nasal and oral swab. The level of innate response chemokine CXCL10 was found to rise at this stage and could be an indication of the disease progression [10]. Based on the emerging data it seems that around 70-80% of COVID-19 disease cases were restricted to the upper respiratory tract and could be self-limiting [11].

In the third stage, about 20% of patients comprises of elderly and immunocompromised individuals with other diseases, the infection spreads to the lower respiratory tract resulting in hypoxia and progression to acute respiratory distress syndrome (ARDS). The existing mortality rate of COVID-19 patients is around 2%, which primarily relies on age and health condition; however, the virus has a high transmission rate and spreads easily from person to person. The pathological changes seen in different organs of COVID-19 patients are summarized in **Table 1.**

Table1. *Pathological changes in various organs infected by COVID-19*

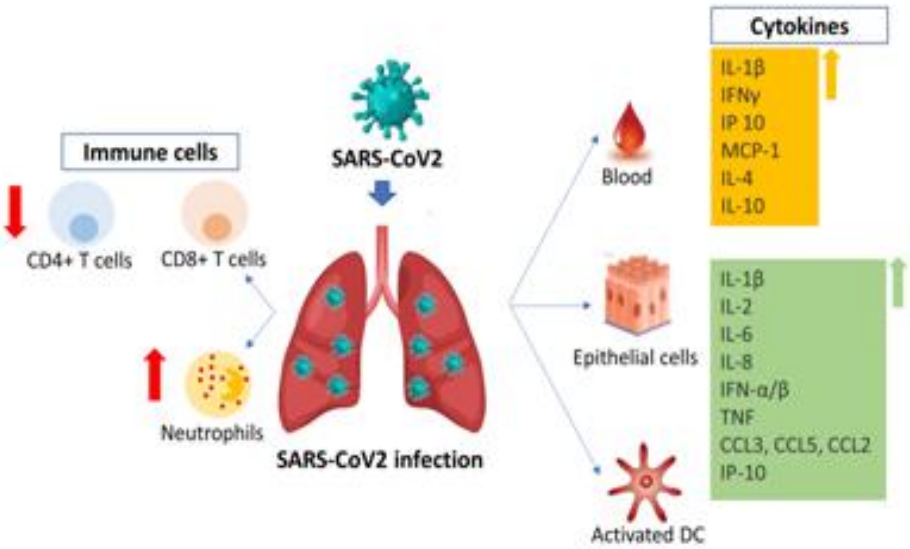
Organ	Clinical Manifestations	Laboratory Findings	Complications	References
Lung	• Pneumonia • Cough, • Coarse breathing sounds of both lungs • Hyperplasia and thickening of alveolar wall	**CT-SCAN** • Ground-glass opacity **Histology** • Diffused alveolar damage • Higher leukocyte numbers • Alveolar edema and proteinaceous exudates • Inspissated spherical secretions or globules • Focally fibrin clusters mixed with mononuclear inflammatory cells and multinucleated giant cells	• Acute respiratory distress syndrome • Cytokine Storm • Activated Notch Signaling • Formation of hyaline membrane	[47] [48]
Brain	• Depression • Anxiety • Anosmia • Ageusia • Acute Neuropsychiatry • Headache • Dizziness • Impaired consciousness • Aataxia • Seizures	-	• Trauma-related disorders • Encephalopathies • Acute cerebrovascular problems	[49] [50] [51]
Heart	• Myocarditis, • Activated notch signaling pathway • Acute cardiac injury • High blood pressure • Arrhythmia	• Increase in the ratio of ANG II: Ang-(1–7) • Increase in high-sensitivity cardiac troponin I levels • High Creatinine Kinase **Histology** • Accumulation of inflammatory cells and apoptotic bodies with endothelium,	Acute cardiac injury	[52] [53] [13] [54]
Kidney	• Acute renal failure (ARF) However, another study by Luwen Wang et al shows COVID-19 does not induce AKI (acute kidney injury)(45)	• Elevated level of plasma creatinine and urea nitrogen **Histology** • Severe acute tubular necrosis • Lymphocyte infiltration • CD68+ macrophages infiltrated into tubulointerstitium • Enhances complement C5b-9 deposition on tubules	Acute kidney injury	[55] [56]
Liver	• Liver injury	• High Bilirubin • High C-reactive protein • Abnormal levels of alanine aminotransferase • Aspartate aminotransferase (AST) • Alkaline phosphatase (ALP) • Gamma-glutamyltransferase (GGT) • Total bilirubin (TBIL) **Histology** • Moderate microvascular steatosis	• Hypoxic hepatitis due to anoxia • Drug induced liver injury (use of potentially hepatotoxic antiviral drugs)	[57] [58] [59]

		• Mild lobular and portal activity		
Blood	• D-dimer • Decreased albumin • High LDH • Lymphopenia • High erythrocyte sedimentation rate • Leukopenia • Leukocytosis • High blood pressure • Thrombocytopenia	• High blood levels of cytokines and chemokines IL1-β, IL1RA, IL7, IL8, IL9, IL10, basic FGF2, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA	RNAemia	[60] [61] [13]
Gut	• Diarrhoea • Anorexia • Nausea • Oesophageal bleeding with erosions and ulcers • Vomiting • Abdominal pain	• CT angiogram of the abdomen and pelvis shows bowel wall thickening, mural hyperenhancement, mesenteric hypervascularity, and pericolic fat • Detection of SARSCoV-2 RNA in stool samples of infected patients	Hemorrhagic colitis	[57] [62]

3. COVID-19 IMMUNE RESPONSE

SARS-CoV2 is a novel coronavirus, and thus there is limited literature about the early events of virus-host interaction and induction of immune responses. SARS-CoV2 causes acute respiratory distress syndrome or septic shock in 10-20% of the patients, due to high levels of pro- inflammatory cytokines [12]. Immuno compromised patients with COVID-19 infection show diffuse intravascular coagulation, severe hepatic dysfunction and increase ferratemia [13]. Multiple findings from different groups have found the up-regulation of cytokine storm in COVID-19 patients [14,15]. The aberrant production of cytokines in COVID-19 patients have been outlined in **Figure 1**. It has been observed that even though there is a substantial increase in neutrophils and leucocytes, but there is a decrease in the total count of lymphocytes; CD4+ and CD8+ T cells. Elevation of type I-IFN response is a common feature among other SARS infection. However, in SARS-CoV2, there is impaired interferon (IFN) type I response (total absence of IFN-II (beta) and very low production and activity of IFN-I (alpha)), which was associated with a persistent blood viral load and an exacerbated inflammatory response. The impaired inflammatory response was due to activation of NF-κB, TNF-α and IL-6 signaling pathways.

Figure1. *SARS-CoV2 infection upregulate inflammatory cytokines in COVID-19 patients which results in severe T cell dysfunction*



4. Lessons Learned from Pathogenesis and Infection Models of the SARS

Since the outbreak of SARS in 2003, several animal models have been studied and developed to evaluate the replication of the virus and its' associated disease biology. Molecular studies demonstrated that SARS virus receptor binding protein (RBD) of surface spike protein mediates coronavirus entry into the host cells by binding to its host receptor, ACE2, and thus facilitating the fusion of the host and viral membranes [16]. Hence, RBD-ACE2 receptor interaction is one of the major host determinants that allow entry and replication of SARS in various host cells that express ACE2. ACE2 is one of the essential host cell receptors that is expressed in humans and other animals such as palm civets, domestic cats, ferrets, hamsters, raccoon dogs, mouse and, bats. The decisive amino acids position in human ACE2 thought to be responsible for its successful binding ability with the RBD are K31, E35, D38, M82 and K353. It has been reported that residual mutations in the functional cellular receptor ACE2 restrict efficient SARS-CoV2 replication in animals **Table 2** [17]. Various animal models have been evaluated to study SARS virus infection and transmission which is summarized below and in **Table 3**.

Table2. *Variation of amino acids in ACE2 resulting in susceptibility or resistance to replicate Sars-Cov-2 infection in different mammals*

Mammal Species	hACE2 Amino Acid Position					Matching Amino acids	Susceptibility	References
	K31	E35	D38	M82	K353			
Macaca mulata	K	E	D	M	K	5/5	Yes	[17,40]
Mustela ermine	K	E	E	T	K	3/5	No	
Camelus dromedarius	E	E	D	T	K	3/5	No	
Procyon lotor	N	E	E	T	K	2/5	No	
Rhinolophus ferrumequinum	D	E	N	N	K	2/5	No	
Rattus norvegicus	K	E	D	N	H	3/5	No	
Mus musculus	N	E	D	S	H	2/5	No	
Ornithorhynchus anatinus	Q	Q	D	K	K	2/5	No	
Loxodonta africana	T	E	D	D	K	3/5	No	
Erinaceus europaeus	D	Q	N	N	N	0/5	No	
Nyctereutes procyonoides	K	E	E	T	R	2/5	No	
Suricata suricatta	Q	E	E	A	K	2/5	No	
Dipodomys ordii	N	E	D	I	K	2/5	No	
Cavia porcellus	E	K	D	A	K	3/5	No	
Manis javanica	K	E	E	N	K	3/5	Yes	
Mustela putorius	K	E	E	T	K	3/5	Yes	
Mesocricetus auratus	K	E	D	N	K	4/5	Yes	

Table3. *Comparison of different animal model studied in human infecting coronaviruses*

Animal Model	Sars-Cov	Mers-Cov	Sars-Cov2
Mouse	Naive BALB/c, C57BL6 and 129S inbred mice are able to replicate virus but lack to exhibit clinical pathology of the disease. Aged inbred mice	Inbred mice are not naturally susceptible to infection.	Young naïve mice are not susceptible for Sars-COv2 infection. Transgenic mice bearing human ACE2 upon infection with SARS-CoV-2 showed weight loss and

	(BALB/c), knockout mice (STAT1, Rag1, CD1, Beige) and transgenic mice (K18-hACE2, A70-hACE2) develop generalized illness, robust viral growth and pronounced lung pathology consistent with pneumonia and acute lung injury.	Transduced mice (Ad5-hDPP4) develop clinical signs and support replication of virus with interstitial pneumonia and viral antigen found in the lungs. Inflammation in the lungs. Lethality was also observed in this model.	virus replication in lung
Rabbits	No study reported	Viral replication observed from nasal swabs, MERS-CoV detected in lungs by RT-qPCR, ISH, and IHC. Similar phenotypes with EMC and England isolates	No study reported
Hamster	Clinical illness (measured by a decrease in activity on the exercise wheel) is accompanied by viral replication and pronounced histopathological changes such as inflammation, pneumonitis and consolidation in the lungs	Hamsters do not support replication.	Golden Syrian hamster is successfully able to replicated Sars-Cov2 and has shown Maximal clinical signs of rapid breathing, weight loss, histopathological changes from the initial exudative phase [63]. High lung viral load (10^5 – 10^7)), spleen and lymphoid atrophy associated with cytokine storming in first week of virus challenge Recovery of all infected hamster within fourteen days post-challenge with serum neutralizing antibody titer of ≥1:427 Viral antigen was also found in the duodenum epithelial cells with viral RNA detected in feces [31].
Ferrets	Clinical illness (fever and sneezing), is accompanied by viral replication and histologic changes in the lungs.	Ferrets do not support replication	Studies on two virus strains; inoculated intranasally with 10^5PFU of F13-E or CTan-H was detected in nasal turbinate, soft palate, and tonsils on day 4 post inoculation (p.i.), but its replication in other organs is undetectable [64], [27].
Common marmoset	Multifocal mononuclear cell interstitial pneumonitis, accompanied by multinucleated syncytial cells, edema, and bronchiolitis in most animals. Hepatic inflammation was observed in most animals, predominantly as a multifocal lymphocytic hepatitis accompanied by necrosis of individual hepatocytes.	Moderate to severe disease with disease signs including loss appetite and lethargy. High viral load in the lung of all animals, some with viremia. Interstitial pneumonia.	Not yet reported
Rhesus	Rhesus macaques, cynomolgus macaques,	Infected monkeys showed clinical signs	SARS-CoV-2 causes respiratory disease in infected rhesus

macaques, cynomolgus macaques, African green monkeys	African green monkeys and common marmosets are susceptible to infection. Clinical signs, viral replication and pathology depend on the species.	of disease, virus replication, increased temperature at 2d p.i., histological lesions, and neutralizing antibody production.	macaques, with disease lasting 8-16 days. Pulmonary infiltrates in lung radiographs. High viral loads were detected in swabs from the nose and throat of all animals as well as in bronchoalveolar lavages.

5. Mouse Model

In the wild type BALB/c or C57BL6 (B6) mouse, the virus replication has been observed both in the upper and lower respiratory tract; however, they did not show any morbidity or mortality [18]. In mouse strain 129S, upon SARS infection, viral replication is seen in the lungs with transient weight loss and development of pneumonitis [18]. Roberts A.et al. have shown clinical signs and symptoms similar to humans in infected old aged BALB/c mouse, however, the mice were recovered by 7 dpi [19]. Several groups have used immune knock out mouse models such as $CD1^{-/-}$, $Rag1^{-/-}$, $STAT1^{-/-}$ to understand the immunopathogenesis of SARS [20]. STAT-1 knockout mice are more susceptible to infection with SARS-CoV as compare to wild type. McCray et al. have developed transgenic mouse expressing in human-ACE2 airway and other epithelial cells under the control of an epithelial cell-specific promoter K18 and have demonstrated intranasal infection of wild type SARS virus strain results in lethal infection [21].

6. Hamster Model

In the golden Syrian hamsters, intranasal infection of SARS virus resulted in high titer viral replication both in the upper and lower track with marked histopathological changes (24). Even though the virus is cleared after 10 days post-infection, but significant histopathological changes were seen in the lungs, nasal mucosa, bronchial epithelial cells and nasal turbinates (28), therefore, they are considered suitable for vaccine efficacy, immunoprophylaxis and treatment studies.

7. Ferret Model

*Mustela furo (*ferret*)* is considered as a popular animal model for the influenza virus and other respiratory viral infections because their lung physiology is similar to that of humans. Due to high susceptibility, ferrets are efficient in transmitting the SARS coronavirus to other animals living in close proximity [22]. Infection of ferrets with SARS virus results in fever and upper respiratory infection and histopathological changes in lungs [23].

8. Non-Human Primates (NHP) Model

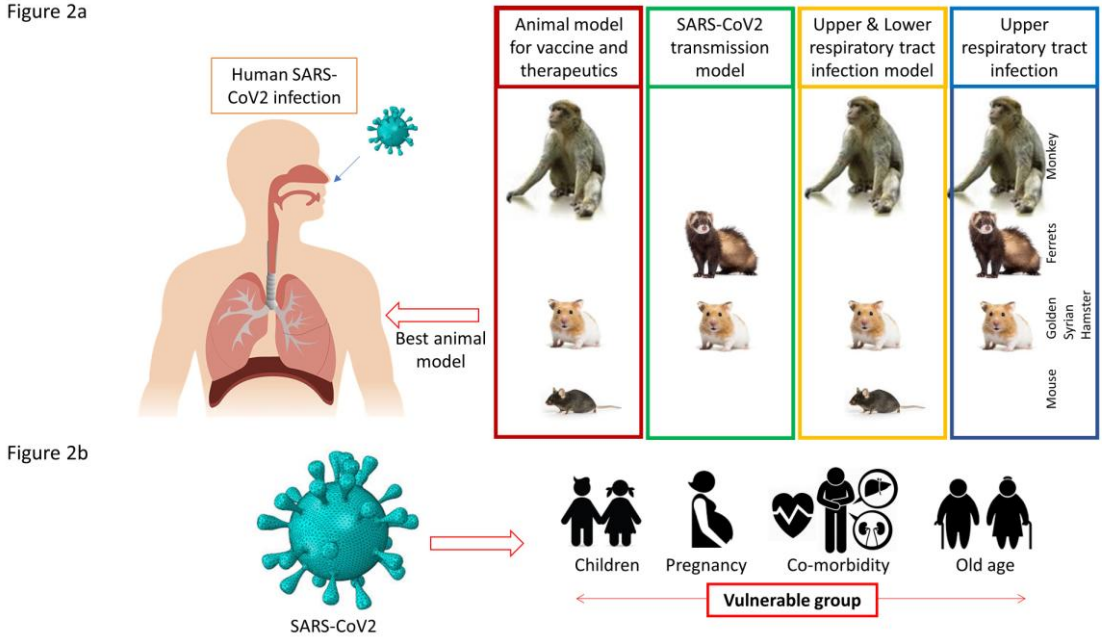
Several non-human primate species Cynomolgus, Rhesus macaques, African green monkeys, Common marmosets were studied for SARS experimental infection model. The disease severity has been seen to vary from moderate infection (Rhesus macaques, African green monkey) to more severe infection and higher virus replication in Marmosets [24]. In monkey's virus was not detected in the respiratory tract and did not develop antibodies against SARS-CoV. Several NHP species studied showed variable degrees of susceptibility to SARS-CoV infection, however none of these susceptible species, could be preferred to other. Nevertheless, costs, limited availability, and individual variation among NHPs make it difficult to conduct studies in large enough sample sizes for statistical evaluation and to draw robust conclusions.

9. Animal Models of SARS-CoV2

Even though the novel SARS-CoV2 share similarity with SARS-CoV, there are striking genetic and clinical symptom differences between the two viruses [25]. Hence, it is important to choose the right animal model which is well characterized so as to advance our understanding of the SARS-CoV2 disease progression and pathogenesis to righteously evaluate vaccine or antiviral efficacy. The first step to identify the animal model is to explicitly define the research objective and specifically determine the expected outcomes from the animal used in the study. Not much information has been available so far on SARS-CoV2 virus replication in different animals. At present, there is an urgent need for animal models mainly to study two important aspects; an animal model to study the disease pathogenesis and transmission, and an animal model to evaluate vaccines, antiviral drugs or immune therapeutics. The basic criteria to choose an animal model for vaccine or antiviral drugs testing is selecting an

animal that allows replication of virus in high titers that correlates with disease severity, so that comparison can be made between immunized and non-immunized control group (**Figure 2A**). To study the disease progression, it is necessary that the animal is able to establish infection when infected by the natural route (intranasal infection) and the virus is able to replicate in both the upper and lower respiratory tract. For transmission studies, it is important to choose an animal model that sneezes and releases virus in air droplets and eventually infect the naïve animal that is housed together.

Figure2. *[A] Available animal models for studying different stages of human SARS-COV2 infection [B] COVID-19: vulnerable and high-risk groups*



10. Infection and Transmission Model to Study Disease Pathogenesis

Recent studies by a few groups suggest mouse, hamsters, ferrets, NHPs, dogs, cats are able to support the SARS-CoV2 virus replication [26]. However, Shi et.al have further shown that other animals/ like chicken, pigs and ducks do not support SARS-CoV2 replication [27]. Mouse models are the most preferable animal models because of their ease to handle, breed and maintain, and also low cost and physiological similarity to humans. However, wild type mouse is not suitable for the COVID-19 infection model because of the difference of mACE2 receptor sequence to hACE2 receptor; hence K18-hACE2 transgenic mice that express hACE2 receptor, might be a good animal model for preliminary studies. Initial results by Bao. L et.al showed virus replication in the lower respiratory tract and virus shedding in fecal however, less disease severity in transgenic mice [28]. Another mouse model developed for SARS-CoV-2 is based on adeno associated virus (AAV)-mediated expression of hACE2. The model is reported for antibody production and viral replication as similar to the pathology found in the COVID-19 patients as well as non-human primate models. Another study reported the successful development of SARS-CoV-2 hACE2 transgenic mouse (HFH4-hACE2 in C3B6 mice) infection model, where mice upon infection showed interstitial pneumonia and pathology similar to human SARS-COV2 infection [29]. Rockx et al. have shown SARS-CoV2 virus replicates in Cynomolgus macaques when infected through combined intranasal and intratracheal administration and virus shedding had been seen in the upper and lower respiratory tract, however mild clinical symptoms were observed in this model [30]. Sia et.al have shown SARS-CoV2 pathogenesis and transmission in the Golden Syrian hamsters [31]. Hamsters showed virus replication in both the upper and lower respiratory tract and shedding of virus through gastro intestinal route. Natural infection with SARS-CoV-2 in hamsters shows typical viral symptoms like weight loss, rapid breathing and could be treated with neutralizing antibodies [26].

Hence, besides vaccine and infection studies, hamsters are also suggested as a good model for immune prophylaxis studies. Ferret is an excellent model for respiratory infections like Influenza; however, they do not support high

titer SARS-CoV2 replication. Shi et.al have demonstrated SARS-CoV-2 can replicate in the upper respiratory tract of ferrets, however they do not able to detect the virus in other organs. In another study by Kim et.al, have detected viral antigens in lungs suggesting the virus replicates in lungs of ferrets though in very low titer [32]. In addition, both hamsters and ferret have shown to transmit the virus from infected to the naïve animals suggesting either of them could be explored further for transmission studies.

11. Animal Model to Study Immunogenicity of Vaccines

Synthetic SARS-CoV2 DNA vaccine has reported to exhibit humoral response in BALB/c mouse and guinea pig when administered to the tibialis anterior muscle [33]. In aged BALB/c mice, SARS-COV2 has shown more successful infection as compared to young mice. Apart from mice and guinea pig; rats and macaques administered with SARS-CoV-2 inactivated vaccine was also able to produce antibodies against SARS-CoV2 vaccine candidate [34]. NHP is also known to be an excellent model for vaccine immunogenicity assessment for SARS and MERS.

12. Animal Challenge Model to Study Vaccine, Antiviral or Monoclonal Antibodies Efficacy

The ultimate goal of any intervention is to elicit a protective response and limit the disease pathogenesis. Using animal model following parameters can be measured; such as vaccine safety, optimal dose and formulation of the vaccine, optimal route of delivery, type of immune response and correlates of protection. For SARS-CoV2 interventions, nonhuman primates are a good model to test vaccines or drugs or antibodies, as the virus replicates both in the upper and lower respiratory tract, though severe clinical symptoms have not been observed. However, the cost, requirement of special infrastructure and expertise limit the use of NHP. Hamsters are another good model to test the efficacy of vaccines and protection as the virus grows both in the upper and lower respiratory tract and shows extensive pathophysiology in different organs mainly lungs as similar to humans. Recently a study has been reported to use golden Syrian hamster model to identify prophylactic vaccine candidates, and passive transfer of convalescent serum from infected animals could restrict viral replication in the respiratory tract of infected animals[35]. Hamsters have been employed for various vaccine studies against SARS-CoV-2 including Ad26-S.PP vaccine which is currently being evaluated in clinical trials. Ad26-S.PP is a replication-incompetent, recombinant Ad26 vector that encodes full length Spike protein of SARS-CoV-2 (S.PP) and was immunized intramuscularly in Syrian hamsters at week 0. Upon infection with high-dose of SARS-CoV-2, Ad26-S.PP provided robust protection against mortality and severe clinical symptoms. Only 4 % of the mean body weight was lost in the vaccinated hamsters. In histopathology, viral interstitial pneumonia, aggregates of lymphocytes and disruption of the bronchiolar epithelium was observed at the minimal level [46]. Another vaccine that was studied in hamsters against COVID-19 is the attenuated SARS-CoV-2 (Del-mut-1). Lau et al. passaged a clinical isolate of SARS-CoV-2 in Vero-E6 cells, lacking interferon production, and observed a series of deleted mutants (Del-muts) and point mutation variants, assuming either these mutations were already present among infected people in a very low level or generated during the virus passage. One of the variants (Del-mut-1) carrying deletion of 10 amino acids (30bp) at the S1/S2 junction when administered into hamsters, did not cause any weight loss and these hamsters developed much milder disease with lower virus replication levels in the lungs. This variant should be evaluated as an attenuated vaccine or laboratory tool (siu lau). Lastly, keeping low cost and global availability along with safety and protection as basis for the SARS-CoV-2 Vaccine, Sun et al. examined an egg-based inactivated Newcastle disease virus (NDV)/SARS-CoV-2 vaccine in hamsters and mice as a potential candidate. The NDV vector vaccine expresses a pre-fusion spike protein, whose transmembrane domain and cytoplasmic tail were replaced with those from the NDV fusion (F) protein (S-F chimera) and it grows well in embryonated chicken eggs. This vaccine is immunogenic, induced stronger binding and/or neutralizing antibodies in hamsters and protected them from SARS-CoV-2 infections. (SRL Sun).Hamster model is cost effective as compared with NHP and Ferret model. The hamster model is suitable pre-clinical model for rapid screening of library of vaccine or antiviral therapy candidates[36]. Narrowed down

selected candidates further can be evaluated in NHP model for validating its usefulness.

13. Current Best Available Experimental Animal Model for SARS-COV2

The host range of animal species which has been reported that can harbor the CoVs are cattle, horses, swine, dogs, cats, camels, rabbits, rodents, birds, ferrets, mink, bats, snakes, frogs, marmots, hedgehogs, pangolins[37]. However, there are very limited options for COVID-19 related animal models. Small animal models are mostly preferred over large animals due to cost effectiveness and the expression of the receptor that facilitates disease progression. Since wild type mouse ACE2 receptor does not support virus attachment, transgenic ACE2 knockout mice, Tmprss2 knockout mMouse, Stat1 knockout mouse, inbred mice, and transgenic HLA Mice are under evaluation for SARS-CoV-2/COVID-19[37]. Cynomolgus macaques, hamster and K18-hACE2 knockout mouse, seem to be promising animal models for COVID-19 as of now. It is also necessary to explore options to establish infection and tests for vulnerable populations (Figure 2B).

Golden hamster (Mesocricetus auratus) is a suitable option for an experimental animal model of COVID-19 because it recapitulates many aspects of SARS-CoV-2 human pathogenesis [31,36]. Hamsters support replication of coronaviruses namely SARS-CoV [38,39] and SARS-CoV-2 [40,41] but not MERS [42]. This replication is host's receptor dependent as computational remodeling suggested an interaction between ACE2 receptor from hamster and Spike (S) glycoprotein of SARS-CoV-2 [42]. Hamster's ACE2 interface, differs only by 3-4 mutations from human ACE2 and may interact with RBD of spike protein. Upon an intranasal infection with SARS-CoV-2, Syrian hamsters closely mimic clinical symptoms, viral kinetics, immune response and histopathological changes as seen in humans. Different modes of transmission of SARS-CoV-2 has been reported in hamsters namely contact, aerosol and fomite transmission. Chan et al. reported the transmission of SARS-CoV-2 virus by close contact when they housed the challenged index hamsters together with the naïve contact hamsters in the same cage. All the naïve contact animals were positive for SARS-CoV-2 infection. In contact hamsters, infectious virus was detected on 1-day post-contact (dpc) [43] and peaked in nasal washes at 3 dpc. Challenged hamsters with SARS-CoV-2 showed loss of maximal mean weight up to ~12% during first week of infection then gradually regained their weight by 14 dpi. They developed lethargy, ruffled furs, hunched back posture, and increased respiration since 2dpi but the behavior was unchanged from naïve hamsters and started to recover at 7dpi. Importantly, none of the challenged hamster died [40], [31].

Histopathological studies on lungs of SARS-CoV-2 challenged hamsters demonstrated the presence of viral antigens in nasal mucosa, bronchial epithelial cells and areas of lung consolidation on second and fifth day after inoculation with SARS-CoV-2, followed by rapid viral clearance and pneumocyte hyperplasia at seventh day. Histopathological examination also detected an increase in inflammatory cells and consolidation from 5–10% of the lungs at 2 dpi (days post infection) to 30–60% at 7 dpi [41]. No significant histopathological change was observed in other organs including brain, heart, liver and kidney at 5 dpi [41]. Importantly, myocardial degenerative changes were also noted both in infected hamster and patient autopsy despite the absence of viral antigen detection in the tissues, which is consistent with the occasional report of heart failure in COVID-19 patients [40,44].

Another important aspect of the SARS-CoV-2 infection study in hamsters is the detection of viral load in affected organs. At 2-3 dpi, peak viral load was detected in the lungs while no infectious virus was present at 7 dpi despite of the continued recovery of high copies of viral RNA [31]. Chan et al. showed that the virus replicated to higher titers in the upper respiratory tract (nasal turbinates) than in the lower respiratory tract (lungs). In contrast, Imai et al. observed efficient replication of the virus in both the respiratory tracts with no difference in its growth, mentioning the possible reason could be the differences in their virus preparation [36,45]. Viruses were also recovered from the infected hamster's brain samples, including the olfactory bulb, which indicates the entry of SARS-CoV-2 in brain. However, absence of viral antigens in the brain sections of challenged hamsters makes it uncertain [46].

14. Conclusion

Current knowledge of SARS-CoV2 virus replication and animal model is limited and largely based on the known information from the closely related virus SARS. The typical and most common characteristic of the SARS-CoV2 infection in humans is development of acute respiratory infection which in some cases developed to severe respiratory disease syndrome and other serious complications. Another distinct feature of SARS-CoV2 is a high transmission rate. Choosing the right animal model for SARS-CoV2, care must be taken that animal is susceptible to COVID-19 infection and replication of the virus should give both qualitative and quantitative readouts which will be useful to evaluate vaccine candidates or anti-viral drugs. Even though clinical signs and symptoms are important to evaluate disease severity, most lab animals do not show typical COVID-19 symptoms though they support viral replication. SARS-CoV2 infection in animals is largely dependent on binding to host cell receptor ACE2. So far, three animal models i.e transgenic hACE2 mouse, golden Syrian hamster and Rhesus macaques have found to be suitable model to study SARS-CoV2 virus replication and evaluation of various interventions. Another, important aspect to consider in evaluating the animal models showing co-morbidity and across the life span as the mortality rate in COVID-19 infection has found to be higher in old age groups and patients with other health complications such as, diabetes, cardiovascular disease, chronic obstructive pulmonary disease, and hypertension. It is important to develop an animal model that will broaden our knowledge of the disease severity in vulnerable patients. SARS-CoV2 is a respiratory tract infection, however the host cell receptor ACE2 is also highly express in other organs such as blood vessels, the kidneys, the neural cortex and brainstem and gastro intestinal tract. It is important to evaluate the viral replication in these organs in animal experimental model to simulate the human infection. Furthermore, the aberrant systemic inflammatory response 'cytokine storm' is an important feature in COVID-19 patient leading to ARDS.

However, most of the required immunological reagents to study the cytokine and chemokine response to COVID-19 is available mostly for mouse, which limits the use of other animal models to study this aspect of immune arm. We have a long way to go in developing a suitable animal model for SARS-CoV2 and more studies are needed using different animal models as a tool which can provide valuable information in this novel emerging disease and filling the limitations and caveats. In conclusion, hamster is a well-suited animal model to study SARS-CoV-2 infection and pathogenesis. By reproducing pathological and clinical symptoms of pneumonia by virus challenge, recovery of pure virus from the infected tissues, and detecting the rise of SARS-CoV-2-specific neutralizing antibody, hamsters completely satisfy Koch's postulates. Working with hamsters is cost-effective when compare with NHP and Ferret model. This model mimics not only the mild disease phenotype of a COVID-19 patient but also severe conditions when challenged with high-dose of SARS-CoV-2, thus competently fitted for assessing therapeutics or vaccines. However, the model failed to develop acute respiratory distress syndrome (ARDS).

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AUTHOR CONTRIBUTIONS

SS conceived the idea SS and PV wrote the original draft. RK, NY, ZAR, GS and AA edited the manuscript.

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