REVIEW

A Comprehensive Review of Animal Models for Coronaviruses: SARS-CoV-2, SARS-CoV, and MERS-CoV

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Abstract

The recent outbreak of coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has already affected a large population of the world. SARS-CoV-2 belongs to the same family of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). COVID-19 has a complex pathology involving severe acute respiratory infection, hyper-immune response, and coagulopathy. At present, there is no therapeutic drug or vaccine approved for the disease. There is an urgent need for an ideal animal model that can reflect clinical symptoms and underlying etiopathogenesis similar to COVID-19 patients which can be further used for evaluation of underlying mechanisms, potential vaccines, and therapeutic strategies. The current review provides a paramount insight into the available animal models of SARS-CoV-2, SARS-CoV, and MERS-CoV for the management of the diseases.

Keywords Coronaviruses · SARS · MERS · COVID-19 · Animal model

Introduction

In the past two decades, two outbreaks of coronaviruses (CoVs) in Asian subcontinent and the Middle East increased the chances of such outbreak in the near future. The severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak at the end of 2002 (November) in China in which 8,098 confirmed cases were reported with 774 total deaths (9.6%) (Ksiazek *et al.* 2003; WHO 2002). Another coronavirus *i.e.* Middle East respiratory syndrome (MERS) occurred in 2012 with a fatality rate of around 35% (WHO 2019; Bermingham *et al.* 2012). Recently, a new coronavirus called as novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak in December, 2019, in China. There is an emergence of global health threats and the situation became worse at the global level

(Rodriguez-Morales *et al.* 2020). The World Health Organization (WHO) termed the syndrome caused by the novel coronavirus as "COVID-19" on 11th, Feb 2020 (Gralinski and Menachery 2020).

Coronaviruses fall in *Coronaviridae* family and subfamily *Coronavirinae*, the members of which infect a broad range of hosts, producing symptoms and diseases of wide spectrum from a flu like to severe acute respiratory infection (SARI). The SARS-CoV-2 is considered to be one of the seven members of the CoV family that infect humans (Zhu *et al.* 2020). Similar to other coronaviruses, the transmission of SARS-CoV-2 among humans occurs through droplets, direct contact, any physical objects, and aerosols (WHO 2020a; Liu *et al.* 2020). Owing to the rapid transmission, SARS-CoV-2 epidemic has become a global public health challenge. On March 11, COVID-19 outbreak was declared as a pandemic by WHO (Cucinotta and Vanelli 2020). By June 10, more than 7 million people have been globally affected, with nearly 408,025 fatalities (WHO 2020b).

Due to the high morbidity and mortality rate of coronaviruses infection, there is an unmet need for therapeutic strategies to treat these diseases (Sarma *et al.* 2020). Currently, there are no recommended therapeutic drugs or

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vaccine for coronaviruses. This emphasizes the surge for a suitable animal model to explore the pathogenesis and evaluation of countermeasures for the disease. With the outbreak of the coronaviruses, numerous animal models were developed in the past (Gralinski and Baric 2015). The current review highlighted the animal models of coronaviruses, summarized the strength and weaknesses of the existing animal models along with their key features.

Animal Models for SARS-CoV-2

Non-Human Primate Models

SARS-CoV-2 was recently recognized as a novel coronavirus causing the symptoms from mild to critical type (SARI). Munster *et al.* used SARS-CoV-2 infected rhesus macaques to study the pathogenesis of COVID-19. They observed high viral titers in nose swabs, throat swabs, and also lung lesions at varying degrees in all animals (Table 1). This model recapitulates COVID-19 symptoms and can be used further to elucidate the therapy for SARS-CoV-2 infection (Munster *et al.* 2020).

Rockx *et al.* compared the pathogenesis of SARS and MERS with COVID-19 by inoculating cynomolgus macaques with virus infection (Rockx *et al.* 2020). The virus samples were taken from the throat and nose. The diffuse alveolar damage was reported. The results of the study thus demonstrated that the infection with SARS-CoV-2 in non-human primates induced signs that resemble COVID-19 like disease, and the severity of virus infection lies in between the MERS and SARS (Mahase 2020).

Yu *et al.* studied the association of virus infection with age. The viral strain was inoculated in rhesus macaques of 3–5 years old and 15 years old through intra-tracheal route. The clinical signs, for instance, viral replication, and histopathological changes were analyzed. Replication of virus was more in lungs and nasopharyngeal swabs of old rhesus macaque as compared to young rhesus macaque after infection. Old rhesus macaques also observed to have diffuse severe interstitial pneumonia (Yu *et al.* 2020).

In a comparative study, three species of non-human primates were infected with SARS-CoV-2 (rhesus macaque, Macaca fascicularis, and common marmoset). Severe gross lesions and histopathological changes in the vital organs of all animals were observed. The study found that the rhesus macaque model was more susceptible to SARS-CoV-2, as compared to *Macaca fascicularis* and common marmoset (Lu *et al.* 2020).

Woolsey *et al.* determined that African green monkeys supported a high level of SARS-CoV-2 replication and developed a respiratory tract related illnesses that might be more substantial than reported for other non-human primate species like cynomolgus and rhesus macaques. The study also reported high viral loads in feces and in mucosal samples of all monkeys after 15 days post-infection (dpi) (Woolsey *et al.* 2020) (Table 1).

Mouse Models

The inoculation of BALB/c mice with mouse-adapted SARS-CoV-2 at passage 6 (MACSp6) significantly affected all groups of mice irrespective of their ages. The infection resulted in moderate pneumonia and inflammation. A study reported higher viral load in lungs along with significant histopathological changes like denatured trachea, inflammation in pulmonary alveoli, detection of viral antigen in the trachea, bronchiole, in type II pneumocytes. There was a significant elevation of inflammatory chemokine and cytokines in sera and lung macrophages similar to clinical symptoms (Gu *et al.* 2020).

Dinnon *et al.* constructed a recombinant virus (SARS-CoV-2 MA) that could replicate in both upper as well as lower airways of all groups of BALB/c mice irrespective of their ages. The results showed that there was more severe infection in aged mice in comparison to young mice (Dinnon *et al.* 2020).

A study has been done based on the human ACE2 (hACE2) transgenic mice to investigate the pathogenesis of COVID-19. Infected mice reported weight loss and increased viral titer in lungs, and histopathological changes revealed the presence of interstitial pneumonia. The mice model seems to be promising for the evaluation of therapeutic measures for COVID-19 (Bao *et al.* 2020).

One of the studies examined the infectivity and pathological changes in SARS-CoV-2 infected transgenic hACE2 mice, which was developed using lung ciliated epithelial cell-specific HFH4/FOXJ1 promoter 81. The model showed a partial simulation of the human COVID-19. The significant decrease in body weight, interstitial pneumonia, and fatality along with the involvement of other organs like heart, brain, and eye depicted its similarity to the human counterpart. The study also showed protection on reinfection in the survived mice (Jiang *et al.* 2020) (Table 1).

Sun SH *et al.* developed the hACE2 mouse model using CRISPR/Cas9 knock in technology to study the SARS-CoV-2 infection and compared it with the wild type C57BL/6 mice model. There was high viral titer in brain, lungs and trachea of hACE2 mice than wild type mice. SARS-CoV-2 infection in hACE2 mice associated with increased cytokine levels and interstitial pneumonia with no mortality. Moreover, intragastric inoculation of the SARS-CoV-2 infection altered significant pathological changes in lungs when compared to wild type mice (Sun *et al.* 2020b).

Table 1 Animal models of SARS-CoV-2.

Sr. No	Animal species	Disease induction (strain and route)	Clinical signs	Advantages	Disadvantages	References
1	Rhesus macaques	SARS-CoV-2, 2.6×10^6 $TCID_{50}$, IT or IN	Virus shedding in upper, lower respiratory tract and intestinal tract	Useful for pathogenesis, vaccines and therapies studies	Small sample size	Munster et al. (2020)
2	Rhesus macaques	3–5 years old and 15 years old, SARS-CoV-2, 1×10^6 $TCID_{50}$, IT	Severe interstitial pneumonia and significantly viral replication in respiratory tract in old monkeys than young monkeys	Useful for pathogenesis, vaccines and therapies studies	Clinical signs were transient	Yu et al. (2020)
3	Rhesus macaques	SARS-CoV-2, 4.75×10^6 PFU, IT and IN	Increased body temperature, progressive pulmonary infiltration, high levels of viral genome RNA, showed progressively abnormal chest radiograph	Suitable for vaccines and therapeutics studies against SARS-CoV-2	Availability, housing cost	Lu et al. (2020)
4	Macaca fascicularis	SARS-CoV-2, 4.75×10^6 PFU, IT and IN	Progressive pulmonary infiltration, abnormal chest radiograph; swab samples collected on 2 dpi from *M. fascicularis* showed surprisingly high levels of viral genome	Mimic pathogenesis closer to clinical disease	Lower level of viral RNA, costly, limited size availability	Lu et al. (2020)
5	Common marmoset	SARS-CoV-2, 1.0×10^6 PFU, IT and IN	One-third of common marmoset had a slightly elevated body temperature, higher viral load in blood, lower levels of viral RNA were detected in swab samples from *C. jacchus*	–	Not showed severe histopathological changes in lung as pneumonia, relatively resistant to SARS-CoV-2 infection	Lu et al. (2020)
6	African green monkeys	SARS-CoV-2, 5.0×10^5 PFU, IT or IN	Pulmonary consolidation with hemorrhage, pronounced viral pneumonia, release of inflammatory mediators with similar immune signatures as human cases	Considered gold standard model for infectious pathogens	Did not develop overt, debilitating clinical illness; not easy to handle and costly	Woolsey et al. (2020)
7	Cynomolgus monkeys	SARS-CoV-2, 2×10^5 $TCID_{50}$, IT or IN	Diffuse alveolar damage in lungs and viral titer in upper and lower respiratory tract	Viral titer remain for long period and histopathological changes in the lungs	No overt clinical signs	Rockx et al. (2020)
8	Transgenic hACE2 mice	SARS-CoV-2 (HB-01), 10^5 $TCID_{50}$/50 μL, IN	Weight loss and increase in virus replication in the lung and interstitial pneumonia also macrophages accumulation alveolar cavities	Fulfilled Koch's postulates; helpful in development of therapeutics and vaccines	Short supply and high cost of hACE2-transgenic mice; mild inflammatory responses and lung damage	Bao et al. (2020)

Table 1 (continued)

Sr. No	Animal species	Disease induction (strain and route)	Clinical signs	Advantages	Disadvantages	References
9	BALB/c mice	SARS-CoV-2 (MACSp6), 7.2×10^5 PFU, IN	Infected all ages of mice; acute inflammatory responses closely related to the damage of lung tissues; levels of chemokines increased significantly in the aged mice as comparison to younger mice	Easy handling breeding, convenient, economical, and effectively used for evaluation of *in vivo* evaluation of vaccines and therapeutics	Exhibited moderate inflammatory responses	Gu *et al.* (2020)
10	BALB/c mice	10-week old and 12 month-old SARS-CoV-2 MA, 10^5 PFU, IN	Age-related increase in pathogenesis	Useful for pathogenesis, vaccine immunogenicity and therapeutic efficacy studies	–	Dinnon *et al.* (2020)
11	Transgenic hACE2 mice HFH4-hACE2 in C3B6 mice)	SARS-CoV-2, 3×10^4 TCID$_{50}$ (for naïve infection) or 7×10^5 TCID$_{50}$ (for the viral challenge), IN	Weight loss, interstitial pneumonia, lymphopenia, gender susceptibility, viral titer in eye, heart & brain apart from lungs	Partially simulated COVID-19 pathology	LD50 of the model is not determined; lethal encephalitis	Jiang *et al.* (2020)
12	hACE2 mice	SARS-CoV-2 4×10^5 PFU-IN, 4×10^6 PFU-IG	High viral titre in lung, brain and trachea; interstitial pneumonia; increase cytokines levels	Helpful in study of transmission, pathogenesis, evaluating of vaccines and therapeutic efficacy	-	Sun *et al.* 2020b
13	Ad5-hACE2-transduced mice	SARS-CoV-2 1×10^5 PFU-IN	Weight loss, high virus titer in lungs, severe pulmonary pathology	Useful for the study of pathogenesis and testing for antiviral therapeutics and vaccines	Absence of critical condition and extra-pulmonary manifestations of infection	Sun *et al.* 2020a
14	HACE2-transduced mice	SARS-CoV-2 1×10^5 FFU- IN & IV	Weight loss, high viral loads in lung, severe lung pathology	Helpful to study pathogenesis, vaccines and therapeutics	Mouse to mouse variation in expression of hACE2, tissue distribution and mild bronchial	Hassan *et al.* 2020
15	Golden Syrian hamster	SARS-CoV-2, 10^5–10^7 TCID$_{50}$, IN	Rapid breathing, loss of weight, diffuse alveolar damage and high lung viral load was observed	Readily available, physiological, and highly similarity with COVID-19 useful for study of pathogenesis, therapeutics and vaccines	There was a different outcomes in this study as comparison to previous study of SARS-CoV, not tested protein expression only tested mRNA of the hamsters cytokine profiles	Chan *et al.* (2020)
16	Golden Syrian hamster	SARS-CoV-2, Beta-CoV/Hong Kong/VM20001061/2020 virus, 8×10^4 TCID$_{50}$, IN	Weight loss, significant viral replication, transmission of infection via aerosols	Useful for immunological studies for vaccine development	Rapid viral clearance on 7 dpi	Sia *et al.* (2020)
17	Ferrets	SARS-CoV-2, $10^{5.5}$ TCID$_{50}$, IN	Showed increased body temperatures and high virus titers in upper respiratory tracts	Viral infection and transmission	Low viral titer in lungs	Kim *et al.* (2020)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IN, intranasal; IT, intratracheal; IG, intragastric; TCID$_{50}$, 50% Median Tissue Culture Infectious Dose; PFU, Plaque forming units; dpi, day post infection

Sun J *et al.* developed a transgenic mice model by delivery of human ACE2 exogenously with a replication deficient adenovirus (Ad5-hACE2). The inoculation of mice with SARS-CoV-2 infection linked to high viral load in lungs along with weight loss and pneumonia. The role of type-1 interferon, signal transducer and activator of transcription 1 (STAT1) signaling is vital in viral clearance of virus and infection. Further, the study concluded the beneficial role of using human convalescent plasma and two antiviral agents (polyinosinic: polycytidylic acid and Remdesivir) (Sun J *et al.* 2020a).

Hassan *et al.* developed hACE2 transduced mice and infected these mice with SARS-CoV-2. The study observed significant weight loss, high viral load in lungs and severe lung pathology in infected mice (Hassan *et al.* 2020).

Hamster Model

Chan *et al.* observed significant binding of the novel coronavirus spike with the ACE2 receptor of the Syrian Hamster by *in silico* study (Table 1). The viral load significantly increased in the hamster which leads to diffuse alveolar damage in the initial stage and extensive apoptosis in the later phase of infection (Chan *et al.* 2020).

The clinical and histopathological observations from SARS-CoV-2 hamster model closely resemble to the clinical condition. The airway involvement is evident from nasal turbinate to the trachea and pulmonary alveoli, associated with changes of inflammation, cellular viral N protein expression, and high viral load during the first week. The disease progressed with increasing respiratory rate, decreasing activity, and progressive weight loss, and was found to be most severe by 6 dpi, which is similar to the disease course of COVID-19 patients (Huang *et al.* 2020).

Sia *et al.* evaluated the transmission and pathogenesis of SARS-CoV-2 infection in the golden Syrian hamster model. The animal groups infected with SARS-CoV-2 showed significant weight loss, and viral replication in the respiratory tract. Viral load was high on 2 dpi and rapid clearance was observed on 7 dpi, which could be attributed to the presence of CD3 positive T-lymphocytes. The involvement of olfactory sensory neurons simulates the anosmia in COVID-19 cases. The transmission of SARS-CoV-2 to naïve hamsters by aerosols can be helpful for further studies (Sia *et al.* 2020) (Table 1).

Ferrets Model

Kim *et al.* used ferrets to study the SARS-CoV-2 infection and its transmission. The study reported that the ferrets were more susceptible to infection and transmission of the virus from one ferret to naive ferrets by direct or indirect physical contact. They showed that infected ferrets exhibited more replication of the virus in the upper respiratory tract and it recapitulates COVID-19 human conditions and can be further used to evaluate the countermeasures for the disease (Kim *et al.* 2020) (Table 1).

Animal Models for SARS-CoV

Non-Human Primate Models

For the development of SARS-CoV infection model, many species, like old and new world primates, were used. There was significant infection observed in cynomolgus (Fouchier *et al.* 2003) and rhesus macaques (Rowe *et al.* 2004), common marmosets (Greenough *et al.* 2005) and African green monkeys (McAuliffe *et al.* 2004), when the virus was inoculated within respiratory tract in those primates. However, in the case of squirrel monkeys and mustached tamarins, investigators were not able to induce infection (Roberts *et al.* 2008; Subbarao and Roberts 2006).

There was significantly high viral replication in cynomolgus, rhesus, and African green monkeys (McAuliffe *et al.* 2004). While in marmosets, diarrhea, pneumonitis, fever, watery stool, and hepatitis were observed (Greenough *et al.* 2005). Investigators have variable findings as a number of factors may affect the results, including age and animal source, dose, route of administration of infection and inoculation of the virus, and their history of the environment (Subbarao and Roberts 2006).

However, results in out-bred species are not consistent because of biological variability. Therefore, it is important to carry large sample sizes for the study to find out the meaningful conclusions (Table 2).

Mouse Models

There were different mice models used for the study of infection. When BALB/c mice were infected intranasally, there was an increase in viral titer in the lungs but no sign of morbidity or mortality was noticed. After 2 to 3 dpi, virus replication significantly increased in the respiratory tract but started decreasing at day 5 dpi (Subbarao *et al.* 2004). This showed that young BALB/c mice failed to exhibit clinical signs of disease but permitted viral replication. On the other hand, a study showed that one-year-old BALB/c mice exhibited critical infection as compared to younger mice after SARS-CoV infection (Vogel *et al.* 2007). Histopathological findings such as bronchiolitis, diffuse alveolar damage, and patchy interstitial pneumonitis were observed in BALB/c mice model of SARS-CoV. It was also observed that old age BALB/C mice were more prone to this disease as similar to the clinical

Table 2 Animal models of SARS-CoV.

Sr. No	Animal model	Disease induction (strain & route)	Clinical signs	Advantages	Disadvantages	References
1	Rhesus macaques	SARS-CoV, Tor2, 10^7 PFU, IV/IT	Significant viral titer in the lungs	Useful for therapeutic evaluation and vaccines immunogenicity	In SARS study limited use	Rowe *et al.* (2004)
2	Rhesus macaques	SARS-CoV, Urbani, 10^6 PFU, IT/ IN	Could not show any clinical sign	Used for Therapeutic evaluation and vaccines immunogenicity	Clinical illness was not present	McAuliffea *et al.* (2004)
3	Rhesus macaques	SARS-CoV, PUMC01, 10^5 PFU, IN	Pulmonary changes were observed on 5–60 dpi; All macaques reported fever 2–3 dpi	Immunological and pathological similarity with clinical condition	The symptoms are less severe as comparison of clinical scenario	Qin *et al.* (2005)
4	Cynomolgus monkeys	SARS-CoV, 10^3 and 10^6 $TCID_{50}$, IN/ IT	Interstitial pneumonia, alveolar macrophages and neutrophils, diffuse alveolar damage	Helpful for the study of SARS pathogenesis and can be used for therapeutic and vaccine studies	Availability and cost	Fouchier *et al.* (2003)
5	Cynomolgus monkeys	SARS-CoV, 1×10^6 $TCID_{50}$, IT/ IN,	Symptoms appeared as difficulty in respiration; more diffuse alveolar damage, type 2 pneumocyte hyperplasia and alveolar macrophages are present in alveolar lumina	Useful for vaccine and therapeutic drug evaluation	There is issue with availability, housing cost; early clearance of virus and pneumonitis occurred	Kuiken *et al.* (2003)
6	Cynomolgus monkeys	SARS-CoV, Tor2, 10^7 PFU, IT	Mild cough; a few scattered pleural adhesions	Presentation of critical disease	Symptoms cleared quickly and animals becomes asymptomatic from 8 to 10 days	Rowe *et al.* (2004)
7	Cynomolgus monkeys	SARS-CoV, Urbani, $3 \times 10^{6.3}$ PFU, IB/ IN,	Reported nasal congestion, mild respiration distress; animals become lethargic; pulmonary related disease	Helpful in immunogenicity of vaccines and therapeutics studies	Lack of apparent clinical illness	McAuliffe *et al.* (2004)
8	African green monkey	SARS-CoV, Urbani, $10^{6.3}$ $TCID_{50}$, IB /IN,	Virologic data and histopathologic findings, focal interstitial pneumonitis was noticed in some African green monkey	Useful for evaluation of vaccine efficacy against infection	Rapid clearance of virus and pneumonitis in African green monkey	McAuliffe *et al.* (2004)
9	Marmoset	SARS-CoV, Urbani, 10^6 PFU, IT	Marmoset reported there is elevation of temperature, interstitial pneumonitis, multifocal lymphocytic hepatitis and diffuse interstitial colitis	Can be used in Pathogenesis, therapeutics and vaccine efficacy studies	This model is not able to explain the viral antigen/viral RNA within hepatic tissues	Greenough *et al.* (2005)
10	BALB/c mice	4–6 week aged SARS-CoV, Urbani, 10^5 $TCID_{50}$, IN	High viral load in respiratory tract	Useful for immunological studies	No overt clinical sign was present	Subbarao *et al.* (2004)
11	BALB/c mice	12–14 month aged SARS-CoV, Urbani, 10^5 $TCID_{50}$, IN	Old aged mice observed significant loss of weight, mild dehydration and alveolar damage; Also reported intra-alveolar edema, perivascular infiltrates	Useful for vaccine evaluation	There is need of further characterization and immune senescence	Vogel *et al.* (2007)

Table 2 (continued)

Sr. No	Animal model	Disease induction (strain & route)	Clinical signs	Advantages	Disadvantages	References
12	129SvEv / STAT 1-/- mice	SARS-CoV, Tor2, 6×10^6 PFU/30 µL, IN	Viral replication, morbidity, mortality and pneumonitis	STAT 1-/- exhibited innate immunity	Defect in innate immunity	Hogan et al. (2004)
13	129SvEv / STAT 1-/- mice	SARS-CoV, Urbani, 10^5 PFU, IN	Reported severity of disease in this study, high virus replication and severe pulmonary lesions	Viral replication, histopathology similar to clinical conditions	Lack of a normal innate immune did not enhance virus pathogenesis	Frieman et al. (2010)
14	C57BL/6 mice	SARS-CoV, Urbani 1×10^4 $TCID_{50}$, IN	Virus infected the respiratory tract (bronchial and bronchiolar epithelium); failure to thrive and also observed infection reached to the brain	Useful for immunological studies	Fail to show clinical signs of disease	Glass et al. (2004)
15	Transgenic hACE2 mice	SARS-CoV, PUMC01 10^5 $TCID_{50}$, IN	Transgenic mice showed severe lung damage, systemic inflammatory reactions, degeneration, and necrosis in many extra-pulmonary organs	More susceptible to infection as comparison to wild type mice, and more closely resemblance to human pathology of SARS	Tissue distribution was limited, decreased expression of hACE2 and lack of lethality	Yang et al. (2007)
16	Golden Syrian hamster	5-week aged, SARS-CoV, Urbani, 10^3 $TCID_{50}$, IN	Increased viral replication in lungs, related interstitial pneumonitis, diffuse alveolar damage	Reproducibility, increased viral replication in lungs which makes it suitable for immunoprophylaxis and immunotherapy and vaccines studies	As virus is cleared rapidly from 7 to 10 dpi, there was no overt clinical illness	Roberts et al. (2005)
17	Ferret and domestic cat	SARS-CoV, 10^6 $TCID_{50}$, IT	Lethargy and mortality in ferrets; histopathological changes including pulmonary consolidation was reported in both animals	Useful for vaccines, immunotherapy and therapeutic studies	Availability; vulnerability to other respiratory viruses	Martina et al. (2003)

SARS, Severe acute respiratory syndrome; IN, intranasal inoculation; IT, intratracheal inoculation; IV, intravenous inoculation; IB, intra-bronchial inoculation; $TCID_{50}$, 50% Median Tissue Culture Infectious Dose; PFU, Plaque forming units.

symptoms observed in elderly people in the 2003 SARS outbreak.

One of the studies suggested that 129S mice infected with SARS-CoV exhibited virus replication and self-limited bronchiolitis. 129S mice strain also reported mild weight loss and pneumonitis after SARS-CoV infection. While on the other side, STAT 1 -/- mice in the 129S background observed sustained replication of the virus and histopathological changes, which were mimicking the human's disease. However, vaccine studies are limited where targeted mice have immune defects (Hogan et al. 2004; Frieman et al. 2010).

Moreover, C57BL/6 mice were also used as a SARS-CoV model. Virus isolates administered intranasal into C57BL/6 mice showed high replication in the respiratory tract of mice with a peak on the 3rd day and clearance on the 9 dpi. The study observed the presence of transient systemic infection in the lungs which ultimately affects the brain (Glass et al. 2004) (Table 2).

Hamster Model

Another model used to study the SARS-CoV infection is a golden Syrian hamster. There was a significantly high titer of viruses in the lungs along with interstitial pneumonitis (Roberts et al. 2005). Because of the clinical signs like pulmonary histopathology and high virus load, the hamster can be used as a model for the study of therapeutics, immunotherapy, and immunoprophylaxis. It was observed that viruses got cleared off by 7dpi. (Table 2).

Table 3 Animal models of MERS-CoV.

Sr. No	Animal model	Disease induction (strain and route)	Clinical Sign	Advantage	Disadvantage	References
1	Dromedary camels	HCoV-EMC/2012, 1×10^7 TCID$_{50}$, IN, IT, CON	Rhinorrhea; mild elevation in body temperature; nasal discharge; mild to moderate acute intraepithelial & sub-mucosal inflammation	Reservoir source of the MERS-CoV	Mild clinical symptoms	Adney *et al.* (2014)
2	Alpacas	Dromedary MERS-CoV Al-Hasa_KFU-HKU13/2013, 1×10^6 TCID$_{50}$, Oronasal	Positive deep nasal swab at 10 dpi	Alpacas as a potential substitute for camels	Small sample size; insufficient observation period of 21 days before rechallenge; mechanism not evaluated;	Crameri *et al.* (2016)
3	Rhesus macaque	HCoV-EMC/2012, 7×10^6 TCID$_{50}$, IT, IN, oral, and ocular routes	Virus shedding via nose, transient LRT infection	Susceptible to infection	Small sample size; transient model; no mortality	de Wit *et al.* (2013a, b)
4	Rhesus macaques	hCoV-EMC, 6.5×10^7 TCID$_{50}$, IT	Increased temperature on 1–2 dpi; multifocal mild-to-moderate interstitial pneumonia; types I and II pneumocytes and alveolar macrophages contain infection	Susceptible to infection	No detectable virus titers	Yao *et al.* (2014)
5	Rhesus monkeys	icMERS-0, 5×10^6 PFU, IT	Lung hyperdensity changes, minimal-to-mild interstitial pneumonia; transient, mild pulmonary pathology	High replication of icMERS-0	Mild infection	Cockrell *et al.* (2018)
6	Rhesus macaques (immunosupressive)	EMC/2012, 7×10^6 TCID$_{50}$, ocular	High viral shedding; mild pulmonary pathology	Immunopathogenic component	Use of immunosuppressive drug is not clinically relevant; immunosuppressive group is not challenged;	Prescott *et al.* (2018)
7	Common Marmoset	HCoV-EMC/2012, 4×10^6 TCID$_{50}$, IN, IT and ocular	Loss of appetite; decreased levels of activity; progressive severe pneumonia; extensive lesions in the lungs; severe, partially lethal disease model	Severe; longer duration; high viral loads in lungs; high viral load both in lower respiratory tract and in blood	Small sample size; lack of additional control animals; subjects euthanized before the disease course could resolve	Falzarano *et al.* (2014)
8	Common Marmoset	MERS-CoV-Jordan-n3/2012, 5×10^7 PFU, IT; MERS-CoV-EMC/2012, 5×10^7 PFU, IT	Respiratory rate increased; interstitial multifocal to coalescing moderate pneumonia	–	No systemic clinical symptoms; limited clinical signs;	Johnson *et al.* (2015)

Table 3 (continued)

Sr. No	Animal model	Disease induction (strain and route)	Clinical Sign	Advantage	Disadvantage	References
9	Yorkshire Landrace pigs	MERS-CoV, 1×10^7 TCID$_{50}$, IN	Viral RNA detected till 7 dpi	Inexpensive and easy to obtain	No animal to animal transmission	Vergara-Alert *et al.* (2017)
10	New Zealand white rabbits	EMC/2012, 1×10^3 TCID$_{50}$, or 1×10^5 TCID$_{50}$, IV	No clinical sign even on exposure to re-infection;	Inexpensive and easy to obtain	Studied asymptomatic infection, non-lethal infections, transient dose-dependent pulmonary infection which is not associated with clinical symptoms	Houser *et al.* (2017)
11	Transgenic mice (Ad5-hDPP4)	EMC-2012, 1×10^5 PFU, IN	Weight loss, virus replication in respiratory tract, interstitial pneumonia	Useful in screening of therapeutics and vaccines efficacy; Efficient and rapid generation of the model within 2–3 weeks; can be used in genetically deficient mice	Level of expression, tissue distribution	Zhao *et al.* (2014)
12	Trangenic C57BL/6 J mice(hCD26/DPP4) CAGG enhancer/promoter, intron sequence, human CD26 cDNA, and rabbit beta globin poly(A) signal	EMC-2012, 1×10^6 TCID$_{50}$, IN	Increase virus titers in lungs and brains; progressive pneumonia; characterized by extensive; significant mortality	Susceptible to the infection	Severe morbidity and mortality; hinders investigations of underlying mechanism	Agrawal *et al.* (2015)
13	Swiss Webstar mice (hDPP4) (VelociGene technology)	MERS-CoV- Hu/Jordan-N3/2012, 2×10^5 PFU, IN	Mild–moderate peribronchiolar & alveolar inflammation	Rapid mouse model production; no prior transduction is required;	No cerebral inflammation	Pascal *et al.* (2015)
14	Trangenic C57BL/6 J mice(hDPP4 model) (hCD26/DPP4) CAGG enhancer/promoter, intron sequence, human CD26 cDNA, and rabbit beta globin poly(A) signal	EMC-2012, 1×10^6 TCID$_{50}$, IN	Persistent inflammatory infiltrates in the lungs; focal infiltrates in brain & liver	Fully permissive to viral infection	Severe morbidity and mortality; hinders investigations of underlying mechanism	Tao *et al.* (2015)
15	Transgenic C57BL/6 mouse (hDPP4) model (purified 5861 bp fragment generated from ApaL1 digestion of pCAGGS-hDPP4)	HCoV-EMC/2012 strain, $1 \times 10^{4.3}$ TCID$_{50}$, IN	Decreased activity; significant weight loss; mortality	Mimic severe MERS pathology	Underlying mechanism; mechanism of lethal which was of aberrant inflammatory response was not studied	Zhao *et al.* (2015)
16	Transgenic C57BL/6 J mice (CRISPR–Cas9 gene editing (homozygous & heterozygous strain))	HCoV-EMC/2012, Camel MERS, icMERS, MERS-0 at 5×10^5 PFU, IN; MERS-15 at 5×10^6 PFU, IN	Mortality and haemorrhage at 6 dpi; 25%–30% weight loss; diffuse alveolar damage and severe respiratory disease; decreased pulmonary function as measured by plethysmography	No confounding effects of CNS infection on mortality	Use of high viral load	Cockrell *et al.* (2016)

Table 3 (continued)

Sr. No	Animal model	Disease induction (strain and route)	Clinical Sign	Advantage	Disadvantage	References
17	Transgenic C57BL/6 mice (hDPP4-KI mice, mouse Dpp4 exons 10–12 replaced with the human DPP4 codons)	Plaque-purified $MERS_{MA}$, 1×10^4 to 1×10^6 PFU, IN	Diffuse alveolar damage; innate immune responses to infection; leukocyte response to infection	Model does not require prior sensitization	Pathological changes associated with species specific $MERS_{MA}$ mutation	Li *et al.* (2017)
18	Transgenic C57BL/6 mouse (R26-hDPP4 mice, insert hDPP4 into the Rosa26 locus using CRISPR/Cas9)	hCoV-EMC, 1.5×10^5 PFU, IN	Severe ARDS; viral titer in CNS	Specificity of the infection in lungs & in CNS	Not severe or lethal	Fan *et al.* (2018)
19	Transgenic mice on a C57BL/6 background express hDPP4	HCoV-EMC 2012 strain, 1×10^5 $TCID_{50}$, IN	Viral titer in lungs	Age related immunopathology	No severe disease and lethal infection, no nasal and brain inflammation; mild infection;	Iwata-Yoshikawa *et al.* (2019)

MERS, Middle east respiratory syndrome; IN, intranasal; IT, intratracheal; CON, Conjuctivital; $TCID_{50}$, 50% Median Tissue Culture Infectious Dose; PFU, Plaque forming units; LRT, lower respiratory tract; ARDS, acute respiratory distress syndrome; CNS, central nervous system; dpi, day post infection.

Ferrets and Domestic Cat Model

Various studies showed that domestic cats and ferrets are prone to SARS-CoV infection because virus replication, as well as specific antibodies, were found in both the species. After indicating drowsiness and epiphysitis 16 to 21 dpi, ferrets died (Martina *et al.* 2003). However, with domestic cats, no further study has been conducted. After the inoculation of infection via intranasal route, the replication of the virus in the lungs and histological changes like pneumonitis were reported (ter Meulen *et al.* 2004) (Table 2).

Animal Models of MERS-CoV

There is a difference in the homology of human and small animals DPP4 molecules which is the main virus receptor of MERS-CoV. Therefore, mice, guinea pig, and ferrets are not usually permissive to the MERS-CoV (Cockrell *et al.* 2014; Coleman *et al.* 2014; de Wit *et al.* 2013a, b; Raj *et al.* 2013). However, non-human primates, rabbits, pigs, and dromedary camels are naturally permissive for the virus.

Dromedary Camel Model

Adney *et al.* studied the pathology and transmission of MERS-CoV among dromedary camels. The animals displayed slight increases in body temperature and rhinorrhea. A large quantity of nasal shedding of the virus is suggestive of an animal-to-animal and animal-to-human transmission which may occur through droplets or direct contact.

MERS-CoV mainly affects the upper respiratory tract and mimics mild clinical signs. Since in clinical settings, the disease shows severe pathology, so camel as a model to study therapeutics is not suitable (Adney *et al.* 2014) (Table 3).

Alpaca Model

MERS-CoV is classified under Biosafety Level 3 which poses difficulty in using camels for infection study. Alpaca, belonging to the *Camelidae* family, provides an alternative to camels. Crameri *et al.* used alpaca as a prospective substitute to a camel for MERS-CoV vaccine testing (Crameri *et al.* 2016). On exposure to MERS-CoV, the study observed dissimilarity between the immune response of two species. However, the pathogenesis of infection in alpaca is unknown (Table 3).

Non-Human Primate Models

de Wit *et al.* used rhesus macaques to develop the MERS model. There was high viral load of MERS-CoV in the lower respiratory tract. The viral strain mainly affected type I and II pneumocytes that formed the architecture of the alveolar space. Since high expression of MERS-CoV was present in the lungs, therefore, there was limited virus shedding. There was no viral replication in the kidney. The study concluded that renal failure in humans might be associated with hypoxia but not with viral dissemination. Due to its transitory nature, this model of rhesus macaques was not found to be exactly mimicking the critical clinical

cases (de Wit *et al.* 2013a). Similarly, Yao *et al.* observed a mild increase in temperature in rhesus monkeys. There was no virus detected in the upper respiratory tract. Significant pneumonia was observed with no systemic dissemination of the virus. The study concluded only lower respiratory infection with no extra-pulmonary effect (Yao *et al.* 2014).

Human exposure to MERS-CoV leads to severe respiratory disease which might result in fatality and it is not easy to attain the same fatality rate in non-human primate models. Cockrell *et al.* used an infectious clone of novel MERS-CoV (icMERS-0) to induce MERS in the rhesus macaque. The clone strain was found to have higher pathogenicity in comparison to its wild type. MERS-CoV peaked at 3 to 5 dpi and resolved to 30 dpi. The respiratory disease was found to be placid and brief which resolves by day 30 dpi. The model mimics only mild infection of MERS in humans (Cockrell *et al.* 2018).

In most of the cases, fatality is associated with comorbidities in MERS affected patients (Hui *et al.* 2014; Saad *et al.* 2014) and severity is linked to the immunocompromised state. In order to study the immune response and disease severity, Prescott *et al.* used the immunosuppressive drugs to down-regulate the immunity of rhesus macaques. The immunosuppressive state was found to be associated with a high titer of MERS-CoV but not supported by the pathology. In lung tissues, inflammatory cells significantly decreased in immunocompromised animals when evaluated histopathologically. This, in turn, shows the effect of the immune status of an individual on the shedding of the viral load. This study depicted the importance of considering the immune response along with using therapy to control infection in clinical settings (Prescott *et al.* 2018) (Table 3).

Marmosets are more prone to MERS-CoV infection than the rhesus macaques and it might be suitable to screen therapeutic strategies for the disease in marmoset (Johnson *et al.* 2015; Falzarano *et al.* 2014). The study by Falzarano *et al.* displayed an increase in severity following exposure to MERS-CoV in marmoset than rhesus macaque. The titer was almost 1000 times greater in marmoset than in rhesus macaque. The infection persisted for a longer duration as compared in rhesus macaque and need euthanasia. In fact, there was a systemic dissemination of the virus. But there was no viral dissemination in the kidney. The study observed that the marmoset model recapitulates more closely to human MERS (Falzarano *et al.* 2014).

Johnson *et al.* used two clones of MERS-CoV *i.e.* MERS-CoV-Jordan-n3/2012 and MERS-CoV-EMC/2012. The inoculation of MERS-CoV into common marmosets resulted in mild to moderate clinical presentation of the disease which might be due to the manipulations of the marmoset to a certain extent (Johnson *et al.* 2015) (Table 3).

Yorkshire Landrace Pig Model

Pigs are naturally permissive to the MERS-CoV infection (de Wit *et al.* 2017). Vergara-Alert *et al.* used HCoVEMC/2012 to infect Yorkshire Landrace pigs. The study observed no animal-to-animal transmission though RNA dissemination of the virus was significantly high. Thus, this model cannot be used to study therapeutic strategies as it does not depict the true clinical condition (Vergara-Alert *et al.* 2017) (Table 3).

New Zealand White Rabbit Model

Houser *et al.* studied the effect of re-infection in the rabbits. The model supported the replication of MERS-CoV-EMC/2012 virus. The model displayed asymptomatic infection which is not associated with any significant clinical sign. The model is not suitable for preclinical screening of therapeutics or vaccines for MERS (Houser *et al.* 2017) (Table 3).

Mouse Models

Mice are easily available, cost-effective, easy to handle, and suitable for initial screening of drug therapies and vaccines. Since, mice are not susceptible to MERS-CoV infection (de Wit *et al.* 2013b; Coleman *et al.* 2014), therefore Zhao *et al.* developed a mouse model for MERS using a recombinant adenovirus that expresses humanized dipeptidyl peptidase-4 (hDPP4) receptor (Zhao *et al.* 2014). The study concluded significant weight loss and virus dissemination in the respiratory tract. Further interstitial pneumonia was observed in the mice. The transient hDPP4 expression, mild respiratory system involvement with no fatality are the drawbacks of the model (Zhao *et al.* 2014).

Though the previous study used an adenovirus vector to develop the DPP4 receptor in mice vulnerable to MERS-CoV infection, it did not mimic more closely to the clinical conditions. Agrawal *et al.* used tissue-specific and inducible promoters, to derive humanized DPP4 transgenic mice. On exposure to MERS-CoV, the model showed high susceptibility to the virus (Agrawal *et al.* 2015). Briefly, the transgenic model depicted progressive pneumonia with fatality.

Agrawal *et al.* developed a hCD26/DPP4 transgenic mice and the receptors were distributed throughout the body which was not physiologically relevant (Agrawal *et al.* 2015). Pascal *et al.* used Veloci Gene technology to rapidly develop a new humanized model. The model showed active viral replication in the respiratory tract without cerebral infection (Pascal *et al.* 2015). Tao *et al.* observed constant inflammatory infiltrates in both brain

and lungs in humanized CD26 (hCD26)/DPP4 mice. The high viral titer was observed in the lungs and in the brain of mice on exposure to MERS-CoV infection. The pathological examination found gross and microscopic inflammation which was not restricted to lungs but a sign of systemic dissemination along with fatality in 4–6 dpi (Tao *et al.* 2015).

Tao *et al.* showed acute severe infection which hampers the opportunity to fully understand the pathogenesis. Zhao *et al.* developed a mouse model that exhibits the codon-optimized- hDPP4 receptor which on exposure to viral strain showed severe disease pathology in lungs which further affected the brain and kidney. The severe infection and aberrant immune response lead to mortality. The exact mechanism of the severity of the disease model requires further evaluation (Zhao *et al.* 2015).

Cockrell *et al.* used CRISPR–Cas9 technology to genetically alter a non-permissive host receptor. The model showed severe respiratory distress and fatality response to MERS-CoV. This was further supported by plethysmography that showed decreased pulmonary function which might be due to the inflammation in pneumocytes and airway epithelial cells. The viral titers were not detected in the brain thereby nullifying any confounding factors which could lead to fatality (Cockrell *et al.* 2016).

Li *et al.* developed transgenic mice, and inoculated with mouse adapted MERS-CoV (MERS$_{MA}$) viral strain which caused weight loss, infection in airway epithelia, pneumocytes, and macrophages. Though the model was rapid to develop, required no prior sensitization, the pathological changes were exclusively associated with the species-adapted mutations of the MERS$_{MA}$ clone. This hinders the model to fully mimic clinical conditions (Li *et al.* 2017).

Fan *et al.* used CRISPR/Cas9 gene-editing technology to develop a knock-in-model of mice. The study displayed a high level of expression of hDPP4 in the respiratory tract and to a lesser extent in the brain. There is a clear site of infection along with acute respiratory distress syndrome (ARDS) in the lungs. There is also the dissemination of viruses into the central nervous system (CNS). The study observed high repeatability and the safety of the model (Fan *et al.* 2018).

Iwata-Yoshikawa *et al.* developed a transgenic mouse model using the endogenous promoter. The hDPP4 mice model showed a high viral titer in the lower respiratory tract. Acute multifocal interstitial pneumonia was observed at 7 dpi. The viral titer was also observed in peripheral blood and lymphoid tissues. There was an age-associated immune response to exposure to the viral strain. The model expressed mild infection pathology. Moreover, after the infection, this model had not shown any signs of lesions in the brain and kidney (Iwata-Yoshikawa *et al.* 2019) (Table 3).

Conclusions

1. The coronaviruses pathology is associated with the involvement of severe acute respiratory infection (SARI) and immune deregulation, thus, it is requisite to perform a study using an assay system that must involve all the cell signalling and thus, using animal model is an unparallel approach.

2. There are some animal models that recapitulate mild to moderate pathology, however, the clinical condition is related to high mortality and morbidity. Therefore, animal models which mimic critical pathologies are much closer to clinical conditions.

3. The novel coronavirus (COVID-19) pathology is linked to viral respiratory infection, hyper-immune response, and coagulopathy (Lin *et al.* 2020; Connors and Levy 2020), therefore, to understand the mechanism or to evaluate therapeutic countermeasures, the animal models should involve all these interplays in a single model. Since with the advent of technology, transgenic mice are the significant tool but the transgenic mice lack the coagulopathy component which is indispensable in order to understand the complete mechanism and to evaluate the countermeasures.

4. Further, validation of the animal model is crucial. The error in the animal experimental study narrows the chances of the potential drugs or repurposing or repositioning drugs or vaccines to translate successfully to clinics and moreover, it is a wastage of resources. Thus, it is the need of the hour to validate the animal model using different criteria, for instance, face, construct, and predictive validity (Denayer *et al.* 2014).

Compliance with Ethical Standards

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