The Pursuit of Covid-19 Animal Models

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a contagious disease instigated by a recently discovered coronavirus, the 2019 novel coronavirus (2019-nCoV). It has infected over 14 million people worldwide from all age groups. This virus is conveyed through big droplets produced during coughing and sneezing by the infected individuals. COVID-19 precipitates acute respiratory distress syndrome which leads to morbidity and mortality in severe cases. To date, there is neither an established specific antiviral treatment for COVID-19, nor vaccine available. Preventive measure of social distancing is deemed to be the most effective way to avoid the spread of the virus. Scientists are currently in search of appropriate COVID-19 animal models for use in research works related to pathogenesis, potential drug treatments and vaccine development. This review delivers a pithy overview of the currently available COVID-19 animal models published in the literatures.

KEYWORDS: COVID-19, animal models

INTRODUCTION

Coronaviruses are pleomorphic enveloped positive sense RNA viruses that are a genus in the *Coronaviridae* family and subfamily *Coronavirinae*.[1,2] They range from 60 nm to 140 nm in diameter with spike-like projections on the surface (crown-like), from which the name coronavirus is derived.[3] They cause infections in both humans and animals, and are commonly associated with self-limiting disease.[4] However, severe acute respiratory diseases are known to occur with zoonotic coronaviruses infections which include the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Most recently discovered is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or also known as the 2019 novel coronavirus (2019-nCoV) causing the coronavirus disease 2019 (COVID-19).[5-7]

The outbreaks of SARS-CoV in 2002 occurred in more than 30 countries with over 8098 cases and 774 deaths whilst those involving MERS-CoV in 2012 had caused 779 deaths of the 2182 infected cases identified in 27 countries.[8] In contrast, the current SARS-CoV-2 infection is more widespread, involving over 215 countries and territories with over 14 million cases and approximately over 613,000 deaths as per 21st of July 2020.[9] Although the SARS-CoV-2 infection demonstrates a lower rate of austere cases and case fatality compared to both SARS-CoV and MERS-CoV,[8] it has a strikingly higher infectivity making it an appalling medical and social burden. Hence on the 11th March 2020, the World Health Organization (WHO) declared it as a worldwide pandemic.[10]

The SARS-CoV-2 infection (COVID-19) was first diagnosed in Wuhan City of Hubei Province, China[11] and subsequently led to widespread infection in Italy.[12] Italian scientists had earlier discovered traces of SARS-CoV-2 virus in the sewage system that were collected in Milan and Turin, although this did not imply that the main transmission chain that led to the development of the epidemic in Italy originated from these findings.[13]

The pathogenesis of COVID-19 is initiated by the receptor-mediated entry of SARS-CoV-2 into the host cells, where the viral S protein receptor binding domain (RBD) of the SARS-CoV-2 binds to the human angiotensin converting enzyme 2 (ACE2) receptor or dipeptidyl peptidase 4 (DPP4) protein receptor.[14]

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Within the cells, the virus is able to reproduce efficiently, mounting the cytopathogenesis and initiates the infection.[8] The clinical features of COVID-19 range from an asymptomatic state to acute respiratory distress syndrome (ARDS) and multiple organ failure.[15] Pneumonia, respiratory failure and death can occur in some patients by the end of the first week of infection.[15,16] This is strongly associated with increase in a variety of inflammatory cytokines.[16] Throat swab, nasopharyngeal swab, sputum, endotracheal aspirates and bronchoalveolar lavage are among the respiratory samples used to make the diagnosis.[15,17] The reverse transcription polymerase chain reaction (RT-PCR) is the current standard diagnostic test for COVID-19.[18]

COVID-19 Animal models

Animal models have been used extensively in biological and biomedical science research to address various pertinent scientific questions for epochs.[19] They are critical to the understanding of disease pathogenesis at molecular, cellular and physiological levels, development and assessment of novel treatments, new surgical techniques and also anaesthesia protocols.[19,20] The findings in animal models played a strikingly important role that have led to a robust input in scientific knowledge and enhancement in life quality.[19] Many Nobel prize winners in the field of Physiology and Medicine have used animal experimentation models for their innovations.[19]

In relation to the current pandemic, among the main focus areas of research where animal models are extremely essential include the study of the viral infection and its pathogenesis which are closely intertwined with development and preclinical evaluation of a vaccine and antiviral drug. The availability of animal models that could provide consistent and reproducible results cannot be overemphasised in these circumstances.[8]

An animal model namely for the study of infectious diseases initiated by viruses has to fulfil a variety of characteristics, the first of which is that the animal model should mimic the human disease under investigation in terms of sharing the route of infection, severity of disease, typical clinical symptoms, host immune responses and has comparable levels of mortality and morbidity.[8,21]

Generally and also specific to SARS-CoV-2 infection, the viral entry and intracellular replication are critical in establishing a successful infection in an animal model[8] and there should be a correlation between the viral load and disease severity.[21] Additionally the viral receptors and their distribution ought to be similar to that of humans.[21] The species-specificity barrier is therefore an important criteria to be considered in selecting animal model for coronaviruses.[8] For example, in mouse model it was found that the mouse dipeptidyl peptidase 4 (DPP4) protein receptors that is critical for the binding of viral S protein differs from that of the human ACE2 or DPP4 receptor.[22] To overcome this problem, the human ACE2 expression is induced experimentally by knocking in or transfecting a human receptor gene into the mouse model, termed as humanized transgenic mouse model.[23] In essence, the animal models must be judiciously assessed and selected to achieve the experimental targets.[21]

This review article provides a pithy overview of the most promising COVID-19 animal models based on their pathogenesis, histological findings, clinical symptoms similarities, the pros and cons of each model. This article will optimistically serve as a guide for scientists to choose the prime model for their research purposes.

Ferret

Ferrets are a well-known model for respiratory infections because of the similarity in their lung physiology to that of humans, where it can cough and sneeze.[24-27] Ferrets infected with SARS-CoV-2 intranasally, showed viral replication limited to the nasal turbinate, soft palate and tonsils by day 4 post inoculation while no replication was detected in the lung lobes even after intratracheal inoculation of the virus.[25] The viral RNA was also detected in the rectal swabs in smaller amount.[25] SARS-CoV-2 gains entry into human body through ACE2 receptors expressed in the lungs, heart, kidneys and gastrointestinal tract,[28-32] whilst in ferrets these receptors are found mainly on the type II pneumocytes and serous epithelial cells of the trachea-bronchial submucosal glands.[33] As such it was concluded that the SARS-CoV-2 virus replicates more efficiently in the upper respiratory tract of ferrets and not in other organs.[25]

The SARS-CoV-2 virus infection in ferrets only increased their body temperature but other

characteristic symptoms were absent. The virus cannot replicate to high levels,[34] making the aforementioned factors as main disadvantages of this animal model.[35] The infected ferrets however could spread the virus with a high transmission rate to adjacent cages,[34] making them an excellent animal model for the study of transmission of SARS-CoV-2[36] and also for antiviral drug treatment and vaccine research works.[25]

Cat

In an experimental study that used this animal model, it was reported that sub-adult cats aged 6 to 9 months which were inoculated with SARS-CoV-2 via intranasal route, had viral replication in the nasal turbinate, soft palates, tonsils, tracheas, lungs, and small intestine at day 3 post inoculation. The viral RNA was also found in the faeces of these animals on days 3 and 5 post inoculation. An uninfected cat that was caged separately adjacent to the infected cat was eventually infected with the virus. This supports the airborne transmission route of this infection.[25]

Scientific studies on transmission of the disease were also carried out in juvenile cats aged between 70 to 100 days old.[25] Massive lesions were observed in the nasal and mucosal epithelium of tracheas and lungs of the cats following intranasal inoculation of SARS-CoV-2. This result indicates that the SARS-CoV-2 virus replicates efficiently in cats, especially the juvenile cats being more permissive via the airborne route.[25] However, the epidemiological data in human shows that the elderly people are more vulnerable to SARS-CoV-2 infection and had higher mortality rate.[37]

Cats may provide an expedient model system in studying the transmissibility of the virus.[38] As most cats in Wuhan have been disclosed to be seropositive for SARS-CoV-2,[39] the surveillance of SARS-CoV-2 in cats ought to be further explored in the efforts to contain COVID-19 in humans.[25]

Dog

The replication and conveyance of SARS-CoV-2 were examined in five 3-month old beagles inoculated intranasally with the virus. They were housed together with two uninoculated beagles in a room. The oropharyngeal and rectal swabs were taken post inoculation every 2 days for two weeks. The viral RNA was detected from the rectal swabs on days 2 and 4

post inoculation. The viral RNA was absent in all organs and tissues of the dogs. In the animals that were not inoculated, all swab results were negative. The SARS-CoV-2 antibody tests by enzyme immunoassay were also negative in the contact dogs. These results suggest that dogs have low susceptibility to infection by SARS-CoV-2.[25]

Livestock animals

Similar experimental studies performed in dogs were carried out in livestock animals such as chickens, ducks and pigs.[25] The viral RNA was not noticed in any of the swabs from both the virally-inoculated and uninoculated animals. All animals were seronegative for SARS-CoV-2 on day 14 post inoculation.[25] These animals are therefore not susceptible to SARS-CoV-2 infection.[8,25] There are no reported investigations of SARS-CoV-2 thus far in rabbits.[8]

Non-human primate (NHP)

Cynomolgus macaque

Cynomolgus macaque is one of the most commonly used NHPs in biomedical research due to its genome being highly homologous to human and the relative ease of carrying out experiments with this animal model. Published findings have shown that cynomolgus macaques are susceptible to SARS-CoV-2 infection, have a prolonged period of viral shedding and exhibit COVID-19-like disease. It has also been proven that SARS-CoV-2 reproduces efficiently in the epithelial cells throughout the respiratory tract. The replication in the upper respiratory tract fits with efficient transmission between hosts, while the replication in the lower respiratory tract fits with the progression of lung disease.[40]

In an experimental study, young (aged 4-5 years) and old adults cynomolgus macaques (15-20 years of age) were inoculated with SARS-CoV-2 strain via a combination of intratracheal and intranasal routes.[40] The viral RNA was detected to peak by days 2 and 4 post inoculation in the young and aged animals respectively. The viral replication was spotted around the nasal cavity, trachea, bronchi and lung lobes with the utmost levels of expression being in the lungs.[40] The RNA level was higher in the nasal swabs of aged as compared to young animals. There was prolonged shedding of the SARS-CoV-2 in the upper respiratory tract of this animal.[40]

There were no clinical signs or weight loss seen in all these animals by day 14 post inoculation except for one old-aged macaque that had serous nasal discharge at day 14 post inoculation.[40] Amongst the histopathological changes observed were consolidation changes of lung tissue, alveoli hyaline membrane formation, diffuse alveoli damage, type II pneumocyte hyperplasia and inflammatory changes in the respiratory tract.[40] Injury to type I pneumocytes can result in pulmonary oedema, and formation of hyaline membranes[40,41] which may explain why hyaline membrane formation is a hallmark for COVID-19.[40,42,43] These changes coupled with expression of SARS-CoV-2 antigen by the types I and II pneumocytes and lung tissues provide strong supporting evidence that the SARS-CoV-2 infection caused the lesions.[40] This study contributed a novel animal model which will be crucial in the assessment and licensure of preventive and therapeutic approaches against SARS-CoV-2 infection for use in humans.[40]

Although the advantage of this model is that the viral titre remains for longer period of time with development of characteristic histopathological changes, the absence of overt clinical signs is deemed to be a disadvantage.[35]

Rhesus Macaque

Rhesus macaque is another non-human primate animal model for COVID-19 that is a closer model to humans.[36] The macaque also has the same amino acid residues of human ACE2.[44] A study was conducted which also used both young (age 3 to 5 years) and old-aged (15 years) macaques where all were inoculated with SARS-CoV-2 via intratracheal route. The body weight was reduced in all macaques except in one young macaque. There was no changed in the body temperature. The viral RNA was detected in the nose, pharynx, and crissum of all the macaques. The viral load peaked on day 3 post inoculation in both the young and old macaques, particularly in the respiratory tract system.[45]

The chest x-rays revealed ground-glass opacity which was more prominent in the older macaque. Histologically both groups of macaque exhibited pneumonic changes with more severe lesions being observed in the old-aged macaques. The changes include inflammatory cells infiltrate in the alveoli interstitium and cavities, thickening of alveolar septum, degeneration of epithelial lining and exudation within the alveoli cavities. Essentially, the inoculation of the older rhesus macaques with SARS-CoV-2 had caused a more serious form of interstitial pneumonia.[45]

The Rhesus macaque can be a promising model to study the efficiency of vaccines as the immune responses in monkeys exhibit similarity to that of the humans.[36] However the clinical signs documented were transient hence posing a disadvantage for this non-human primate model.[35]

African Green Monkey

African Green monkeys (AGMs) were also used as COVID-19 research animal model. These monkeys were inoculated with intratracheal and intranasal low doses of SARS-CoV-2 virus that was isolated from the first case in Italy. There were no overt clinical signs observed in these monkeys except for decreased in appetite and transient fever. Histological findings at day 5 post inoculation revealed marked pneumonia characterised by pulmonary consolidation and haemorrhage. The inflammatory mediator profiles were similar to that seen in the human immune response to SARS-CoV-2. High viral loads were spotted in mucosal samples of all animals including faeces of several animals on day 15 post inoculation.[46]

This model is considered a gold standard animal model for the study of pathogenesis of COVID-19 in comparison to other NHP models predominantly as the model requires a lower dose of inoculation to exert the effects. The drawback of this model however is that the animals did not develop debilitating clinical signs and symptoms as in the human counterpart. This model is costly and difficult to handle the animals.[35]

Golden Syrian Hamster

A recent animal model of COVID-19 was developed using the Golden Syrian hamster *(Mesocricetus auratus)* to study the pathogenesis, transmission and passive immunization effects of COVID-19.[47] The *in silico* structural analysis hypothesises that the hamster ACE2 will bind to SARS-CoV-2 spike glycoprotein receptor-binding domain at high binding affinity. The ACE2 proteins of the hamster are highly similar to that of the human.[36]

A study that utilized this animal model inoculated with SARS-CoV-2 intranasally revealed that the animals became symptomatic with features that include ruffled furs, hunched back posture, lethargy and dyspnoea on day 2 post inoculation.[47] The histopathological changes ranged from diffuse alveolar damage with extensive apoptosis to proliferative phase of tissue repair in the later stage. The lymphoid organs also exhibited atrophic changes which were attributed to cytokine activation that occurred within the first week post inoculation. Naïve hamsters which were housed in the same cage as the infected hamsters also developed similar pathology. All infected hamsters ameliorated and developed a mean serum neutralising antibody titre of ≥1:427 by day 14 post inoculation with a significant decrease in the lung viral load. There was no mortality reported throughout the study.[47] This model is deemed to be appropriate for studying the pathogenesis, transmission, immune responses, treatments and vaccines for COVID-19.[36,47]

Human Angiotensin-converting enzyme 2 (hACE2) transgenic mice

In a study, both male and female 11-month-old hACE2 transgenic mice, were inoculated intranasally with 2019-nCoV stock virus. The transgenic mice had significant reduction in body weight. The lung and liver tissues showed the presence of the virus. The histopathological examination demonstrated that the bronchioles, blood vessels, alveolar interstitium and the alveolar epithelial cells of the transgenic mice were infected with 2019-nCoV.[48] However there were no significant histopathological lesions or viral antigens observed in the myocardium, liver, spleen, kidney, cerebrum, intestine and testis. The pneumonia became mild with only focal lesions at day 7 post inoculation, signifying a non-lethal and self-limiting infection course.[48] Thus; this model does not mimic the austere and lethal cases of COVID-19 in humans.[36]

Advantages and disadvantages of animal models

Animals are excellent research subjects as they have biological similarities to that of humans and are susceptible to diseases.[36] In contrast to humans, the progress of a disease can be studied and documented in animal models due to their shorter life-cycles and under a controlled environment.[36] COVID-19 research works involving animal model development would allow for the understanding of the disease mechanisms prior to the development of vaccines and other therapeutic modalities namely drugs.[49]

However, some of the results generated from animal studies were unable to be translated to humans, such as human trial of COVID-19 drug Remdesivir which yielded better outcomes in rhesus macaque model.[50] Animal studies have been shown to not predict the outcomes in humans despite them sharing biological similarities since the final response to disease depends partly on the epigenome and environmental variations.[36]

CONCLUSION

There are many animal models to date that can be used to study the SARS-CoV-2 infection. Ferrets, non-human primates (cynomolgus macaques, rhesus macaques and African green monkey), Golden Syrian hamster and hACE2 transgenic mice are deemed to be the most promising animal models in COVID-19 research works, namely in areas related to pathogenesis, anti-viral treatment and vaccine development. Livestock animal such as chickens, ducks and pigs are considered to be not suitable models for COVID-19 due to its insusceptibility to SARS-CoV-2 infection.

All models have their pros and cons based on the findings of published studies. Finally, it is important to emphasise that experimental outcomes attained in animal studies were not necessarily confirmed in further human studies despite large similarities between human and the chosen animals. This is because there are some differences between a given animal species and humans.

CONFLICT OF INTERESTS

All the authors declare no financial conflict of interests upon the preparation of this manuscript.

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