EXPERT OPINION ON DRUG DISCOVERY
https://doi.org/10.1080/17460441.2022.1995352

Taylor & Francis
Taylor & Francis Group

REVIEW

Using *in vivo* animal models for studying SARS-CoV-2

Camila B. P. Da Costa [a,b], Ana Claudia De Menezes Cruz [b], Julio Cesar Q Penha [b], Helena C Castro [b], Luis E. R. Da Cunha [a], Norman A Ratcliffe [b,c], Rafael Cisne [b] and Francislene Juliana Martins [b]

[a]Technological Development and Innovation Laboratory of the Industrial Board, Instituto Vital Brazil, Rio De Janeiro, Brazil; [b]Programa de Pós-graduação em Ciências e Biotecnologia, IB, UFF, Rio de Janeiro, Brazil; [c]Department of Biociences, College of Science, Swansea University, Swansea, UK

ABSTRACT

Introduction: The search for an animal model capable of reproducing the physiopathology of the COVID-19, and also suitable for evaluating the efficacy and safety of new drugs has become a challenge for many researchers.

Areas covered: This work reviews the current animal models for *in vivo* tests with SARS-CoV-2 as well as the challenges involved in the safety and efficacy trials.

Expert opinion: Studies have reported the use of nonhuman primates, ferrets, mice, Syrian hamsters, lagomorphs, mink, and zebrafish in experiments that aimed to understand the course of COVID-19 or test vaccines and other drugs. In contrast, the assays with animal hyperimmune sera have only been used in *in vitro* assays. Finding an animal that faithfully reproduces all the characteristics of the disease in humans is difficult. Some models may be more complex to work with, such as monkeys, or require genetic manipulation so that they can express the human ACE2 receptor, as in the case of mice. Although some models are more promising, possibly the use of more than one animal model represents the best scenario. Therefore, further studies are needed to establish an ideal animal model to help in the development of other treatment strategies besides vaccines.

ARTICLE HISTORY
Received 4 May 2021
Accepted 15 October 2021

KEYWORDS
COVID-19; coronavirus; mammals; *in vivo* model; safety

1. Introduction

In late November 2019, a new disease of unknown etiology emerged in Wuhan, China, causing several unidentified cases of pneumonia in humans, and spreading rapidly to many countries around the world [1–4]. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was subsequently designated as the causative agent of coronavirus disease 2019 (COVID-19) [2,5] and the Director-General of the World Health Organization (WHO) in January 2020 declared COVID-19 as a worldwide public health emergency [1]. By October 2021, the disease had affected more than 233 million people, with about 4.7 million deaths in more than 220 countries and the numbers continue to grow [6].

Currently, many drugs and therapies, including vaccines and hyperimmune sera, are being researched [7–9] with more than 40 vaccines in clinical trials, and more than 150 in preclinical studies [10,11]. Despite the efforts of researchers worldwide, few official therapies have been recognized as effective [3,12–15].

One of the greatest difficulties faced during preclinical trials is the selection of the most appropriate animal model relevant to the scope of the research. Several studies, with advantages and disadvantages, have been conducted, but no animal model, so far, reproduces accurately, the serious symptoms of COVID-19 in humans [8,13,14,16,17].

Issues related to infection rates, mechanisms of action, the possibility of reinfection, and potential therapeutic approaches require the use of experimental models as previously used for other coronaviruses, and assisting the development of strategies to understand COVID-19 [4].

Until 2019, SARS-CoV-2 had not been detected in humans or animals, regardless of whether this virus shows about 96% genetic similarity with the coronavirus that was detected in 2013, in China, in *Rhinolophus* spp. (horseshoe bats). Whether other animal species could become hosts for a virus that is widespread and whether the clinical manifestation of SARS-CoV-2 infection in humans behaves in the same way in other animals is questionable. In addition, in efforts to develop vaccines and antiviral drugs, which animals are most relevant for experimental tests to increase the effectiveness of such control measures in humans [2,18].

The present study, therefore, aimed to compile and analyze studies of the suitability of which animal models are more useful in preclinical trials with SARS-CoV-2, showing the pathophysiology of the disease in animals, as well as the advantages and disadvantages in relation to their use in research with the new virus.

2. Animal models

Finding an animal model to study the new coronavirus and test vaccines or new forms of therapies, as well as understand

CONTACT Francislene Juliana Martins francislenemartins@yahoo.com.br; Rafael Cisne rafaelcisne@id.uff.br Programa de Pós-graduação em Ciências e Biotecnologia, IB, UFF, Rio de Janeiro 24210-201, Brazil

Article highlights

- The emergence of COVID-19 in 2019 represented/respresents a global concern.
- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was designated as the causative agent of COVID-19 disease.
- The search for an animal model to study SARS-CoV-2 is a challenge.
- Infections in animals still do not reproduce all the characteristics of COVID-19 in humans.
- Using more than one model can guarantee more robust results.
- In vitro assays are essential to study new anti-SARS-CoV-2 drugs.

This box summarizes key points contained in the article.

the molecular mechanisms of COVID-19 is not an easy task and has attracted the interest of researchers worldwide [19,20]. These models may be experimentally induced so that the study of the disease becomes possible, or they can be spontaneous models, including naturally existing genetic variants, genetically modified models, negative models, resistance to some diseases, or even orphan models, which suffer from certain natural disorders [21,22]. As the virus-host interaction is very complex, it may require the use of more than one animal model, since the chances of a single model reproducing all aspects related to the disease in humans are low [23].

The coronavirus incubation period in humans is approximately 5 days, with the possibility of developing symptoms until day 14 [24]. Predicting the course of the disease in animals is challenging because the highest level of pathology mainly occurs in one week, which can be caused by variable immune responses, and the way the infection occurs and the virus replicates. When looking for an ideal model, we need to ensure that at the end of the experimental trials, the differences in the pathophysiology are not in doubt when extrapolating to the human situation.

To assess the course of COVID-19 in the proposed animal models, histological, radiological, and visual inspection tests should be performed. The animal model must be able to indicate the presence of lung tissue damage and the development of an inflammatory process. In addition, changes in the function of the alveolar-capillary barrier and physiological should be detectable so that, the effectiveness of the therapy implemented can be evaluated and determined [16].

Studies have shown that civet cats, camelids, monkeys, mice, hamsters, ferrets, and rabbits can be considered as animal models for coronavirus [3,25–31]. In studies with SARS-CoV-2, in some of these animals, viral replication was observed with limited histopathological changes and signs of clinical disease. However, the symptoms of the disease were not consistent and there was no pattern of immune response or mortality [32]. Furthermore, receptors in the Fc region of antibodies differ between animal and human models, compromising the evaluation of vaccines for human use in tests in animal models [33].

Preclinical studies for the development of new anti-SARS-CoV-2 drugs can select suitable animal models and feasible infection neutralization pathways, detecting viral tropism and binding to ACE2 [34,35]. ACE2 represents the main receptor for SARS-CoV-2 entry into cells whereas lungs and bronchi are the main targets for viral infection. However, the heart, kidneys, liver, brain, gastrointestinal tract, and upper respiratory tract can also be affected [36]. Zeiss et al. [34] signaled that some amino acid sequences present in ACE2, considered critical residues, are more important than the total number of similar amino acids when comparing the animal model to the human. Apparently, they have a direct influence on the susceptibility to infection. It was verified that, when comparing the virus binding region to ACE2 in marmosets and pigs, the critical residues are absent or in a lower number than necessary for a perfect viral coupling. Differently, Syrian hamsters and ferrets show these residues favoring viral tropism. This revealed Syrian hamsters and ferrets as better model animals for preclinical research studies [28].

Another factor that directly impacts ACE2 affinity and differs from one species to another is the tissue distribution of ACE2 together with TMPRSS2 and furin. The TMPRSS2 cellular serine protease and the furin proprotein convertase act as cofactors for this binding, which can be considered as a target for the action of inhibitory drugs and a difference among animal models facing infection [34]. The fact that the main targets (lungs and bronchi) express low levels of ACE2 pointed to the existence of other molecules involved in cell invasion. In addition, the presence of such molecules directly impacts viral tropism, including the AXL receptors (present lung), CD147, and ASGL1, independent of ACE2, which facilitate viral entry into cells. Furthermore, heparan sulfate, sialic acid, lectin receptors, Neuropilin 1, and CD4 act as co-receptors while SR-B1/cholesterol, Furin, PC-1, trypsin, matriptase, TMPRSS2, and cathepsin are cofactors, demonstrating that the engineering of the infection of SARS-CoV-2 in organs is a quite complex process, and the virus uses more than one pathway to cross the cell barrier [36].

After a careful review of the course of COVID-19, and the post-infection characteristics (virological, histological, and immunological), non-human primates (NHPs), ferrets, and hamsters have been considered to be potential natural hosts of SARS-CoV-2. However, the tree shrew, woodchuck, pangolin, rat, guinea pig, cotton mouse, cats [2], and dogs might also be potential hosts for SARS-CoV-2 infection [26].

2.1. Non-human primates

Due to their pathophysiological similarity to humans, studies with non-human primates have great clinical relevance for several diseases, especially for emergent pathogens such as coronaviruses. Some studies using these animals and performed with SARS-CoV-2 failed to reproduce all the human characteristics of infections. However, such models had sufficient similar characteristics and were used in the development of new vaccines against SARS-CoV-2 [13,37–39].

Studies involving SARS-CoV-2, carried out in monkeys, showed that infected animals had pulmonary infiltrates, with the presence of virus along the upper respiratory tract [22]. African green monkeys, cynomolgus, and rhesus exhibited mild symptoms of the disease but were able to produce neutralizing antibodies that could help fight the virus [16,23,40]. Elevated levels of chemokines and decreased levels

of TGFα were detected in the serum of these animals in the first 3 days after infection, but the difference was not statistically significant. The non-significance may be related to the fact that men and monkeys share the main amino acid residues present in the angiotensin-2 converting enzyme [22].

There are many studies involving rhesus monkeys in the literature where researchers have been analyzing the distribution and pathological changes caused by SARS-CoV-2, but many gaps remain in relation to immune responses induced by the infectious process [37,40,41]. Intranasal, intratracheal, oral, and ocular routes of administration were used for viral inoculation in these animal models [16,37], where the viral peak was detected between the third and fifth days of infection [42], and the symptoms observed with the aid of Radiology and histology that monitored alveolar infiltrates, irregular breathing patterns, and increased respiratory rates [16]. Transient severe pneumonia [43], with pulmonary, infiltrates, histological lesions, and seroconversion has been observed in several studies [13,37], confirming the viral capacity to replicate throughout the respiratory tract, facilitating transmission between hosts [38,44], in addition to reduced appetite, transient hyperthermia, increased body temperature, and slight weight loss [23,44]. Zheng et al. [45] reported the functioning of Treg cells in disease progression, summarizing the T cell response and local cytokine/chemokine changes, important features in an animal model for application in vaccine evaluations and treatments against COVID-19.

In another study, Aid and colleagues [46] used rhesus monkeys, aged 6 to 12 years, to assess the development of vascular changes in animals affected by SARS-CoV-2, changes commonly seen in human patients. Histopathological analysis of lung tissue in animals on days 2 and 4 post-infection showed endothelial inflammation, similar to that observed in humans, with endothelial hyperplasia, tunica intima proliferation, in addition to macrophage and lymphocyte adherence. The proteomic and transcriptomic study was also carried out, which demonstrated the recruitment of macrophages and neutrophils, with platelet aggregation, culminating in endothelial alterations and thrombosis, as it happens in humans [47]. Jiao et al. [47] evaluated the pathogenesis of COVID-19 in a rhesus monkey model using the gastrointestinal tract as an alternative route for infection. Changes observed in the gastrointestinal and respiratory tracts from intranasal and intragastric virus inoculation were compared. After intranasal inoculation, no symptoms such as weight loss, hyperthermia, or diarrhea were observed, although the virus was detected in lung and intestinal tissue samples. Histopathological changes were observed in samples from lung, trachea, and bronchi, which included inflammatory cell infiltrate, congestion, edema, changes in epithelial cells, and vascular changes. In tissue samples from the gastrointestinal tract, infiltrates of inflammatory cells and changes in epithelial cells in the stomach, duodenum, jejunum, ileum, descending colon and rectum could also be identified [47]. In animals inoculated by the intragastric route, the authors observed in macroscopic analyzes that the lungs showed hyperemia in the first days after infection and consolidation on the 7th day. In the microscopic analysis, as well as in animals inoculated by the intranasal route, an inflammatory cell infiltrate was observed in the lungs, in addition to vascular congestion and hemorrhage. In the gastrointestinal tract, inflammatory cell infiltration and loss of epithelial cells were observed, similar to the changes observed in animals inoculated by the intranasal route. The findings show that SARS-Cov-2 can affect both the respiratory tract of affected animals, as well as producing enteropathogenic effects, as observed in human patients [47].

Research has shown that older animals were more susceptible and developed stronger symptoms than younger animals, and there was no reinfection [16]. Although the condition presented is extensive, it is not common for these animals to evolve to death after the onset of the infectious condition [22,44,48]. To assess the impact of age on disease progression, Song et al. [49] used Chinese rhesus monkeys (*Macaca mulatta*) of various ages. The authors observe that young monkeys, after infection, have altered respiratory function, a high rate of viral replication, severe lung damage with cellular infiltrates ($CD11^+$ and $CD8^+$). Aged monkeys showed delayed immune responses, with a more severe cytokine storm. Furthermore, there was an increase in pulmonary infiltration of $CD11b^+$ cells and persistent infiltration of $CD8^+$. There was greater inflammation and chemotaxis of peripheral blood T cells from elderly monkeys. However, the antiviral response was less than that seen in young monkeys. Thus, the late and more severe cytokine storm and cellular infiltration help to elucidate the worse prognosis of elderly patients with SARS-CoV-2 infection [49]. Deng et al. [50] evaluated whether a previous SARS-CoV-2 infection would protect rhesus monkeys from reinfection, where the results demonstrated that infected animals were able to produce an immune response that protected them during the initial period after recovery.

Lu and collaborators [39] conducted a comparative study between three species of non-human primates, *M. mulatta and Macaca fascicularis* and New World monkey *Callithrix jacchus*. Among all species studied, *M. mulatta* showed the strongest symptoms in the initial phase of the infection and was more similar to humans. According to their findings, as well as the studies previously presented, an increase in body temperature, as well as abnormal patterns in the lungs and presence of infiltrates, were detected. Early in the infection, viral RNA could be found in the respiratory system, spleen, lymph nodes, digestive and intestinal systems, as well as bladder and uterus. In the final stages, the location was restricted to the spleen, bronchi, and laryngopharynx. This study brings the oro-fecal transmission as one of the forms of transmission of this disease. For immunological evaluation, the production of specific antibodies and inflammatory cytokines were analyzed. As for antibodies, all species produced antibodies, but young *M. mulata* produced less when compared to older animals and *M. fascicularis* produced higher titers, and these two species also had the highest rate of production of inflammatory cytokines [39].

Alluwaimi et al. [21] and Salguero et al. [51] evaluated the susceptibility of cynomolgus monkeys to SARS-CoV-2 infection. This animal model also has pathological lung lesions, albeit mildly but quite aggressive, slightly more pronounced

in the elderly [21]. Viral RNA was found in the respiratory tract, conjunctiva, oral cavity, and rectal smears and no viral RNA was observed in the animals' nervous system or lymphoid tissues, in addition to the animals seroconverting on the fourteenth day after infection [21,52]. According to Ishigaki et al. [52], viral RNA gradually decreases over the 28 days observed in the experiment. The most important clinical signs were a significant increase in body temperature in the first 3 days, weight loss, lungs with a ground-glass appearance during the first 7 days, and pneumonia with the presence of infiltrates, in addition to an increase in the production of inflammatory cytokines. The authors analyzed the production of neutralizing antibodies and found that after 28 days there was no more detection, unlike other studies in the literature, but justified by the use of elderly animals [52]. According to Salguero et al. [51], cynomolgus monkeys are as important a model as the rhesus monkeys for evaluating the efficacy and safety of drugs to be developed for therapies against SARS-CoV-2.

A therapy that has been widely discussed lately in the treatment of COVID-19 is the use of convalescent plasma. Cross et al. [53] carried out tests using African green monkeys (*Chlorocebus aethiops*). It was carried out in a cohort study with a group infected with SARS-CoV-2 by intratracheal and nasal routes versus not infected, and the efficacy of using convalescent plasma as therapy against untreated animals. The symptoms presented by the infected animals were mild, with coagulopathy, lack of appetite, presence of viral RNA in the airways, severe pneumonia with the presence of infiltrates, hemorrhage, and slow recovery. However, the difference between the group treated with a convalescent low-titer plasma and the untreated group was not as marked. In the group that received a plasma with a higher titer, the severity of the pulmonary manifestation of the disease, decrease in coagulopathy and the inflammatory processes resulting from the infection were more expressive when compared to the untreated groups, highlighting the importance of administering this treatment of early form with plasmas that have high levels of antibodies [53].

Greenough [43] and colleagues studied the course of SARS-CoV-2 infection in young marmosets and young adults in the first 7 days of infection. According to the results after nasal infection, the animals presented fever, diarrhea, pneumonia with the presence of infiltrates and edema, hepatitis, and multiple organ failure from the fourth day after the viral infection. Viral RNA could be detected in the lungs, liver, heart, and lymphatic system, showing that the ACE2 and virus interaction may be similar to the interaction that occurs in the human host. However, the researchers recognize that the results in marmosets are promising, but that further studies need to be carried out to better analyze this model over the entire period of viral infection and symptomatological manifestation [43].

2.2. Ferrets

Ferrets have been studied as an animal model for human respiratory viruses [54–60] like influenza virus A [8,13,57] and syncytial respiratory virus [8] owing to their pulmonary morphological similarity to humans. Animals are susceptible to coronavirus, are able to cough and sneeze, manifesting symptoms such as increased body temperature [22] because of their morphological similarity to humans. To assess the susceptibility of these animals to infection with SARS-CoV-2, Shi and collaborators [2] inoculated intranasal pairs of ferrets with virus samples collected from the environment and human patients. Components of the upper and lower respiratory tracts of each animal were collected, in addition to organs such as lung, heart, liver, spleen, kidneys, pancreas, small intestine, and brain. This sampling aimed to quantify viral RNA by qPCR and virus titration in Vero E6 cells. The authors noted that SARS-CoV-2 only replicated in the upper respiratory tract of animals, without causing serious illness or death. These data differ from what has been verified for the influenza virus and other coronaviruses, which are able to replicate in the upper and lower respiratory tract of animals [55].

Type II pneumocytes and serous epithelial cells of the tracheobronchial submucosal glands are the main cells that express ACE2 in ferrets [56]. These animals differ in only 2 amino acids in the binding region of SARS-CoV-2 Spike protein with ECA2 [2,13] and the mechanism that inhibits viral replication in the lower respiratory tract of ferrets is still not well understood. However, the fact that the virus replicates in the upper respiratory tract of these animals may make them important candidates for animal models for testing new drugs against COVID-19 [2,13].

According to Cleary et al. [16], when inoculated with the coronavirus, ferrets show symptoms between the second and sixth days, and histological tests showed severe lymphoplasmacytic pulmonary perivasculitis and vasculitis on the thirteenth day of infection. According to Khoury et al. [42], the animals had vasculitis on the third day and bronchiolitis on the fourth day. When exposed to SARS-CoV-2, ferrets show hyperthermia and allow viral replication, characteristics that allow this animal to be useful for the development of drugs and vaccines [2,61].

Richard et al. [62] and Fenollar et al. [63] verified the airborne transmission of SARS-CoV-2 between ferrets. This fact highlights the importance of community-level social distancing, which has been applied in many countries around the world, and the adoption of infection control measures in healthcare settings [62]. Ferrets were able to transmit the virus through direct contact with other animals or by indirect contact [8,16,42,62]. This form of transmission reinforces that ferrets can be considered good models for studying the transmission of SARS-CoV-2 [22].

2.3. Mouse *(Mus musculus)*

Mouse models exhibit an important role in drug development [17,64,65]. It is a reproducible, low-cost model that allows genetic manipulations [66,67] and is considered one of the main models used in the study of human diseases [68,69].

SARS-CoV-2 has a low affinity for mouse tissues, being inefficient to infect these animals. This is justified by the difference between mouse ACE2 compared to human ACE2 proteins [17,70]. Due to this low affinity of the virus for mice tissue, the wild-type mouse strains were not considered ideal

for studying SARS-CoV-2 infections [17,70,71]. According to Deb et al. [22], 11 of 29 amino acids in mice and 13 of 29 amino acids in rats differ in the ACE2 binding region compared to human ACE. This means that the ACE2 of these animals is unable to bind to the virus.

McCray et al. [72] analyzed the introduction of a vector carrying a human ACE2-coding sequence into wild-type mice and the development of a hACE2 transgenic mouse strain. These mice may be useful in assessing the pathogenesis of SARS-CoV-2 and in the development of antivirals and vaccines against COVID-19 [32,73]. Humanized and genetically modified mice responded to viral infections. They present with weight loss, viral pulmonary replication, interstitial pneumonia, positive exudation, macrophage-rich infiltrates, and T and B lymphocytes, but these animals are unable to transmit the disease. Munshi et al. [74] reported that SARS-CoV-2 has tissue tropism for the lungs of these animals. However, an abnormal expression of receptors in different tissues can modify viral tropism. In addition, the maintenance cost of these mice can be high and the demand in the world market has increased due to the pandemic, which has hampered their acquisition [22].

The strategy of genetic modification of mice has also been studied by other authors [8,16,19,75,76]. Genetic modification, which makes it possible to introduce hACE2 in mice, allows these animals to manifest human-like symptoms of SARS-CoV -2 infection [21,44,77]. The human cytokeratin 18 (K18) promoter in epithelial cells regulates ACE2 expression and has been observed in infected airway epithelial cells as well as the liver, kidney, spleen, heart, and intestine [13,75,76]. Studies have demonstrated that the K18-hACE2 transgenic mouse infected with a human SARS-CoV strain by the intranasal route would not survive [32]. The infection begins in the airway epithelium, spreads to the lungs and brain with pulmonary infiltration of macrophages and lymphocytes and upregulation of pro-inflammatory cytokines and chemokines in the lungs and brain [32]. Subsequently, the mice began to lose weight and became lethargic, presenting difficulty breathing. All animals died by the seventh day. This information reinforce that the expression of the hACE2 transgene in epithelial cells can transform a SARS-CoV infection initially considered moderate to severe and potentially fatal [17,71,78]. These findings are corroborated by Moreau et al. [79].

Some authors noted that a peak of pulmonary pathology occurred around the seventh day with abnormalities in the brain, heart, and kidney [42]. The animals had a high viral load in the lungs, in addition to weight loss and morbidity [13], inflammation and pulmonary infiltration developing severe lung disease, with thrombosis and anosmia, and mortality around the fourth day of infection [16,44,78].

Likewise, Jiang et al. [80] also evaluated the response of transgenic animals (HFH4-hACE2 in C3B6 mice) to SARS-CoV-2 infection, in which the animals had high levels of receptors in the lungs and other tissues (brain, liver, kidney, and tract gastrointestinal). Infected animals developed typical interstitial pneumonia; a picture similar to that seen in human infections. The authors also concluded that pre-exposure to the virus may protect against the development of severe pneumonia.

Other techniques used with mice are collaborative crossing and the use of mice transplanted with human cells [8]. To produce AdV-hACE2 mouse adenovirus vectors are used to insert human hACE2 receptors into the animal DNA [13].

Zhang et al. [68] reported that mice susceptible to SARS-CoV-2 infection could be obtained by producing mice that express the hACE2 receptor, using recombinant adenovirus vectors, and through the adaptation of the virus to the respiratory tract of animals through sequential passages. Another technique is the viral passage in the mouse lung to develop a viral adaptive process, which can cause lung damage, infiltration of monocytes and lymphocytes, and death of the animal [21,44,77].

Israelow and collaborators [81] elaborated a model based on the expression of hACE2 mediated by an associated adenovirus. They mentioned that the transgenic models presented some obstacles, such as limited availability, being restricted to a single genetic fund. The animals used were inoculated intranasally and had viral replication and pathological characteristics similar to those presented in human infection [81].

Wang et al. [17] found that the viruses replicated efficiently in the lungs of BALB/c mouse-adapted virus model; in a reverse genetically modified SARS-CoV-2 infection model, and in a recombinant adenovirus-mediated transient expression of human ACE2 mouse models. It is important to considerer that none of these animal models developed an infection considered significant and stable in the upper respiratory tract [17].

It was observed that humanized mice exhibited a high viral load in the lungs, trachea, and brain by using CRISPR/Cas9 technique. Elderly mice manifested pulmonary neutrophilia, extensive alveolar thickening, vascular injury, and focal hemorrhage [18,76].

The infection of the upper respiratory tract in humans caused by SARS-CoV-2 is related to the initial infection, the spread of the virus, and ability to transmit the disease. Thus, one of the desirable aspects of the development of antiviral and vaccines against COVID-19 is the prevention of viral replication in the respiratory tract [17]. Wang et al. [17], using BALB/c and C57BL/6 J mice, suggested that combined intranasal and intramuscular administration of Remdesivir treatment efficiently inhibited viral replication in the upper and lower respiratory tracts of mice.

Another important point in understanding the pathogenesis of COVID-19 is an intragastric infection, which leads to the development of gastrointestinal symptoms in humans. This was also investigated by Sun et al. [82], and mice inoculated orally did not show the symptoms commonly seen in humans infected by this route, such as diarrhea, abdominal pain, and vomiting [82].

Dinnon et al. [70] remodeled the Spike protein-binding domain of the virus in a mouse model adapted for SARS-CoV -2, which was not genetically modified, thus facilitating its binding to the mouse ACE2 receptor. According to the

authors, this is a high-performance model for evaluating therapeutic measures.

In a study published by Leist et al. [83], the authors reported a model that could help elucidate acute lung damage and acute respiratory syndrome, being useful for the study of new drugs. This model of lethal pathogenesis was adapted for BALB/c mice, using the SARS-CoV-2 MA10, and the severity of the disease was similar to that observed in older humans.

Gurumurthy et al. [19] believe that mice expressing the hACE2 receptor do not faithfully reproduce the course of human disease. In addition, the technique has limitations, since the mice continue to express the mACE2 receptor, reducing the physiological concentrations of hACE2 available for binding to the virus. In addition, different promoters involved in the expression of transgenic cassettes can lead to the expression of proteins in different cells and at different concentrations of endogenous concentrations of mACE2. They are also not suitable for studies that require the expression of hACE2 in specific cells or at certain periods.

However, Hewitt et al. [23] reported that the use of NOD SCID gamma mice could be an important tool for studying the evolution of the disease in the presence of comorbidities, which can be induced or obtained by genetic crosses. These models can also help to understand why certain individuals may develop a severe form of the disease, while others may have mild symptoms or even be asymptomatic.

Zhu et al. [84] evaluated the virus induces a response by interferon (IFN) type I, which promotes the migration of monocytes and macrophages to the lungs the immune response to SARS-CoV-2 in mice. Regarding the innate immune response, the authors noted that SARS-CoV-2 is sensitive to IFN-β, leading to a reduction in viral load. IFN has been used in the treatment of patients affected by Covid-19. Regarding the humoral response, immunoglobulins (IgG) specific for the Spike protein (S) of the virus were detected in the blood of mice 21 days after infection. The authors also evaluated the cellular immune response in these animals and observed the presence of different types of T lymphocytes, as well as B lymphocytes and natural killer cells [84]. In another study, Hassan and colleagues [85] demonstrated the importance of IFN in the immune response to SARS-Cov -2. Infected mice showed greater weight loss and lung involvement due to the absence of signaling or partial signaling generated by this protein in response to the virus [85]. Leist and colleagues [83] observed the presence of cytokines and chemokines in the lungs and blood of experimentally infected mice. Like what is observed in humans, the authors found high levels of interleukins, tumor necrosis factor α (TNF-α), monocyte chemoattractant protein 1 (MCP-1), and IFN-γ [83].

2.4. Hamster *(Mesocricetus auratus)*

Hamster has been used as an animal model in other viral researches [16,86–91]. A comparative *in silico* study between human ACE2 and the hamster enzyme suggested that this animal may show symptoms similar to those presented by humans 1 or 2 days after SARS-CoV-2 infection [8], with pulmonary pathology, with peak load on the seventh day and transmission by direct contact [8,13,87]. Hewitt et al. [23] also reported that after intranasal infection, the animals showed clinical symptoms, histopathology, and viral kinetics similar to human disease.

The ACE2 amino acid sequence of these animals is very similar to the human sequence, differing only in 4 of the 29 amino acids. They are susceptible to infection, with weight loss, presence of virus in the nose, lung, and trachea, with a lethality rate of 5%, exudative inflammation with hemorrhage and alveolar necrosis, inflammation of the intestinal mucosa epithelium with diarrhea, and myocardial degeneration. The pathological picture seen in hamster lungs by SARS-CoV-2 infection may be the result of an intense immune reaction, as is observed in humans [8]. The unfolding of this immune response is the production of interferon γ, for macrophage recruitment, and pro-inflammatory cytokines two days after viral infection, which culminated in an acute inflammatory picture marked by cell death [22,23,86]. According to Muñoz-Fontela et a [8]., it was possible to detect neutralizing agents in the blood of these animals, 7 days after the onset of infection, a period that coincides with the increase in TGF-β and reduction of interferon II and IL6, alleviating the inflammatory picture [22,86]. By 14 days after infection, a humoral immune response with high neutralizing titers of can be observed, especially in young animals [92]. The fact that Syrian hamsters can transmit the disease under challenge with SARS-CoV-2, demonstrating to be an ideal animal model [22,87].

A recent study using a Syrian hamster as a model clearly demonstrated the need for the coexistence of the presence of ACE2 and the transmembrane serine protease 2 for SARS-CoV -2 to be able to invade the target cell. The first is responsible for binding with protein S while the second cleaves it, fusing it to the cell membrane. It was observed that in the lung of the hamster these proteins had a different distribution, and one was not always close to the other. Transcriptomic results suggested that transmembrane serine protease 2 is expressed in higher concentrations and is more widely distributed than ACE2. Only in places where both coexisted, pulmonary middle lobe, tertiary bronchioles, and in places at the interface between bronchioles and alveoli, infection was observed [93].

Cleary et al. [16] also observed that the animals presented pulmonary viral replication with edema, inflammation, cell death, animal weight loss, and increased respiratory rate. According to Johansen et al. [13], the animals still had goosebumps, protein-rich lung exudates, mononuclear cell infiltrates, cell death, hemorrhage in the alveoli, and recovery after 10 or 12 days of infection.

According to Muñoz-Fontela et al. [8], these animal models may present mild to moderate symptoms with weight loss, difficulty breathing, lethargy, unkempt hair, and stooped posture. The most severe symptoms appeared after the fourth day, with the lung acquiring the appearance of ground glass, infiltration with cellular apoptosis in the lower respiratory tract, and lung damage. It was found that males and the elderly are more susceptible to the severe form of the disease, related to the viral load to which they are exposed. The

infectious condition ended on the fourteenth day, and it was not necessary to euthanize the animal to assess the course of the infection, as the evolution of the disease was monitored by computed tomography.

The pathogenesis of the disease in hamsters was studied by Sia et al. [88], used animals from 4 to 5 weeks of age, with viral replication was detected on the second day after infection, with a decrease on the fifth and absence on the seventh day. Histopathology revealed the presence of inflammatory cell infiltrate, with the presence of mononuclear cells in areas in which the presence of the virus was detected, with the consolidation of lung tissue whose proportion ranged between 5 and 60% between days 2 and 7 post-infection. On the seventh day of infection, the authors no longer observed the presence of viral antigens in the lungs, with significant type II pneumocyte hyperplasia [83,88]. The authors also observed the presence of the virus in epithelial cells of the duodenum, which is similar to that observed in human patients, even without the existence of inflammatory changes in these animals.

Regarding the oral inoculation of the virus, Lee et al. [94] reported that no clinical symptoms were observed, unlike animals inoculated intranasally. In the histopathological analysis, the authors observed mild inflammatory infiltration in the tongue and oral and stomach mucosa. In the intestine, a greater inflammatory cell infiltrate was observed when compared to the anterior portions of the gastrointestinal tract and significant changes in the villi of the intestinal epithelial cells [94].

Chan et al. [86] isolated viral strains, and the animals were inoculated intranasally. The clinical and histopathological findings observed in the infected hamsters proved to be similar to those observed in humans, and the authors concluded that the Syrian hamster model might be an important alternative for understanding the disease, its form of transmission, possible treatments, and vaccine for COVID-19. Hewitt et al. [23] considered the Syrian golden hamster as an ideal model for evaluating new drugs, with animals with compromised immune systems developing the severe form of the disease, and human antibodies could act as protectors against infection. This can be considered a stable model that is capable of predicting infection and is important in biological tests [95].

Contrary to other published studies [86,88,92], Osterrieder et al. [92] analyzed the impact of age differences in the course of SARS-CoV-2 infection in Syrian hamsters, establishing a model that resembles the most serious SARS-CoV-2 infection in elderly patients. Imaia et al. [87] also addressed the different age groups and noted that older hamsters had more marked weight losses.

Furthermore, through *in situ* hybridization, these authors showed that viral genetic material was detected in cells of the lower respiratory tract and macrophages, that were potential targets of SARS-CoV-2 in human lung tissue. Therefore, the infection of hamsters seems to reflect what has been reported for human patients [92]. Du et al. [96] evaluated the effectiveness of the administration of antibodies (BD-368-2) in hamsters infected with SARS-CoV-2. The data demonstrated weight loss of the animals and reduction in viral load.

Suresh et al. [97] evaluated the proteomic alteration in SARS-CoV-2 infection using this animal model. Although the study had some limitations, such as sample size and experimental conditions, they found alterations in the complement system pathway, coagulation dysregulation and programmed cell death. Besides alterations in the extracellular matrix, and the organization of the actin cytoskeleton in the animals' lungs, abnormal expression of pulmonary surfactants was also detected. It affected the gas exchange whereas the Scgb1a1 protein, a surfactant component, was downregulated. This protein has anti-inflammatory, immunomodulatory, and antifibrotic activities and this downregulation may be related to the respiratory difficulty observed in lung injury.

2.5. *Lagomorphs*

ACE2 is considered the main receptor for SARS-CoV-2 and other beta coronaviruses such as SARS-CoV, which caused an epidemic, in 2002–2003, in 26 countries, although other entry mechanisms and cell receptors are under investigation [98–100].

According to Preziuso [100], further studies are needed on the interaction between SARS-CoV-2 and ACE2 of lagomorphs, despite the common use of these animals in laboratories. This work investigated the ACE2 sequences of lagomorphs and the binding of the complexes SARS-CoV-2-ACE2, showing a high affinity of the ACE2 to the viral Spike protein [100]. According to Maurin et al. [101] viral RNA was detected in nasal and throat smears with pulmonary, peribronchiolar, and peribronchial infiltration with lymphoid tissue growth. It is worth mentioning that the role of these animals in SARS-CoV-2 epidemiology should be investigated [98].

2.6. Mink *(Neovison vison)*

In this model, the animals can develop pneumonia with infiltration, when infected with SARS-CoV-2, which can evolve to death [13]. They present moderate symptoms with respiratory difficulty with viral detection in the respiratory and intestinal tracts. It was also used in studies with SARS-CoV-1 by Muñoz-Fontela and collaborators [8].

Recently, SARS-CoV2 infection has severely affected mink farms in countries such as Denmark, Netherlands, the USA, and Spain. Transmission of the infection between animals and from animal to human has been suggested [63,102,103]. These animals may represent important reservoirs of SARS-CoV-2 [104]. Evaluation of genetic and epidemiological data from this event showed zoonotic transfer with a variant SARS-CoV-2 strain [102,104,105].

In a study published in 2020, Oude Munnink et al. [105] observed a high diversity in the SARS-CoV-2 genomic sequences obtained from some mink farms. This data may represent prolonged circulation of the virus between minks, which may have led to virulence of SARS-COV-2 due to the accumulation of mutations [105].

Since its emergence, SARS-CoV-2 has continued to accumulate mutations [106]. A total of 214 human cases of COVID-19 have been reported in Denmark with different variants of SARS-CoV-that may come from cultured mink [107]. The Statens Serum Institut (State Serum Institute), Denmark, has

identified seven mutations in the Spike protein of SARS-CoV-2 variants found co-circulating in mink and humans [108].

It is evident that mink farms will still play an important role in the emergence of newer and more virulent variants of SARS-CoV-2 [106].

Hoffman and collaborators [109] evaluated the reduced response to therapies involving antibody-mediated viral neutralization caused by SARS-Cov-2 mutations in minks. This work reported the mutation in protein S leading to greater efficiency in the invasion of human cells, whereas reduces viral neutralization by antibodies in use in therapeutic protocols, due to the $Y453F^{4}$ mutation. On the other hand, in a study carried out by Bayarri-Olmos and collaborators [110] on the Y453F variant, it was shown that there was no reduction in humoral immunity or viral neutralization by antibodies. Similar to the study of Hoffman and collaborators, Bayarri-Olmos and collaborators observed a greater affinity of the virus to the human cell receptors, which may increase its transmissibility potential [109,110].

2.7. Non-mammalian animals

The use of zebrafish (*Danio rerio*) for testing new drugs could be an alternative to the development of humanized mice. They are small animals, easy to handle, that have become a model of choice for studying human viral diseases. It is noteworthy that zebrafish have a well-defined and human-like immune system. In addition, it is possible to produce humanized models by xenotransplantation to study respiratory diseases with COVID-19 [111].

Fernandes et al. [112], using zebrafish, sought to test the safety of vaccines and the immune response developed against the SARS-CoV-2 virus. The study comparing the human genome with that of the zebrafish showed 70% similarity. In order to allow the animal to present a natural and acquired immune response, in addition to adverse effects, an N-terminal region of the recombinant SARS-CoV-2 Spike protein was inoculated into the zebrafish. Although these animals do not have a lung, the inflammatory responses were similar to those presented by humans with the infection. Seven days after the immunization process, IgM could be detected in the plasma, reaching up to twice the value presented by the control animals. Many organs present inflammatory changes, such as the brain, with the presence of macrophages, ovarian stroma, renal thrombosis, impaired blood filtration, among other symptoms forming a pattern similar to that of humans [112].

In Table 1, it is possible to observe the main differences between animal models, advantages and disadvantages in their use in preclinical trials.

3. The role of other animals in the epidemiology of COVID-19

During the SARS-CoV-2 outbreak in Wuhan, infection was observed in cat populations. This finding was based on the detection of specific antibodies to SARS-CoV-2 in approximately 14% of the animals evaluated [102]. Zhang et al. [122] show that a higher antibody titer was detected in cats that lived in close contact with owners infected with SARS-CoV-2. In addition, the US Centers for Disease Control and Prevention announced SARS-CoV-2 infection in two pet cats for the first time at two separate sites in New York [102]. In addition, SARS-CoV-2 was detected in the feces and vomit of two infected cats that also lived with infected owners in Belgium and Hong Kong, indicating active virus replication [123]. In this regard, it has been reported that SARS-CoV-2 replicates only in the upper respiratory tract of cats and this replication has not been associated with severe illness or death [2]. It is worth mentioning that younger cats were more tolerant to SARS-CoV-2 infection [2]. In addition, cats can transmit the infection to other cats [124]. Therefore, pet cats are more susceptible to SARS-CoV-2 than dogs, but with mild symptoms and virus shedding [125].

Although dogs have low susceptibility to SARS-CoV-2 infection [2], two pet dogs from Hong Kong and one from northern Italy were infected with this virus, without symptoms, due to contact with people infected [102]. Dogs have ACE2 receptors similar to humans ACE2 (hACE2), which function as SARS-CoV receptors, raising the possibility that dogs may be a potential intermediate host [126]. Although there is no evidence that infected dogs can transmit the virus to animals or humans [127]. Recently, Freuling et al. [128] showed the susceptibility of raccoon dogs to SARS-CoV-2 infection after intranasal inoculation. Virus shedding was detected in nasal and oropharyngeal smears from infected dogs on the 2nd day post-infection. In addition, infected dogs were able to transmit the virus to contact animals, suggesting that raccoon dogs may be a potential reservoir for SARS-CoV-2 [128].

A Malay tiger at the Bronx Zoo, New York, USA was tested positive for SARS-CoV-2 as the first case of animal infection in the USA [102]. This tiger was the first infected tiger in the world and the first case of transmission from humans to non-domestic animals [129,130]. Later, infection was detected in four tigers and three lions [102], indicating that different feline species are susceptible to SARS-CoV-2 infection [130]. It was suspected that the tiger was infected from an asymptomatic site worker [102,129].

4. Tests of vaccines in *in vivo* mammal models

Many anti-SARS-CoV-2 vaccines are in the process of developing worldwide. Each has a particularity, based on viral vectors, mRNA, DNA, subunit vaccines, nanoparticles, or inactivated whole virus [35,131,132].

Studies with a human adenovirus with a type 5 replication defect were used to test a vaccine proposal in mice and ferrets (Ad5-nCoV). A single dose of the vaccine protected ferrets and mice from viral infections. The intramuscular and intranasal routes were analyzed and considering that SARS-CoV-2 causes a serious respiratory disease, the vaccine applied to the mucosa can lead to the best efficacy, but must be well monitored, as it can induce risks of asthmatic crises [131].

In both ferrets and mice, after vaccination, challenge tests showed that no virus was found in the lungs and nasal shells, unlike the control group that had a detectable viral load. This result was observed for animals vaccinated intramuscularly and intranasally. In tests with ferrets, intranasally and intramuscularly, it was shown that all the animals that received the

Table 1. Main animal models: general aspects of SARS-CoV-2 infection, advantages and disadvantages of their research applications.

Animal model	General aspects and immunology	Advantages	Disadvantages	References
 Nonhuman primates (NHP)	Non-human primates, due to their high physiological and phylogenetic similarity with humans, are excellent models for studying the effects of SARS-CoV-2 and present symptoms ranging from mild to moderate. Seroconversion, production of neutralizing antibodies, and pro-inflammatory cytokines, and development of cellular immunity. Presence of pulmonary infiltrates in contaminated animals, as well as endothelial inflammation, endothelial hyperplasia and proliferation of the tunica intima.	Rhesus monkeys: most affected by SARS-COV-2 among NHP. Older animals may be more susceptible. Cynomolgus monkeys: viral RNA was found in the respiratory tract, conjunctiva, oral cavity and rectal smears and no viral RNA was observed in the animals' nervous system or lymphoid tissues. Slightly more pronounced in the elderly. It is possible to observe pathogenicity for up to 4 weeks after viral infection. African green monkeys: they allow a higher rate of viral replication, and may present more severe conditions than other NHP. Presence of viral RNA in the airways. Marmoset: viral RNA can be detected in lungs, liver, heart and lymphatic system, fever, diarrhea.	Rhesus monkeys: In the final stages of infection, viral detection was restricted to the spleen, bronchi and laryngopharynx. Slower playback rate than cynomologus. Slow reproduction rate They are animals that require a greater infrastructure for maintenance, with a higher cost involved, which can make their use impossible in some cases.	[8,13,17,22,23,39–42,45,46,53,109,113,114]
 Ferrets	Severe lymphoplasmacytic pulmonary perivasculitis and vasculitis, bronchiolitis, hyperthermia. Seroconversion, production of neutralizing antibodies and development of cellular immunity	Advantageous model for viral transmission studies. Ferrets are models that share important binding residues with SARS-Cov-2 on ACE2 receptors with humans, which strengthens their use as an animal model for Covid 19.	SARS-CoV-2 only replicated in the upper respiratory tract without causing serious illness or death. They are larger animals when compared to rodents and, for this reason, its use in research presents greater difficulty about installation and handling needs. These models also lack some symptoms commonly observed in humans, which can range from non-existent to mild.	[8,16,42,56,62,115–117]
 Mouse (Mus musculus)	Seroconversion, production of neutralizing antibodies, and pro-inflammatory cytokines, and development of cellular immunity. Interstitial pneumonia, positive exudation, infiltrates rich in macrophages and T and B lymphocytes	Mice, in general, are very docile animals, with small size and easy handling, short life cycle, high productivity and short gestation period, known genetic background, and low cost. The genetic similarity between mice and humans can vary between 70 and 90%, which may explain the fact that this is the most used model in research. High reproduction rate. Wild-type: no symptoms. K18-hACE2: The infection starts in the airway epithelium, spreads to the lungs and brain, heart and kidney, and the animal's death occurs. HFH4-hACE2 – C3B6 mice: It affects brain, liver, kidney and gastrointestinal tract. High reproduction rate.	Wild-type: SARS-CoV-2 has low affinity for ACE2. K18-hACE2: females have a stronger immune response than males, but are more resistant to the virus, with a lower mortality rate. They do not transmit the disease to other animals. HFH4-hACE2 – C3B6 mice: The infected mice that survived become resistant to new infections.	[8,17,22,32,70,80,115,117–119]

(*Continued*)

Table 1. (Continued).

Animal model	General aspects and immunology	Advantages	Disadvantages	References
 Hamster	Seroconversion and production of neutralizing antibodies. Exudative inflammation with hemorrhage and alveolar necrosis, lung acquiring ground-glass appearance, infiltration with cell apoptosis and lung injury.	They reproduce quickly. Direct-contact transmission was significant, but indirect-contact transmission was not.	The pneumonia is very fast, 2 weeks, and the antibodies titer rapidly reduces, being a poor model for studying the pathogenicity of severe forms of COVID-19. They are less docile animals, which can make their handling a little difficult. Furthermore, as in mice, these models do not present some symptoms commonly observed in humans and may be mild to moderate	[8,13,16,22,87,115–118]
 Lagomorphs	Rabbits are known to produce high titers of antibodies against infectious agents. Presence of pulmonary, peribronchiolar and peribronchial infiltration with lymphoid tissue growth.	Viral RNA was detected in nasal and throat swabs.	Transmission between animals may be less effective. Although animals are susceptible to the virus, the disease is usually asymptomatic.	[100,101,113,120]
 *Neovison vison*	SARS-CoV-2 can mutate in these hosts. Lung lesions similar to those seen in humans with COVID-19. Presence of pulmonary infiltrate, fibrinous necrosis of the blood vessels, hemorrhage, exudation, vasculitis and perivasculitis. In addition to fibrin formation in blood vessels and bronchial injuries.	Viral detection in the respiratory and intestinal tract. They are able to transmit to other animals. Its high transmission capacity can lead to large outbreaks among animals, as happened in Denmark in 2020.	There is evidence of transmission of the virus from these animals to humans.	[102,104–109,114,121]
 *Danio rerio*	The inflammatory responses remaining at the brain level, with the presence of macrophages, ovarian stroma, renal thrombosis with difficulty in blood filtration.	Seven days after the immunization process, IgM could be detected in plasma.	They do not have lungs.	[111,112]

vaccine produced specific antibodies against glycoprotein S, in addition to presenting cellular immunity and some cytokines, which were not detected in the control group. Preexisting human immunity to viral vector vaccines is a concern for developers, as a weak humoral and cellular response may occur, which makes it necessary to choose the second route of administration [131].

Another vaccine study, using an adenovirus vector, was carried out by Van Doremanlen et al. [133], which showed that the vaccine administered intramuscularly was immunogenic in mice, generating a robust humoral response mediated by cells, mainly type 1 T helper cells, in addition to IgG and some cytokines. This scientific finding is extremely relevant in the current scenario, in which researchers need to focus on developing vaccines that can protect against mutant strains of SARS-CoV-2 in which helper T cells can maintain lasting immunity [134]. When tested in rhesus monkeys, there was a balance between the humoral and cellular immune responses, and when challenged with the virus after vaccination, the pulmonary viral load was reduced, without pneumonia, without lung damage, without viral detection, and without detectable adverse effects [131]. Jia et al [135]. also reported that DNA vaccine conferred protection to rhesus monkeys against SARS-CoV-2 infection. It is worth mentioning that the use of adjuvants can favor the durability and intensity of the antibody response induced in vaccines that are based on proteins, but also modulate the cytokines produced by T cells [136].

Sun et al. [76] used BALB/C mice genetically modified to express hACE2 using adenovirus vectors. These mice were immunized with Venezuelan equine encephalitis replicon particles, expressing the SARS-CoV-2 Spike protein, a transmembrane protein, nucleocapsid, and envelope proteins. Only immunization with SARS-CoV-2 Spike protein reduced viral titers and the protection provided by anti-S antibodies that prevented the virus from fixing.

In an attempt to select an animal model for tests to assess the effectiveness of vaccines, GU et al. [137] used isolates of human SARS-CoV-2 to perform intranasal inoculation in older BALB/c mice. This method aimed to adapt this strain to the animal and allow the manifestation of infectious symptoms of the disease, with inflammatory responses and moderate pneumonia. There were six passages, in which the viral strain mutated amino acids Asn501 to Tyr (N501Y), inside the receptor-binding domain (RBD), increasing its virulence. Elderly animals showed more severe symptoms, but no visible clinical symptoms or weight loss were observed in any of the groups studied. The most common symptoms were denatured epithelial cells, alveolar damage. The animals were challenged with the virus intramuscularly, hemorrhage, and adherent

inflammatory cells, which appeared 3 days after inoculation. However, the symptoms regressed after 5 days, suggesting a process of self-recovery. These animals were tested to assess the protective action of a possible vaccine candidate. Subcutaneous immunization was performed with doses of recombinant RBD-Fc from mice that when challenged with the viral strain, showed high levels of specific antibodies against the virus and neutralizing antibodies, with reduced viral load and an extremely small number of infected cells when compared to the control group [137].

Yu et al. [138] assessed the impact of a candidate DNA vaccine, which encodes Spike protein, on the immunization of rhesus monkeys. Animals that were vaccinated showed cellular and humoral immune responses, similar to humans recovered from COVID-19 and monkeys infected with SARS-CoV-2. After immunization and challenge with the virus, their ability to respond to infection was demonstrated, suggesting the efficacy of this vaccine, which generated neutralizing antibodies in monkeys, proving to be promising.

Mercado et al. [139] studied the effectiveness of administering a single dose of an adenovirus-based vaccine against SARS-CoV-2 in nonhuman primates. The Ad26-S.PP vaccine (which encodes a pre-fused stabilized S immunogen), with a single administration, induced robust neutralizing antibody responses, with detectable S-specific antibodies (IgG and IgA). The animals also showed cellular immune responses and, when challenged with SARS-CoV-2, no virus replication was detected in the lungs.

Vaccine trials should consider the possibility of antibody-dependent enhancement (ADE). This event occurs when non-neutralizing antibodies or in subneutralizing concentrations bind to the virus, facilitating its entry into cells expressing the Fc receptor, such as macrophages. This process requires prior awareness, such as the use of inactivated whole virus vaccines, and an increase in viral infectivity may occur during subsequent exposure to the virus. When the virus-antibody complex is internalized by endocytosis, the production of high amounts of viral particles is stimulated, in addition to inflammatory mediators, which contribute to the serious evolution of cases [136,140]. Although there is no scientific evidence to confirm the occurrence of ADE in SARS-CoV-2 infection, studies using animal models have shown its occurrence in SARS and MERS-CoV, which will require monitoring of post-vaccination individuals [141].

5. Immunotherapies tests

Many studies aim to discover therapies that prevent Spike protein binding to the ACE2 receptor, to prevent the development of infection. In this context, the role of heterologous antibodies was studied, providing immediate immunity to the patient and preventing the binding of Spike protein to the ACE2 receptor [7,77].

For over 120 years, serotherapy has been the only officially recognized therapy for the treatment of venomous animal poisoning. The use of antivenoms in therapies started at the beginning of

the 20th century with Albert Calmette. At that time, first-generation hyperimmune sera were produced from immunized horses whose blood serum was free of red blood cells, but the purification process was practically nonexistent, which favored the manifestation of adverse reactions that were often fatal. Clinical trials and pharmacological research involving hyperimmune sera have only emerged since the 1950s. The therapeutic value outweighed the risk of adverse drug reactions, a concept that has been changing over the years, causing producers to modernize their production route, leading to the emergence of the second and third generation of hyperimmune sera [142,143].

Many producers of hyperimmune sera already have an antiviral product, anti-rabies serum, on their technological platform [7]. This serum neutralizes rabies virus particles, preventing their entry into cells, in addition to promoting antibody-dependent cell-mediated cytotoxicity [144]. In Brazil, Instituto Vital Brazil [145], Instituto Butantan [146], and Ezequiel Dias Foundation [147] are the three official laboratories that manufacture anti-rabies serum [7].

The search for a hyperimmune serum for the treatment of COVID-19 has led countries such as Brazil, Argentina, and Costa Rica to a new scenario in their research [148]. Argentina has already started phase I clinical studies, while in Brazil and Costa Rica, the studies are still in the preclinical phase [7]. Recently, the Brazilian National Health Surveillance Agency (ANVISA) authorized the Butantan Institute to start its clinical studies in humans, changing the status of this research in the country [149].

As established in the Brazilian Pharmacopoeia, sixth edition [150], for hyperimmune serums of general use already established in therapeutics for other diseases where it is used, the tests to determine the protective potency of the hyperimmune serum aim to determine the median effective dose (ED_{50}) capable to protect mice against lethal viral effects. For this, a statistical calculation was performed to compare the number of dead and alive animals in each test. This is a major difficulty in using this technique for anti-SARS-CoV-2 sera, as the ideal animal model that evolves to death spontaneously has not yet been defined.

Thus, *in vitro* plaque reduction neutralization test (PRNT) to quantify antibody titers, in equine plasma and hyperimmune serum was expressed as the dilution factor of the formulations in which 50% of the virus was detected. Neutralization was expressed as the ratio of protein concentration/ED_{50} [151,152].

According to Guimarães [153], among the available serological tests, the PRNT is the most specific when it comes to viruses. It is considered a gold standard for serology and requires BSL-3 laboratories to function [154]. This methodology exposes a cell monolayer to the study target virus and quantifies the neutralizing antibodies produced against this stimulus. When not neutralized, viruses invade the cell, causing cell lysis, which is visible, forming plaques of dead cells that can be observed. Antibodies added by the introduction of hyperimmune serum neutralize the virus, preventing these reactions [153]. In the Guimarães study, virus concentrations were kept constant, varying only the serum samples per dilution, which allowed the final result of the PRNT to be calculated for each serum sample dilution tested [153].

Cunha et al. [155] conducted two different studies, but the antigen used in both was the trimeric Spike protein. First, they

performed a bench test to assess the possibility of producing an equine anti-SARS-CoV-2 hyperimmune serum. In this test, equine plasma was purified, generating a concentrated $F(ab')_2$ solution with PRNT50 1:32.000 and PRNT90 1:16.000. In the second experiment, a pilot industrial batch was produced, in which the equine plasma purification process generated a concentrated $F(ab')_2$ solution PRNT50 1: 65.556 and PRNT90 1:16.384. The product was compared to plasma obtained from three convalescent patients for PRNT results, and the product was found to be approximately 150 times more potent than human plasma.

Herrera et al. [156] also performed two experiments, but each group received a type of antigen. The recombinant protein SARS-CoV-2 S1 Group 1 was used to immunize group 1 and group 2 received a mixture (anti-Mix) of S1, SEM (SPIKE-EM), and N protein. mean effective dose (ED_{50}) was 1:29.108 for anti-S1 and 1:25.355 for anti-Mix. Thus, when compared to convalescent plasma, the anti-S1 and anti-Mix formulations were considered 80 times more potent.

Zylberman et al. [152] and Pan et al. [157] used RBD as an immunogen. In the study by Zylberman et al. [152], the results of the in vitro neutralization tests were 1:10.240 (complete neutralization dilution) of the titer and 30 mg mL^{-1} of protein in the final product, concluding that it was 50 times the neutralizing potency of the convalescent serum used as a control [152]. The results obtained by Pan et al. [158] revealed that the neutralization rate for SARS-CoV-2 was greater than 90% by treatment with $F(ab')_2$ at a concentration of 31,15 µg mL^{-1} and more than 50% at a concentration of 7,81 µg mL^{-1} Sapkal et al. [159] used SARS-CoV-2 inactivated by gamma radiation to prepare the antigen, which generated a final product with neutralizing titers of PRNT90 above 20.000.

It is not possible to make a direct comparison between these studies, as they differ in several aspects, such as antigen, adjuvant, immunization plan, and plasma purification process. Each one, in its own way, managed to develop a hyperimmune plasma with a superior neutralizing capacity when compared to the plasma of a convalescent patient, regardless of the chosen production route. Lopardo et al. [160] recently published a clinical study with anti-SARS-CoV-2 hyperimmune serum in Argentina. Although this study has many limitations, the authors suggested that the serum was more promising in severe cases and indicated the need for further studies.

Research using animals to verify the potency of hyperimmune sera has not been published yet. However, Pakdemirli et al. [161] used animals to assess nonspecific toxicity. In these assays, mice were intraperitoneally inoculated with the hyperimmune serum and monitored for seven days to verify weight loss. The product is considered nontoxic when no animal has reduced weight. In addition to these tests, it is also possible to analyze the presence of pyrogen in the samples using rabbits, following the current Pharmacopoeia [150].

Although the study with monoclonal antibodies also finds difficulties in establishing a unique animal model for evaluating SARS-CoV-2, Baum et al. [162] analyzed the efficacy of a two-human monoclonal antibodies cocktail in hamsters and rhesus monkeys. The trials aimed to investigate the neutralizing capacity of antibodies in these models that exhibited mild (rhesus monkeys) or severe (hamsters) symptoms. This allowed a broader understanding of the evolution of this disease with the use of this therapy, the impact on the viral load, and the pathological manifestation of the infection. Jia et al. [135] reported that the CB6 monoclonal antibody reduced the viral load in rhesus monkeys, whereas inhibited pathological pulmonary manifestations. Furthermore, the MD65 antibody protected transgenic mouse hACE2 against infection, and CC12.1 protected the Syrian hamster against weight loss and lung disease. These studies demonstrated the need for more studies involving preclinical *in vivo* evaluation of new immunological therapies against COVID-19.

6. Expert opinion

The search for animal models capable of faithfully reproducing the course of COVID-19 in humans or allowing trials of new therapies against this new disease is complex and requires efforts by many researchers.

The use of animals in preclinical trials is a controversial issue, especially with regard to the question of whether these models produce reliable results. On the other hand, one should consider the ease of using animal models to assess the course of the disease, since they can be sacrificed, allowing a more in-depth analysis of some parameters, such as histopathology.

A single model is unlikely to have all the characteristics related to the disease in humans, and it may be necessary to use more than one model. In addition, given the current global health emergency scenario, which requires the rapid development of new therapies to treat COVID-19, more researches are required to establish which animal model is best suited for use in experiments with the disease.

One case that deserves to be cited is that of hyperimmune serum, under development in countries such as Brazil, Argentina, and Costa Rica, which have not yet established the ideal animal model for use in their tests. As a result, there are still no publications referring to its pre-clinical animal tests. Generally, the potency of the antivenoms is tested in mice and analyzed for the relationship between live and dead animals at each dilution challenged. Unlike conventional antivenom tests, in the case of SARS-CoV-2, wild mice are no longer the ideal model, as animals do not die easily due to infection with this virus. Thus, studies to evaluate the efficacy of these products have been carried out and presented by means of plaque sero-neutralization techniques *in vitro*.

This unprecedented pandemic has highlighted a problem that consists of the availability of animals for clinical trials with SARS-CoV-2. In view of the increase in the development of new vaccines and antiviral drugs development, the supply of ideal animals has not kept up with demand. This had a direct impact on the price and availability of these animals, favoring countries with more financial resources and greater purchasing power to the detriment of the poorest countries.

The use of animals in pre-clinical tests is still part of an ethical discussion, in the scope of animal protection. In this sense, alternative techniques should be encouraged and employed, whenever possible. It is worth mentioning the need to develop validated *in vitro* methodologies, capable of replacing in vivo

tests in safety and efficacy tests for vaccines, hyperimmune and antiviral serums. This thinking reinforces the ideology of cruelty free, where products are developed without the need for testing on animal models. The cosmetic industry has already evolved a lot in this aspect, making us think that the pharmaceutical industry also needs to move forward.

Despite this, the current pandemic scenario has made ethical question regarding animal research temporarily less relevant, for the rapid and effective development of vaccines. Thus, the use of *in vitro* assays in combination with *in vivo* assays, is still important as a confirmatory test or guideline for animal testing. This approach can improve the toxicological, safety, and efficacy assessment in the development of new drugs, as well as to reduce the number of animals used in tests. Furthermore, *in vitro* assays are generally accessible, have good reproducibility and have fewer ethical limitations.

All of these factors reflect the importance of international collaborations, which allow the exchange of knowledge and equity in scientific research. This is more evident at this time, when countries with less financial resources have difficulties developing research for prophylactic treatments or new therapies, but have high rates of new cases and deaths, in addition to new variants of the virus. An example of this successful collaboration is the Covax Facility program, which reduces disparities in the acquisition of vaccines, created by the World Health Organization with some philanthropic entities. This program aims to distribute vaccines equally, without neglecting low-income nations.

Funding

This work was supported by the Pro-Rectory of Research, Graduate Studies and Innovation (PROPPI), the Universidade Federal Fluminense, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) [FAPERJ - E-26/202.447/2019] and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)- Finance Code 001, both financially and with fellowships.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

ORCID

Camila B. P. Da Costa http://orcid.org/0000-0002-6926-7956
Ana Claudia De Menezes Cruz http://orcid.org/0000-0002-4520-6884
Julio Cesar Q Penha http://orcid.org/0000-0002-5249-9742
Helena C Castro http://orcid.org/0000-0001-5283-1541
Luis E. R. Da Cunha http://orcid.org/0000-0003-1381-0611
Norman A Ratcliffe http://orcid.org/0000-0001-8538-0636
Rafael Cisne http://orcid.org/0000-0002-4624-3935
Francislene Juliana Martins http://orcid.org/0000-0002-6149-8258

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. MacKenzie JS, Smith DW. COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. Microbiol Aust. 2020;41(1):45–50.
2. Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 2020;368(6494):1016–1020.
3. Wang Q, Zhang Y, Wu L, et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell. 2020;181(4):894–904.e9.
4. Sanclemente-Alaman I, Moreno-Jiménez L, Benito-Martín MS, et al. Experimental models for the study of central nervous system infection by SARS-CoV-2. Front Immunol. 2020;11:1–12.
5. World Health Organization. Coronavirus disease (COVID-19). 2020. [cited 2021 Oct]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019?gclid=Cj0KCQjwt-6LBhDIARIsAIPRQcJfRW8z2zccy8yXZnrdbNAR1IcgQgN6lOaFisxgvfy-4urMrGPn2swaAnITEALw_wcB.
6. World Health Organization. WHO coronavirus disease (COVID-19) dashboard. 2021. [cited 2021 Oct]. Available from: https://covid19.who.int/
7. Da Costa CBP, Martins FJ, Da Cunha LER, et al. COVID-19 and Hyperimmune sera: a feasible plan B to fight against coronavirus. Int Immunopharmacol [Internet]. 2021:90:107220.
•• **This article is a review that describing the development of a hyperimmune serum in the treatment of coronavirus infections over the past few years emphasizing its effectiveness in treatment.**
8. Muñoz-Fontela C, Dowling WE, Funnell SGP, et al., Animal models for COVID-19. Nature. 2020;586(7830):509–515.
•• **This review covers preclinical trials using animal models on possible vaccine candidates.**
9. Ku M-W, Bourgine M, Authié P, et al. Intranasal vaccination with a lentiviral vector protects against SARS-CoV-2 in preclinical animal models graphical. Cell Host Microbe. 2021;29(2):236–249.
10. Teerawattananon Y, Dabak SV. COVID vaccination logistics: five steps to take now. Nature. 2020;587(7833):194–196.
11. World Health Organization. WHO: draft landscape of COVID-19 candidate vaccines – 12. 2020.
12. Ahangarzadeh S, Payandeh Z, Arezumand R, et al. An update on antiviral antibody-based biopharmaceuticals. Int Immunopharmacol [Internet]. 2020;86:106760.
13. Johansen MD, Irving A, Montagutelli X, et al. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunol [Internet]. 2020;13(6):877–891.
•• **This article presents important aspects of the pathogenesis of COVID-19 in animal models.**
14. Park SJ, Yu KM, Kim Y Il, et al. Antiviral efficacies of FDA-approved drugs against SARS-COV-2 infection in ferrets. MBio. 2020;11(3):1–10.
15. Wang G, Yang ML, Duan ZL, et al. Dalbavancin binds ACE2 to block its interaction with SARS-CoV-2 spike protein and is effective in inhibiting SARS-CoV-2 infection in animal models. Cell Res [Internet]. 2021;31(1):17–24.
16. Cleary SJ, Pitchford SC, Amison RT, et al. Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. Br J Pharmacol. 2020;177(21):4851–4865.
17. Wang J, Shuai L, Wang C, et al. Mouse-adapted SARS-CoV-2 replicates efficiently in the upper and lower respiratory tract of BALB/c and C57BL/6J mice. Protein Cell. 2020;11(10):776–782.
18. Lam SD, Bordin N, Waman VP, et al. SARS-CoV-2 spike protein predicted to form complexes with host receptor protein orthologues from a broad range of mammals. Sci Rep [Internet]. 2020;10(1):1–14.
19. Gurumurthy CB, Quadros RM, Richardson GP, et al. Genetically modified mouse models to help fight COVID-19. Nat Protoc. 2020;15(12):3777–3787.

20. Pujhari S, Rasgon JL. Mice with humanized-lungs and immune system - an idealized model for COVID-19 and other respiratory illness. Virulence. 2020;11(1):486–488.
21. Alluwaimi AM, Alshubaith IH, Al-Ali AM, et al. The coronaviruses of animals and birds: their zoonosis, vaccines, and models for SARS-CoV and SARS-CoV2. Front Vet Sci. 2020;7:1–12.
22. Deb B, Shah H, Goel S. Current global vaccine and drug efforts against COVID-19: pros and cons of bypassing animal trials. J Biosci. 2020;45(1):1–10.
- **This review presents aspects of the pathogenesis of COVID-19 in animal models and makes some comparisons with humans.**
23. Hewitt JA, Lutz C, Florence WC, et al. ACTIVating resources for the COVID-19 pandemic: in vivo models for vaccines and therapeutics. Cell Host Microbe [Internet]. 2020;28(5):646–659.
24. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of coronavirus disease 2019 (CoVID-19) from publicly reported confirmed cases: estimation and application. Ann Intern Med. 2020;172 (9):577–582.
25. Browne SK, Beeler JA, Roberts JN. Summary of the vaccines and related biological products advisory committee meeting held to consider evaluation of vaccine candidates for the prevention of respiratory syncytial virus disease in RSV-naïve infants. Vaccine [Internet]. 2020;38(2):101–106.
26. Yuan L, Tang Q, Cheng T, et al. Animal models for emerging coronavirus: progress and new insights. Emerg Microbes Infect [Internet]. 2020;9(1):949–961.
27. Speranza E, Williamson BN, Feldmann F, et al. Single-cell RNA sequencing reveals SARS-CoV-2 infection dynamics in lungs of African green monkeys. Sci Transl Med. 2021;13(578):1–13.
28. Bertzbach LD, Vladimirova D, Dietert K, et al. SARS-CoV-2 infection of Chinese hamsters (Cricetulus griseus) reproduces COVID-19 pneumonia in a well-established small animal model. Transbound Emerg Dis. 2021;68(3):1075–1079.
29. Qiao J, Li Y, Zeng R, et al. SARS-CoV-2 M pro inhibitors with antiviral activity in a transgenic mouse model. Science. 2021;371 (6536):1374–1378.
30. Driouich JS, Cochin M, Lingas G, et al. Favipiravir antiviral efficacy against SARS-CoV-2 in a hamster model. Nat Commun [Internet]. 2021;12(1):1–13.
31. Blair RV, Vaccari M, Doyle-Meyers LA, et al. Acute respiratory distress in aged, SARS-CoV-2–infected African green monkeys but not rhesus macaques. Am J Pathol. 2021;191(2):274–282.
32. Lutz C, Maher L, Lee C, et al. COVID-19 preclinical models: human angiotensin-converting enzyme 2 transgenic mice. Hum Genomics. 2020;14(1):1–11.
33. Eroshenko N, Gill T, Keaveney MK, et al. Implications of antibody-dependent enhancement of infection for SARS-CoV-2 countermeasures. Nat Biotechnol. 2020;38(7):788–789.
34. Zeiss CJ, Compton S, Veenhuis RT. Animal models of COVID-19. I. comparative virology and disease pathogenesis. ILAR J. 2021;7:1–13.
•• **This article describes the interaction between SARS-CoV-2 and ACE2, the importance of the amino acid sequence of this enzyme in this process and its influence on the manifestation of symptoms in several animal models.**
35. Wang Y, Yang C, Song Y, et al. Scalable live-attenuated SARS-CoV-2 vaccine candidate demonstrates preclinical safety and efficacy. Proc Natl Acad Sci U S A. 2021;118:1–7.
36. Peng R, Wu LA, Wang Q, et al. Cell entry by SARS-CoV-2. Trends Biochem Sci [Internet]. 2021:xx:1–13.
- **This article describes the interaction between SARS-CoV-2 and ACE2 and the infection process in organs and tissues.**
37. Munster VJ, Feldmann F, Williamson BN, et al. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. Nature. 2020;585 (7824):268–272.
38. Shan C, Yao YF, Lou YX, et al. Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in rhesus macaques. Cell Res [Internet]. 2020;30(8):670–677.
39. Lu S, Zhao Y, Yu W, et al. Comparison of nonhuman primates identified the suitable model for COVID-19. Signal Transduct Target Ther [Internet]. 2020;5. DOI:10.1038/s41392-020-00269-6.
40. McAuliffe J, Vogel L, Roberts A, et al. Replicarion of SARS coronavirus administered into the respiratory tract of African green, rhesus and cynomolgus monkeys. Virology. 2004;330(1):8–15.
41. Rockx B, Kuiken T, Herfst S, et al. SARS-CoV-2 causes COVID-19-like disease in cynomolgus macaques. Science. 2020;368:1012–1015.
42. Khoury DS, Wheatley AK, Ramuta MD, et al. Measuring immunity to SARS-CoV-2 infection: comparing assays and animal models. Nat Rev Immunol. 2020;20(12):727–738.
43. Greenough TC, Carville A, Coderre J, et al. Pneumonitis and multi-organ system disease in common marmosets (Callithrix jacchus) infected with the severe acute respiratory syndrome-associated coronavirus. Am J Pathol [Internet]. 2005;167(2):455–463.
44. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. Trends Immunol. 2020;41 (12):1100–1115.
45. Zheng H, Li H, Guo L, et al. Virulence and pathogenesis of SARS-CoV-2 infection in rhesus macaques: a nonhuman primate model of COVID-19 progression. PLoS Pathog [Internet]. 2020;16(11):1–23.
46. Aid M, Busman-Sahay K, Vidal SJ, et al. Vascular disease and thrombosis in SARS-CoV-2-infected rhesus macaques. Cell [Internet]. 2020;183(5):1354–1366.e13.
47. Jiao L, Li H, Xu J, et al. The gastrointestinal tract is an alternative route for SARS-CoV-2 infection in a nonhuman primate model. Gastroenterology. 2021;160(5):1647–1661.
48. Bao L, Deng W, Huang B, et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. Nature. 2020;583(7818):830–833.
49. Song TZ, Zheng HY, Han JB, et al. Delayed severe cytokine storm and immune cell infiltration in SARS-CoV-2-infected aged Chinese rhesus macaques. Zool Res. 2020;41(5):503–516.
50. Deng W, Bao L, Liu J, et al. Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. Science. 2020;369(6505):818–823.
51. Salguero FJ, White AD, Slack GS, et al. Comparison of rhesus and cynomolgus macaques as an infection model for COVID-19. Nat Commun. 2021;12(1). DOI:10.1038/s41467-021-21389-9.
52. Ishigaki H, Nakayama M, Kitagawa Y, et al. Neutralizing antibody-dependent and -independent immune responses against SARS-CoV-2 in cynomolgus macaques. Virology. 2020;554:97–105.
53. Cross RW, Prasad AN, Borisevich V, et al. Use of convalescent serum reduces severity of COVID-19 in nonhuman primates. Cell Rep. 2021;34(10):108837.
54. Stittelaar KJ, De Waal L, Van Amerongen G, et al. Ferrets as a novel animal model for studying human respiratory syncytial virus infections in immunocompetent and immunocompromised hosts. Viruses. 2016;8(6):168.
55. Zhang Q, Shi J, Deng G, et al. H7N9 influenza viruses are transmissible in ferrets by respiratory droplet. Science. 2013;341 (6144):410–414.
56. Van Der Brand JM, Haagmans BL, Leijten L, et al. Pathology of experimental SARS Coronavirus infection in cats and ferrets. Vet Pathol. 2008;45(4):551–562.
57. Cohen J. From mice to monkeys, animals studied for coronavirus answers. Infected lab animals can assess drugs and vaccines. Science. 2020;368(6488):221–222.
58. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol [Internet]. 2020;5(4):562–569.
59. Ryan KA, Bewley KR, Fotheringham SA, et al. Dose-dependent response to infection with SARS-CoV-2 in the ferret model and evidence of protective immunity. Nat Commun [Internet]. 2021;12 (1):1–13.
60. De Vries RD, Schmitz KS, Bovier FT, et al. Intranasal fusion inhibitory lipopeptide prevents direct-contact SARS-CoV-2 transmission in ferrets. Science. 2021;1382:1379–1382.
61. Kim Y-I, Kim S-G, Kim S-M, et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. Cell Host Microbe. 2020;27(5):704–709.
62. Richard M, Kok A, De Meulder D, et al. SARS-CoV-2 is transmitted via contact and via the air between ferrets. Nat Commun [Internet]. 2020;11(1):1–6.

63. Fenollar F, Mediannikov O, Maurin M, et al. Mink, SARS-CoV-2, and the human-animal interface. Front Microbiol. 2021;12. DOI:10.3389/fmicb.2021.663815.
64. Rosenfeld R, Noy-Porat T, Mechaly A, et al. Post-exposure protection of SARS-CoV-2 lethal infected K18-hACE2 transgenic mice by neutralizing human monoclonal antibody. Nat Commun. 2021;12 (1):2–10.
65. Pagliano P, Sellitto C, Scarpati G, et al. An overview of the preclinical discovery and development of remdesivir for the treatment of coronavirus disease 2019 (COVID-19). Expert Opin Drug Discov [Internet]. 2021:1–10. DOI:10.1080/17460441.2021.1970743.
66. Magadum A, Kishore R. Cardiovascular manifestations of COVID-19 infection. Cells. 2020;4:1–21.
67. Arce VM, Costoya JA. SARS-CoV-2 infection in K18-ACE2 transgenic mice replicates human pulmonary disease in COVID-19. Cell Mol Immunol [Internet]. 2021;18(3):513–514.
68. Zhang YN, Li XD, Zhang ZR, et al. A mouse model for SARS-CoV-2 infection by exogenous delivery of hACE2 using alphavirus replicon particles. Cell Res. 2020;30(11):1046–1048.
69. Colunga Biancatelli RML, Solopov PA, Sharlow ER, et al. The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in K18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells. Am J Physiol Cell Mol Physiol. 2021;321 (2):L477–L484.
70. Dinnon KH, Leist SR, Schäfer A, et al. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. Nature. 2020;586 (7830):560–566.
71. Körner RW, Majjouti M, Alejandre Alcazar MA, et al. Of mice and men: the coronavirus mhv and mouse models as a translational approach to understand SARS-CoV-2. Viruses. 2020;12 (8):1–26.
72. McCray PB, Pewe L, Wohlford-Lenane C, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol. 2007;81(2):813–821.
73. Rathnasinghe R, Strohmeier S, Amanat F, et al. Comparison of transgenic and adenovirus hACE2 mouse models for SARS-CoV-2 infection. bioRxiv Prepr Serv Biol. 2020;9(1):2433-2445.
74. Munshi I, Khandvilkar A, Chavan SM, et al. An overview of preclinical animal models for SARS-CoV-2 pathogenicity. The Indian Journal of Medical Research 2020;153(1-2):517–520.
75. Winkler ES, Bailey AL, Kafai NM, et al. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. Nat Immunol [Internet]. 2020;21 (11):1327–1335.
76. Sun J, Zhuang Z, Zheng J, et al. Generation of a broadly useful model for COVID-19 pathogenesis, vaccination, and treatment. Cell [Internet]. 2020;182(3):734–743.e5.
77. Datta PK, Liu F, Fischer T, et al. SARS-CoV-2 pandemic and research gaps: understanding SARS-CoV-2 interaction with the ACE2 receptor and implications for therapy. Theranostics. 2020;10 (16):7448–7464.
78. Golden JW, Cline CR, Zeng X, et al. Human angiotensin-converting enzyme 2 transgenic mice infected with SARS-CoV-2 develop severe and fatal respiratory disease. JCI Insight. 2020;5(19):e–142032.
79. Moreau GB, Burgess SL, Sturek JM, et al. Evaluation of K18-hACE2 mice as a model of SARS-CoV-2 infection. Am J Trop Med Hyg. 2020;103(3):1215–1219.
80. Jiang RD, Liu MQ, Chen Y, et al. Pathogenesis of SARS-CoV-2 in transgenic mice expressing human angiotensin-converting enzyme 2. Cell. 2020;182(1):50–58.e8.
81. Israelow B, Song E, Mao T, et al. Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. J Exp Med. 2020;217(12). DOI:10.1084/jem.20201241.
82. Sun SH, Chen Q, Gu HJ, et al. A mouse model of SARS-CoV-2 infection and pathogenesis. Cell Host Microbe [Internet]. 2020;28 (1):124–133.e4.
83. Leist SR, Dinnon III KH, Sachäfer A, et al. A mouse-adapted SARS-CoV-2 induces acute lung injury and mortality in standard laboratory mice. Cell. 2020;183(4):1070–1085.
84. Zhu A, Chen Z, Wang Y, et al. Immune responses to SARS-CoV-2 infection in humans and ACE2 humanized mice. Fundam Res [Internet]. 2021;1(2):124–130.
85. Hassan AO, Case JB, Winkler ES, et al. A SARS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies. Cell [Internet]. 2020;182(3):744–753.e4.
86. Chan JF, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility authors: Jasper Fuk-Woo Chan. Clin Infect Dis. 2019;2019:1–50.
87. Imai M, Iwatsuki-Horimoto K, Hatta M, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. Proc Natl Acad Sci U S A. 2020;117:16587–16595.
88. Sia SF, Yan LM, Chin AWH, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature [Internet]. 2020;583 (7818):834–838.
89. Haagmans BL, Noack D, Okba NMA, et al. SARS-CoV-2 neutralizing human antibodies protect against lower respiratory tract disease in a hamster model. J Infect Dis. 2021;223(12):2020–2028.
90. Brocato RL, Kwilas SA, Kim RK, et al. Protective efficacy of a SARS-CoV-2 DNA vaccine in wild-type and immunosuppressed Syrian hamsters. Npj Vaccines [Internet]. 2021;6(1):1–7.
91. Nambulli S, Xiang Y, Tilston-Lunel NL, et al. Inhalable nanobody (PiN-21) prevents and treats SARS-CoV-2 infections in Syrian hamsters at ultra-low doses. Sci Adv. 2021;7(22):eabh0319.
92. Osterrieder N, Bertzbach LD, Dietert K, et al. Age-dependent progression of SARS-CoV-2 infection in syrian hamsters. Viruses. 2020;12(7):1–11.
93. Tomris I, Bouwman KM, Adolfs Y, et al. 3D visualization of SARS-CoV-2 infection and receptor distribution in Syrian hamster lung lobes display distinct spatial arrangements. bioRxiv Prepr Serv Biol. 2021;1–27. DOI: https://doi.org/10.1101/2021.03.24.435771
94. Lee ACY, Zhang AJ, Chan JFW, et al. Oral SARS-CoV-2 inoculation establishes subclinical respiratory infection with virus shedding in golden Syrian hamsters. Cell Rep Med [Internet]. 2020;1 (7):100121.
95. Cimolai N. Defining protective epitopes for COVID-19 vaccination models. J Med Virol. 2020;92(10):1772–1773.
96. Du S, Cao Y, Zhu Q, et al. Structurally resolved SARS-CoV-2 antibody shows high efficacy in severely infected hamsters and provides a potent cocktail pairing strategy. Cell. 2020;183(4):1013–1023.e13.
97. Suresh V, Mohanty V, Avula K, et al. Quantitative proteomics of hamster lung tissues infected with SARS-CoV-2 reveal host factors having implication in the disease pathogenesis and severity. FASEB J. 2021;35(7):1–15.
98. Daly JL, Simonetti B, Klein K, et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science. 2020;370(6518):861–865.
99. Cantuti-Castelvetri L, Ojha R, Pedro L, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 2020;6518(370):856-860
100. Preziuso S. Severe acute respiratory syndrome coronavirus 2 (Sars-cov-2) exhibits high predicted binding affinity to ace2 from lagomorphs (rabbits and pikas). Animals. 2020;10(9):1–11.
101. Maurin M, Fenollar F, Mediannikov O, et al. Current status of putative animal sources of SARS-CoV-2 infection in humans: wildlife, domestic animals and pets. Microorganisms. 2021;9(4):868.
102. Mahdy MAA, Younis W, Ewaida Z. An overview of SARS-CoV-2 and animal infection. Front Vet Sci. 2020;7. DOI:10.3389/fvets.2020.596391
103. Prince T, Smith SL, Radford AD, et al. Sars-cov-2 infections in animals: reservoirs for reverse zoonosis and models for study. Viruses. 2021;13(3):494.
104. Hammer AS, Quaade ML, Rasmussen TB, et al. SARS-CoV-2 transmission between Mink (Neovison vison) and humans, Denmark. Emerg Infect Dis. 2021;27(2):547–551.
105. Munnink BBO, Sikkema RS, Nieuwenhuijse DF, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science. 2021;371(6525):172–177.

106. Sharun K, Tiwari R, Natesan S, et al. SARS-CoV-2 infection in farmed minks, associated zoonotic concerns, and importance of the one health approach during the ongoing COVID-19 pandemic. Vet Q [Internet]. 2021;41(1):50–60.
107. World Health Organization. SARS-CoV-2 mink-associated variant strain – Denmark (November 6, 2020). 2020.
108. World Health Organization. SARS-CoV-2 mink-associated variant strain – Denmark (December 3, 2020). 2020.
109. Hoffmann M, Zhang L, Krüger N, et al. SARS-CoV-2 mutations acquired in mink reduce antibody-mediated neutralization. Cell Rep. 2021;35(3):109017.
110. Bayarri-Olmos R, Rosbjerg A, Johnsen LB, et al. The SARS-CoV-2 Y453F mink variant displays a pronounced incresase in ACE-2 affinity but does not challenge antibody neutralization. J Biol Chem. 2021;296:100536.
111. Balkrishna A, Solleti SK, Verma S, et al. Application of humanized Zebrafish model in the suppression of SARS-CoV-2 spike protein induced pathology by tri-herbal medicine coronil via cytokine modulation. Molecules. 2020;25(21):5091.
112. Fernandes BHV, Feitosa NM, Barbosa AP, et al. Zebrafish studies on the vaccine candidate to COVID-19, the spike protein: production of antibody and adverse reaction. bioRxiv. 2020. DOI: https://doi.org/10.1101/2020.10.20.346262
113. Mykytyn AZ, Lamers MM, Okba NMA, et al. Susceptibility of rabbits to SARS-CoV-2. Emerg Microbes Infect. 2021;10(1):1–7.
114. Larsen HD, Fonager J, Lomholt FK, et al. Preliminary report of an outbreak of SARS-CoV-2 in mink and mink farmers associated with community spread, Denmark, June to November 2020. Eurosurveillance [Internet]. 2021;26(5). DOI:10.2807/1560-7917.ES.2021.26.5.210009.
115. Lee C-Y, Lowen AC. Animal models for SARS-CoV-2. Curr Opin Virol. 2021;48:73–81.
116. Winkler MS, Skirecki T, Brunkhorst FM, et al. Bridging animal and clinical research during SARS-CoV-2 pandemic: a new-old challenge. EBioMedicine. 2021;66:103291.
117. Michelitsch A, Wernike K, Ulrich L, et al. SARS-CoV-2 in animals: from potential hosts to animal models [Internet]. 1st ed. Adv Virus Res Elsevier Inc. 2021. DOI:10.1016/bs.aivir.2021.03.004.
118. Takayama K. In vitro and animal models for SARS-CoV-2 research. Trends Pharmacol Sci [Internet]. 2020;41(8):513–517.
119. Jarnagin K, Alvarez O, Shresta S, et al. Animal models for SARS-Cov2/Covid19 research-A commentary Jarnagin.pdf. Biochem Pharmacol. 2021;188:114543.
120. Pavia CS, Plummer MM. Impact of the COVID-19 pandemic on Easter. Wien Klin Wochenschr; 2021. DOI: 10.1007/s00508-021-01853-8.
121. Shuai L, Zhong G, Yuan Q, et al. Replication, pathogenicity, and transmission of SARS-CoV-2 in minks. Natl Sci Rev. 2021;8(3). DOI:10.1093/nsr/nwaa291.
122. Zhang Q, Zhang H, Gao J, et al. A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg Microbes Infect. 2020;9(1):2013–2019.
123. Garigliany M, Van Laere AS, Clercx C, et al. SARS-CoV-2 natural transmission from human to cat, Belgium, March 2020. Emerg Infect Dis. 2020;26(12):3069–3071.
124. Mallapaty S. Coronavirus can infect cats - dogs, not so much. Nature. 2020. DOI: https://doi.org/10.1038/d41586-020-00984-8
125. Abdel-Moneim AS, Abdelwhab EM. Evidence for SARS-COV-2 infection of animal hosts. Pathogens. 2020;9(7):1–27.
126. Goumenou M, Spandidos DA, Tsatsakis A. Possibility of transmission through dogs being a contributing factor to the extreme Covid-19 outbreak in North Italy. Mol Med Rep. 2020;21:2293–2295.
127. Sit THC, Brackman CJ, Ip SM, et al. Infection of dogs with SARS-CoV-2. Nature [Internet]. 2020;586(7831):776–778.
128. Freuling CM, Breithaupt A, Müller T, et al. Susceptibility of raccoon dogs for experimental SARS-CoV-2 infection. Emerg Infect Dis. 2020;26(12):2982–2985.
129. Rodriguez-Morales AJ, Dhama K, Sharun K, et al. Susceptibility of felids to coronaviruses. Vet Rec. 2020;186(17). DOI:10.1136/vr.m1671.
130. Csiszar A, Jakab F, Valencak TG, et al. Companion animals likely do not spread COVID-19 but may get infected themselves. GeroScience. 2020;42(5):1229–1236.
131. Wu S, Zhong G, Zhang J, et al. A single dose of an adenovirus-vectored vaccine provides protection against SARS-CoV-2 challenge. Nat Commun [Internet]. 2020;11. DOI:10.1038/s41467-020-17972-1.
132. Russo G, Di Salvatore V, Caraci F, et al. How can we accelerate COVID-19 vaccine discovery? Expert Opin Drug Discov [Internet]. 2021;16(10):1081–1084.
133. Van Doremalen N, Lambe T, Spencer A, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. Nature. 2020;586(7830):578–582.
134. Tarke A, Sidney J, Kidd CK, et al. Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. Cell Rep Med [Internet]. 2021;2(2):100204.
135. Jia W, Wang J, Sun B, et al. The mechanisms and animal models of SARS-CoV-2 infection. Front Cell Dev Biol. 2021;9:1–13.
136. Graham BBS. Rapid COVID-19 vaccine development availability includes the avoidance of safety pitfalls. Science. 2020;368(6494):945–946.
137. Gu H, Chen Q, Yang G, et al. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. Science. 2020;369(6511):1603–1607.
138. Yu YZ, Zhang SM, Ma Y, et al. Development and evaluation of candidate vaccine and antitoxin against botulinum neurotoxin serotype F. Clin Immunol [Internet]. 2010;137(2):271–280.
139. Mercado NB, Zahn R, Wegmann F, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature [Internet]. 2020;586(7830):583–588.
140. Taylor A, Foo SS, Bruzzone R, et al. Fc receptors in antibody-dependent enhancement of viral infections. Immunol Rev. 2015;268(1):340–364.
141. Malik JA, Mulla AH, Farooqi T, et al. Targets and strategies for vaccine development against SARS-CoV-2. Biomed Pharmacother. 2021;137:111254.
142. Chippaux JP, Stock RP, Massougbodji A. Antivenom safety and tolerance for the strategy of snake envenomation management. Snake Venoms. 2015;1–16. DOI: https://doi.org/10.1007/978-94-007-6648-8_25-1.
143. Boyer L, Degan J, Ruha AM, et al. Safety of intravenous equine F(ab')2: insights following clinical trials involving 1534 recipients of scorpion antivenom. Toxicon [Internet]. 2013;76:386–393.
144. Squaiella-Baptistão CC, Magnoli FC, Marcelino JR, et al. Quality of horse F(ab')2 antitoxins and anti-rabies immunoglobulins: protein content and anticomplementary activity. J Venom Anim Toxins Incl Trop Dis. 2018;24(1):1–9.
145. Vital-Brazil I. Serums [Internet]. 2020. [cited 2021 May]. Available from: http://www.vitalbrazil.rj.gov.br/soros_produzidos.html.
146. Institute Butantan. Serums [Internet]. 2020. [cited 2021 May]. Available from: https://www.butantan.gov.br/soros-e-vacinas/soros.
147. Ezequiel-Dias F. Products [Internet]. 2020. [cited 2021 May]. Available from: http://www.funed.mg.gov.br/produtos-2/.
148. Alape-Girón A, Moreira-Soto A, Arguedas M, et al. Heterologous hyperimmune polyclonal antibodies against SARS-CoV-2: a broad coverage, affordable, and scalable potential immunotherapy for COVID-19. Front Med. 2021;8:1–5.
149. Brazil ANDVS. Anvisa autoriza testes do soro do Butantan. 2021.
150. Agência Nacional De Vigilância Sanitária. Farmacopeia Brasileira. Farm. Bras. 6ª edição. 2019.
151. León G, Herrera M, Vargas M, et al. Development and pre-clinical characterization of two therapeutic equine formulations towards SARS-CoV-2 proteins for the potential treatment of COVID-19. bioRxiv. 2020;21:1–9.
152. Zylberman V, Sanguineti S, Pontoriero AV, et al. Development of a hyperimmune equine serum therapy for covid-19 in Argentina. Medicina (B Aires). 2020;80:1–6.

•• **This article describes the development of a highly potent hyperimmune serum for the treatment of SARS-CoV-2 infections.**

153. Guimarães DDF. Desenvolvimento e padronização de ensaio imunoenzimático para detecção de anticorpos contra os vírus do Oeste do Nilo e da encefalite de São Luís. Desenvolvimento e padronização de ensaio imunoenzimático para detecção de anticorpos contra os vírus do Oes. 2017.
154. Musso D, Desprès P. Serological diagnosis of flavivirus-associated human infections. Diagnostics. 2020;10(5):1–13.
155. Cunha LER, Stolet AA, Strauch MA, et al. Equine hyperimmune globulin raised against the SARS-CoV-2 spike glycoprotein has extremely high neutralizing titers. bioRxiv [Internet]. 2020;2020.08.17.254375. DOI:10.1101/2020.08.17.254375.
•• **This article describes the development of a highly potent hyperimmune serum for the treatment of SARS-CoV-2 infections.**
156. Herrera M, León G, Segura A, et al., Factors associated with adverse reactions induced by caprylic acid-fractionated whole IgG preparations: comparison between horse, sheep and camel IgGs. Toxicon. 2005;46(7):775–781.
•• **This article describes the development of a highly potent hyperimmune serum for the treatment of SARS-CoV-2 infections.**
157. Pan X, Zhou P, Fan T, et al. Immunoglobulin fragment F(ab')2 against RBD potently neutralizes SARS-CoV-2 in vitro. Antiviral Res [Internet]. 2020;182:104868.
158. Pan X, Wu Y, Wang W, et al. Development of horse neutralizing immunoglobulin and immunoglobulin fragments against Junín virus Xiaoyan. Antiviral Res. 2020;174:104666.
159. Sapkal G, Yadav A, Deshpande GR, et al. Development of equine antisera with high neutralizing activity against SARS-CoV-2 (Preprint). Res Sq. 2020;1–16. DOI: https://doi.org/10.21203/rs.3.rs-83582/v1
160. Lopardo G, Belloso WH, Nannini E, et al. RBD-specific polyclonal F (ab´)2 fragments of equine antibodies in patients with moderate to severe COVID-19 disease: a randomized, multicenter, double-blind, placebo-controlled, adaptive phase 2/3 clinical trial. EClinicalMedicine. 2021;34:100843.
161. Pakdemirli A, Çaliskan E, Hacioglu S, et al. History repeats itself: horse originated Hyperimmune sera production against SARS CoV-2. Turk J Med Sci. 2021;51(5):2263-2273.
162. Baum A, Ajithdoss D, Copin R, et al. REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamster. Science. 2020;370(6520):1110–1115.