

## **Commentary**

### **Recent Advancements in Developing Animal Models for COVID-19**

#### **Abstract**

The SARS-CoV-2 virus's prominence, severity, and unique characteristics—including its ability to mutate quickly and cause idiosyncratic symptoms—has prompted researchers to fully focus on understanding the pathological process behind infection and developing an effective vaccine. To achieve this objective, several animal models, from small animals to non-human primates (NHPs), have been developed to identify different immunizing agents, which can provide protection against coronavirus disease (COVID-19). In this review, we discuss the possible advantages and drawbacks of these animal models including their susceptibility to infection, and pathological manifestations. While vaccination efforts have been, for the most part, successful, there still remain several limitations and areas for improvement. High-risk people may need individualized immunization plans and newer antigenic targets must be discovered to combat the virus's mutations. Animal models can serve as a valuable tool to develop better vaccines that can provide protection against COVID-19.

**Comment [WU1]:** please add keywords

#### **Introduction**

The COVID-19 pandemic, caused by the novel Coronavirus SARS-COV-2, has burdened the world with its presence, afflicting 486 million people and claiming the lives of 6.1 million, as of April 2022 (covid19.who.int). The rapid emergence of this coronavirus, coupled with the existence of its ever-changing variants, has necessitated a concentrated effort in understanding the pathogenesis of viral infection as well as developing a safe and effective vaccine. Most

current COVID-19 vaccines approved by North America and Europe target the SARS-CoV-2 full-length spike (S) protein, which allows the virus to attach to ACE2 receptors and enter host cells. These vaccines use either inactivated virus, protein, or virus vectors with nucleic [1] and induce an immunoglobulin G (IgG) response.

Before releasing a vaccine to the public, however, several challenges have to be addressed. Researchers must understand potential pathways for SARS-CoV-2 infection and how an infection causes immunopathogenesis in the respiratory system and must keep in mind that different host immune responses might induce different outcomes of an infection. As studies suggest that protection from a vaccine can be present as early as 10 days after the first shot, understanding immunity after a single dose is important. Furthermore, mutations can increase transmission, infectivity, and severity of viral variants, as well as increase resistance to vaccine-induced neutralizing antibodies (NAbs) [2].

All of these questions require an appropriate animal model to understand viral evolution, mutation sites, and off-target effects of current vaccines. Indeed, one central focus of vaccine research has been finding a model that recreates the pulmonary changes of human infections [3]. Small animal models (rabbits, mice, hamsters) as well as non-human primate (NHP) models have been used to study viral replication, transmission, pathology, and vaccine efficacy. Assessing how COVID vaccines work in NHP models is especially useful due to the phylogenetic closeness of NHPs to humans, translating into a better understanding of how COVID interacts with humans than smaller animal models would provide [4]. Generally, these animal models demonstrate a reduction of viral load and efficient augmentation of NAbs. Specific details of vaccine testing in various animal models have been outlined in **Table 1**.

**Table 1: Pre-clinical trials of COVID vaccines using animal models**

Small Animal Models					
Animal Model	Vaccine Type	Dose	Route	Results	References
BALB/c mice	Rhesus adenovirus serotype 52 vector (encodes variations of SARS-CoV-2 S protein)	10 <sup>9</sup> viral particles	Intramuscular or intranasal	<ul style="list-style-type: none"> <li>- 100% of mice exhibited SARS-CoV-2 S-specific binding antibodies by the ELISA assay</li> <li>- Mice that received two doses exhibited one log higher median antibody titers compared with mice receiving one dose</li> <li>- Weights of vaccinated mice remained generally stable (regardless of whether they received one or two doses) compared with a median loss of 15.2% of body weight in control group</li> </ul>	Tostanoski et al., 2021 [5]
	CoviVac, β-propiolactone-inactivated whole virion vaccine	5-6 μg in safety study and 1.5-6 μg in efficacy study	Intramuscular	<ul style="list-style-type: none"> <li>- 40% developed NAbs 14 days after first dose, and 100% developed NAbs 14 days after second dose</li> <li>- Mice in both control and experimental groups gained weight, and their behavior did not differ significantly</li> <li>- Vaccination of mice resulted in the formation of SARS-CoV-2-specific lymphocytes</li> <li>- No significant decrease in NAb titers</li> </ul>	Kozlovskaya et al, 2021 [6]

				over one-year period	
	Antigens based on RBD in S protein (NG19)	20 µg	Intramuscular	IgG in serum of immunized mice with neutralization values of 17.1-66.9% against 2.1-9.6% in control	Núñez-Muñoz L et al., 2021 [7]
hACE2 mice	h11B11 monoclonal antibody (MAb)	5 or 25 mg/kg	Intraperitoneal; followed by intranasal exposure to virus (preventive or treatment)	<ul style="list-style-type: none"> <li>- Viral titers were under the detection limit in the lungs of mice in preventive group regardless of dose</li> <li>- 10-fold reduction in viral titres in the lung tissue of treatment group compared with controls</li> <li>- Mild interstitial pneumonia, infiltration, and alveolar thickening in controls, but minimal pneumonia in the antibody group; even more limited changes at higher dose of antibody</li> </ul>	Du et al., 2021 [8]
K18-hACE2 transgenic mice	DNA plasmid encoding a secreted monomeric form of SARS-CoV-2 S protein receptor-binding domain (RBD)	20 µg	Intramuscular	<ul style="list-style-type: none"> <li>- Positive anti-RHD IgG after the first dose, further boosted by second dose</li> <li>- Stable body weight in immunized mice but 20% weight loss in controls</li> </ul>	Conforti et al., 2021 [1]
Syrian hamsters	COH04S1, a synthetic multiantigen modified	1×10 <sup>8</sup> plaque forming units	Intramuscular or intranasal	- High binding antibodies to both Spike (S) and Nucleocapsid (N)	Chiuppesi et al., 2021 [9]

<p>Vaccinia Ankara-based SARS-CoV-2 vaccine</p>	<p>(PFU)</p>		<p>antigens, and S-receptor binding domain (RBD) after first vaccine, further boosted by the second shot</p> <ul style="list-style-type: none"> <li>- Vaccinated hamsters did not have a severe weight loss after being exposed to SARS-Cov-2 virus, compared with controls</li> <li>- <math>10^3</math>-<math>10^4</math> lower viral gRNA levels seen in lung tissues of immunized animals</li> <li>- Viral sgRNA was undetectable in lung tissue in vaccinated hamsters (<math>10^3</math>-<math>10^6</math> decrease compared with controls)</li> <li>- Nasal viral load not significantly affected with either vaccine route</li> <li>- Inflammation, consolidation and hyperplasia on histopathology of lungs in control animals; no pathology noted in vaccinated ones</li> </ul>	
<p>ChAdOx1 nCoV-19, adenovirus genome vector (encoding human optimized S protein)</p>	<p>100 <math>\mu</math>l of <math>2.5 \times 10^8</math> infectious units of vaccine intramuscularly or 50 <math>\mu</math>l of <math>2.5 \times 10^8</math></p>	<p>Intramuscular or intranasal</p>	<ul style="list-style-type: none"> <li>- High IgG titers with no difference observed between vaccination routes</li> <li>- High neutralizing antibody titers for vaccinated hamsters, higher in those that received intranasal inoculation compared</li> </ul>	<p>van Doremalen et al., 2021 [10]</p>

		infectious units of vaccine intranasally		<p>with intramuscular</p> <ul style="list-style-type: none"> <li>- Control group started losing weight at 3 days post inoculation (dpi) and didn't regain it until 8 dpi. None of the vaccinated animals lost weight</li> <li>- Much less infectious virus detected in swabs of intranasal- and intramuscular-vaccinated animals compared to controls</li> <li>- Pneumonia and pulmonary lesions found in control animals (40-70% of tissue) but no lesions found in lung tissue of vaccinated hamsters</li> <li>- Amount of viral RNA detected in nasal swabs of vaccinated animals lower than that of control animals, and no viral RNA or infectious virus found in pulmonary tissue after direct contact with infected hamsters</li> </ul>	
	CoviVac, $\beta$ -propiolactone-inactivated whole virion vaccine	5-6 $\mu$ g in safety study and 1.5-6 $\mu$ g in efficacy study	Intramuscular	<ul style="list-style-type: none"> <li>- 87% developed NAbs 14 days after first dose, and 100% developed NAbs 14 days after second dose</li> <li>- Vaccinated hamsters gained weight, whereas unvaccinated hamsters lost weight</li> <li>- Inflammation in lungs in all infected animals. However,</li> </ul>	Kozlovskaya et al, 2021 [6]

				control group experienced tissue damage, loss of alveolar structure, pneumonia, and mild perivascular fibrosis while vaccinated hamsters saw minor tissue damage and no pneumonia - No significant decrease in NAb titers over one-year period	
New Zealand rabbits	Antigens based on receptor binding domain (RBD) in S protein (NG06 and NG19)	200 µg	Intramuscular	- Detection of antigen with IgG at 1:10 <sup>6</sup> dilution; even stronger detection after a second dose	Núñez-Muñoz L et al., 2021 [7]
	Spike (S1+S2) ectodomain, S1 domain, RBD domain, and S2 domain (control)	50 µg	Intramuscular	- After second vaccination, all rabbit sera contained anti-spike antibodies that were at least 80% IgG - Sera from S ectodomain, S1, and RBD (but not S2) showed 50 to 60% virus neutralization after a first dose and 93 to 98% virus inhibition after second	Ravichandran et al., 2021 [11]
Ferrets	SARS-CoV-2 RBD-ferritin nanoparticles	15 µg	Intramuscular or intranasal	- Vaccinated ferrets produced strong neutralizing antibodies after booster - Control ferrets saw an increase in body temperature and a	Kim et al, 2021 [12]

				<p>decrease in body weight whereas vaccinated ferrets saw no change in either</p> <ul style="list-style-type: none"> <li>- Immunized ferrets showed rapid viral clearance in the nasal washes and lungs</li> <li>- Immunized ferrets showed significant reduction of viral RNAs in lungs compared to control ferrets. At 6 dpi, lung tissues of vaccinated ferrets showed complete clearance of viral RNAs</li> </ul>	
Ferrets	RBD vaccine	400 µg	Intramuscular	<ul style="list-style-type: none"> <li>- After an intranasal infection with SARS-Cov-2, viral subgenomic RNA in nasal washes and throat swabs at day 7 was significantly lower in vaccinated ferrets compared with controls.</li> </ul>	Conforti et al., 2021 [1]
<b>NHP Models</b>					
<b>Animal Model</b>	<b>Vaccine Type</b>	<b>Dose</b>	<b>Route</b>	<b>Results</b>	<b>References</b>
Rhesus macaques	Stabilized, recombinant, full-length SARS-CoV-2 S glycoprotein (NVX-CoV2373)	5 or 25 µg	Intramuscular	<ul style="list-style-type: none"> <li>- Lower replicating virus (sgRNA) in all animals regardless of dose, in all tissues except nasopharyngeal swabs</li> <li>- 25 ug dose cleared sgRNA in nasopharyngeal swabs and bronchoalveolar</li> </ul>	Gorman et al., 2021 [13]

				<p>lavage (BAL), but 5 ug dose cleared sgRNA only in BAL</p> <ul style="list-style-type: none"> <li>- No detectable viral load in any tissue on any day and with any dose in prime/boost regimen</li> <li>- No gRNA in nasal cavity, lungs or trachea tissue sample on necropsy, in prime/boost regimen irrespective of dose</li> <li>- Few animals with single vaccine had detectable gRNA in the tissues</li> <li>- Elevated anti-S IgG after a single vaccine (any dose)</li> <li>- 21-35x increase in anti-S IgG titers after a second shot (any dose)</li> </ul>	
ChAdOx1 nCoV-19, adenovirus genome vector (encoding human optimized S protein)	$2.5 \times 10^{10}$ virus particles	Intramuscular or intranasal	<ul style="list-style-type: none"> <li>- Reduced virus concentrations in nasal swabs and reduction in viral loads in bronchoalveolar lavage and lower respiratory tract tissue</li> <li>- 3 of 4 control animals developed viral interstitial pneumonia and pulmonary lesions</li> <li>- No pulmonary pathology or COVID antigen detected in vaccinated animals</li> <li>- High IgG titers found in nasosorption samples from animals</li> </ul>	van Doremalen et al., 2021 [4]	

				<p>that received two doses</p> <ul style="list-style-type: none"> <li>- SARS-CoV-2 specific IgA antibodies weakly detected in samples upon first vaccination but further increased upon second vaccination</li> <li>- Viral load in lungs significantly lower for vaccinated animals than for control animals</li> <li>- No SARS-CoV-2 antigen detected by immunohistochemistry in vaccinated animals, but viral antigen observed in pneumocytes in all control animals</li> </ul>	
	mRNA vaccine	4 µg	Intramuscular	<ul style="list-style-type: none"> <li>- No adverse events observed</li> <li>- No remarkable differences noted between control and vaccinated group</li> <li>- No binding antibodies detected 14 or 28 days after first dose</li> <li>- 14 days after second dose, low levels of spike-specific binding but not NAbs detected in 4 of 6 vaccinated monkeys</li> </ul>	van Doremalen et al., 2021 [10]
Common marmosets	CoviVac, β-propiolactone-inactivated whole virion vaccine	5-6 µg in safety study and 1.5-6 µg in	Intramuscular	<ul style="list-style-type: none"> <li>- 83% developed NAbs 14 days after first dose, and 100% developed NAbs 14 days after second dose</li> </ul>	Kozlovskaya et al, 2021 [6]

		efficacy study		- No significant decrease in NAb titers over one-year period	
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**Table 2: Advantages and Disadvantages of COVID-19 animal models**

Animal Model	Advantages	Disadvantages
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Mice	<ul style="list-style-type: none"> <li>• COVID infection can induce pulmonary, immunological, chemical, and weight(-loss) changes similar to those in humans with mild COVID</li> <li>• Show efficacy of neutralizing antibodies (Nabs) and inactivated vaccines</li> <li>• Small size, established understanding of genome, and ease of genetic manipulation</li> <li>• Demonstrates different severities of infection based on age differences</li> <li>• Viral DNA was consistently seen in the mice tissue</li> <li>• Reproduce fast</li> </ul>	<ul style="list-style-type: none"> <li>• Low affinity of mouse ACE2 for S protein, so mice cannot be efficiently infected with wild-type viruses</li> <li>• Risk of misplaced expression of hACE2</li> <li>• Low expression of hACE2</li> <li>• Difficult to replicate role of hACE2 in humans with other medical conditions like hypertension, diabetes, cardiac disease etc.</li> <li>• Limited availability</li> </ul>
Syrian Hamsters	<ul style="list-style-type: none"> <li>• S protein binds tightly to hamster ACE2 receptors</li> <li>• Show similar pathology as pneumonia caused by COVID in humans (progression of symptoms and clearance of the virus during the first week after inoculation)</li> <li>• Model appears to mimic gender- and age-dependent differences in human patients <ul style="list-style-type: none"> <li>• susceptible to virus</li> <li>• Highly reproductive</li> <li>• Requires less space to breed</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Anatomy and structure of the hamster lower respiratory tract differs from that of humans</li> <li>• No histopathological changes seen in brain, kidney, heart or liver in infected animals.</li> <li>• Virus is quickly cleared out of their bodies.</li> </ul>
New Zealand Rabbits	<ul style="list-style-type: none"> <li>• Docile</li> <li>• Widely bred</li> <li>• Economical</li> <li>• Frequently used to assess pharmacology/toxicology of vaccine antigens</li> </ul>	<ul style="list-style-type: none"> <li>• None found in COVID literature</li> </ul>
Ferrets	<ul style="list-style-type: none"> <li>• Naturally susceptible to COVID infection</li> <li>• Infected ferrets show clinical symptoms similar to those of</li> </ul>	<ul style="list-style-type: none"> <li>• Viral replication only in upper respiratory tract; low viral load in lungs</li> <li>• Relatively more expensive</li> </ul>

	<p>humans due to similar structure of the respiratory tract</p> <ul style="list-style-type: none"> <li>• High transmission between animals</li> <li>• Good to study asymptomatic carriers due to low viral load and prolonged lung infection</li> </ul>	<p>than other small animal models</p>
Rhesus macaques	<ul style="list-style-type: none"> <li>• Clinical symptoms, immune responses, and pathology is similar to those of humans</li> <li>• Evolutionarily close to humans</li> <li>• Demonstrate different severities of infection based on age differences</li> </ul>	<ul style="list-style-type: none"> <li>• Variables such as age, gender, species, and host immune statuses need to be tested in a larger sample size to make a better conclusion on accuracy of animal model</li> <li>• Ethic restriction, high costs, low breeding efficiency, large size, individual variation among NHPs</li> <li>• NHP models infected with highly infective pathogens such as SARS-CoV-2 must be operated in isolation</li> <li>• High cost</li> </ul>
Cynomolgus macaques	<ul style="list-style-type: none"> <li>• Disease pattern similar to humans</li> <li>• Evolutionarily close to humans</li> </ul>	<ul style="list-style-type: none"> <li>• High cost</li> </ul>
Common marmosets	<ul style="list-style-type: none"> <li>• Evolutionarily close to humans</li> <li>• Small; reproduce well in captivity</li> </ul>	<ul style="list-style-type: none"> <li>• Somewhat resistant to COVID infection</li> </ul>

All vaccine candidates mentioned in this review have been efficacious to some extent—in reducing viral shedding, transmission, tissue lesions, and preventing weight loss—in their respective animal models (Table 1).

The various animal models used in preclinical testing have their advantages and disadvantages (Table 2). While mice are smaller, less expensive, and easy to reproduce and work with, their ACE2 receptor does not have high affinity for the SARS-CoV2 spike protein.

The need to introduce a genetically engineered hACE2 limits their utility. Syrian hamsters, besides being small, less expensive, and quick to reproduce, also have another advantage that their ACE2 receptor which easily binds to the S-protein. The natural history of the disease in hamsters also resembles that of humans. But the limitation of this model is that they clear the virus quickly from their bodies. It is difficult to study the extrapulmonary disease in Syrian hamsters due to lack of any tissue changes in their brain, heart, liver or kidneys. Ferrets have a natural susceptibility to SARS-CoV2 infection, and their pattern of infection is similar to humans. They also form a good model to study asymptomatic carriers due to low viral load in lungs and milder infection in the lungs and lower respiratory passages. Ferrets, however, are more expensive, and have a limitation of infecting only the upper respiratory passages. Macaques and marmosets are closer to humans genetically as well as physiologically. The infections (clinical as well as pathological) in them and other non-human primates (NHPs) resemble those observed in humans. While they may form the best models to study the disease and vaccines, their use is affected by greater ethical and financial concerns.

While comparing various vaccines and animal models studied so far, we observed wide variations not only in the types of vaccines used, but also in the dosage administered and routes of administration. Using the same vaccine and similar doses to immunize NHPs and humans may allow us to better demonstrate vaccine efficacy and similarity in the immune response among animal models [12,14]. Furthermore, observed results from NHP models do not always directly translate to humans in clinical trials. In fact, several clinical symptoms such as asymptomatic infection, gastrointestinal infection, diarrhea, and vomiting were reported in human patients but not observed in NHP models. Patients with pre-existing conditions and diseases have higher severity and mortality after COVID infection. Therefore, these individuals may need a different

immunization strategy—consisting of more boosters or higher doses—that can prevent SARS-CoV-2 infection. There is a need to establish animal models which very closely recapitulate these human clinical conditions for the development of appropriate immunization paradigms for these high-risk populations. The present mRNA-based vaccines have proven to be very useful and provides protection against viral infection, but the immunity they induce is short lasting. There also exists the issue of frequent mutations in the virus, bringing us face to face with new variants of the virus every few months. In order to have a continued edge over the spread of the infection, the scientific community needs to search for new antigenic targets, in order to obtain more sustainable immunity. The road to achieving an effective vaccine has been a long one, filled with difficulties and successes, and the use of these animal models for vaccine research is a major stepping-stone in our journey of defeating COVID for good.

**Comment [WU2]:** the background is less conical, the purpose is not clear, please add research methods, discussions and conclusions

#### **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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**Comment [WU3]:** please add literature