Animal Hosts and Experimental Models of SARS-CoV-2 Infection

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Abstract

Viruses arise through cross-species transmission and can cause potentially fatal diseases in humans. This is the case of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which recently appeared in Wuhan, China, and rapidly spread worldwide, causing the outbreak of coronavirus disease 2019 (COVID-19) and posing a global health emergency. Sequence analysis and epidemiological investigations suggest that the most likely original source of SARS-CoV-2 is a spillover from an animal reservoir, probably bats, that infected humans either directly or through intermediate animal hosts. The role of animals as reservoirs and natural hosts in SARS-CoV-2 has to be explored, and animal models for COVID-19 are needed as well to be evaluated for countermeasures against SARS-CoV-2 infection. Experimental cells, tissues, and animal models that are currently being used and developed in COVID-19 research will be presented.

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Introduction

A novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, Hubei Province, China, in December 2019 causing a syndrome termed by the World Health Organization (WHO) "COVID-19" on February 11, 2020 [1–4] and recognized as a pandemic on March 11, 2020 [5]. Coronaviruses are enveloped viruses with a positive sense single-stranded RNA genome (26–32 kb) [6], one of the largest known genomes among the RNA viruses [7]. Within the last 2 decades, 2 coronaviruses have been introduced into the human population, the SARS-CoV and the Middle East respiratory syndrome-CoV (MERS-CoV) and, by crossing the species barrier and causing severe disease spread from person to person. SARS-CoV emerged in China in 2002 from Chinese horseshoe bats and transmitted to human by human civet intermediate reservoirs and resolved within a year, causing 8,437 infected people with 813 deaths in 27 different countries worldwide, and resulting in a 9.6% fatality rate [8]. Bats may be the primary reservoir, and camels the intermediate host for MERS-CoV, originated in the Middle East and remaining largely restricted in the Arabian peninsula, resulting in 2,229 cases

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and 858 deaths with a case mortality rate of 35% [9]. Human coronaviruses (HCoVs), namely, HCoV-E299 (-CoV), HCoV-NL63 (-CoV), HCoV-OC43 (-CoV), and HCoV-HKU1 (-CoV), usually cause mild, both upper and lower respiratory tract infections in humans. HCoVs are thought to have emerged from an animal source, and then, other amplifier hosts might have played a role of becoming epidemiological animal reservoirs. Interestingly, evidence indicates 95% genetic homology of RNA sequence of HCoV-OC43 to the one of the bovine BCoV, speculating transmission from cattle, the zoonotic ancestor, to humans 100 years ago [10]. The identification of animal reservoirs plays a crucial role in effective disease control.

Animal Reservoirs of SARS-CoV-2

Most of viral diseases are caused by zoonotic pathogens maintained by wildlife reservoir hosts [11]. Bats are a natural reservoir host of different families of viruses, many of which cause severe human diseases [12, 13]. Viruses can spill over from the host to other animals and humans, causing disease outbreaks worldwide. The bat *Rhinolophus* (horseshoe bat) is speculated as the source of SARS-CoV-2 [2]; however, the possibility exists for additional interspecies transmissions, and the precise route of transmission of the virus into the human population has not been established yet. Phylogenetic analysis revealed SARS-CoV-2 as a newly emerged strain of β-coronavirus that shared 79.6 and 96.2% sequence identity with SARS-CoV-1 and bat Co-RaTG13, respectively, suggesting a zoonotic origin from the likely bat reservoir [14, 15]. RaTG13 was detected in bat *Rhinolophus affinis* from Yunnan Province, far away from Hubei Province. Divergence dates between SARS-CoV-2 and RaTG13 were estimated between 40 and 70 years ago in horseshoe bats. Similar to what was observed with SARS and MERS, the possible involvement of an intermediate host as a plausible conduit for transmission to humans has been considered. Specifically, Guangdong Pangolin-CoV genome is very closely related to SARS-CoV-2, sharing 92.4% sequence similarity; thus, pangolin could be responsible for the zoonotic event [16, 17]. SARS-CoV-2 could have been for decades in bats and been transmitted to other hosts such as pangolins. Unlike SARS-CoV, the spike (S) glycoprotein of SARS-CoV-2 harbors a unique furin cleavage site (PRRA) before the S1/S2 region, which increases the transmissibility of this virus that is absent in bats and pangolins [15, 18]. Although the exact origin of the PRRA motif from an animal virus has not yet been determined, this insertion is an independent natural event, probably due to a recombination, and fixed by the natural selection [18]. The currently available data do not clarify whether the origin of the virus is due to a natural selection in the wild animal reservoir before zoonotic transfer or natural selection in humans following zoonotic transfer.

Animals Naturally Infected with SARS-CoV-2

Dogs and Feline
Naturally infections with SARS-CoV-2 have been documented in various animal species. SARS-CoV-2 infection in dogs occurred in Hong Kong after close contact with people infected with the virus developing detectable antibodies against SARS-CoV-2 [19, 20]. In addition, genetic analysis revealed that dog and human viral sequences were closely related. Other human-to-dog transmissions have been reported in the Netherlands and in New York State confirmed by analysis of neutralizing antibodies against SARS-CoV-2 [21]. Conversely, neither France nor Spain tested positive dogs living with an individual infected with SARS-CoV-2 [22, 23]. Several human-to-feline transmissions have been documented [24–26]. In January 2020, in Wuhan, antibodies against SARS-CoV-2 were detected in domestic cats using ELISA and/or neutralization assay. Between March and June 2020, in Hong Kong, New York State in the USA, Belgium, and France, asymptomatic and/or symptomatic cats were tested positive for SARS-CoV-2 by RT-qPCR. Cats may infect other cats in close contact. In April 2020, in the Bronx Zoo in New York City, 1 tiger and 3 lions were found positive after interaction with SARS-CoV-2-infected zookeeper [27].

Minks
In April 2020, minks in 2 separate farms in the Netherlands developed signs of breathing and gastrointestinal disorders, with a mortality of 1.2–2.4% for a presumed human-to-mink transmission of SARS-CoV-2, workers at the farm having been previously tested positive for coronavirus disease 2019 (COVID-19). In November 2020, the Danish National Institute of Public Health announced the alert for back spillover of SARS-CoV-2 from mink farms into the community. During the passage through minks, the virus mutated in the S protein-encoding gene. In the viral samples from minks and humans, researchers identified mutations that were then used to

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confirm that the people on the farm were infected by the viruses from the animals, and the mutations were suggestive of the virus adaptation to this new host [28, 29]. It is unclear how SARS-CoV-2 has been introduced in the farms. A likely scenario for the infection of minks is human-to-animal transmission, although virus could potentially be introduced by wild mustelids or other wildlife, as shown by other virus infections like influenza viruses. Minks are *Mustelidae*-like ferrets that have been used as animal models of COVID-19 owing to their susceptibility to the virus and transmission between ferrets. The virus's rapid spread in mink makes this animal a potential nonhuman reservoir of viral source that can easily infect humans, and surveillance at the human-animal interface is highly recommended. Natural or experimental animal host susceptibility to SARS-CoV-2 is shown in Figure 1.

Animal Models of COVID-19

Animal models are important tools both for studying the pathogenesis of infectious diseases and for the preclinical evaluation of vaccines and antivirals against virus infections [30, 31]. Several animal models have been used to study COVID-19 with varying susceptibility of the host to SARS-CoV-2 infection, suggesting a species-specific role for angiotensin-converting enzyme 2 (ACE2). The ACE2 is the host cell receptor responsible for mediating infection of SARS-CoV-2. The S glycoprotein mediates binding to ACE2, followed by proteolytic cleavage by protease serine 2 (TMPRSS2), which triggers fusion of viral and cellular membranes. In this regard, species-specific differences in the ability of ACE2 to bind the S protein for virus entry has been predicted in silico and demonstrated experimentally in vitro and correlated with susceptibility to SARS-CoV-2 infection of some species tested in experimental protocols [32–34]. In accord with this, mice are resistant to SARS-CoV-2 infection [35]. Animal models currently being used in COVID-19 research are shown in Table 1.

Mouse
Mice are widely used in infectious disease research, even though often, in order to be susceptible to the infection, they need to be genetically modified. Otherwise, the virus needs to be adapted for the growth in different species. SARS-CoV-2 uses cellular surface ACE2 protein as a primary receptor for cell attachment and entry, and mouse ACE2 does not bind efficiently to the viral S protein [33,
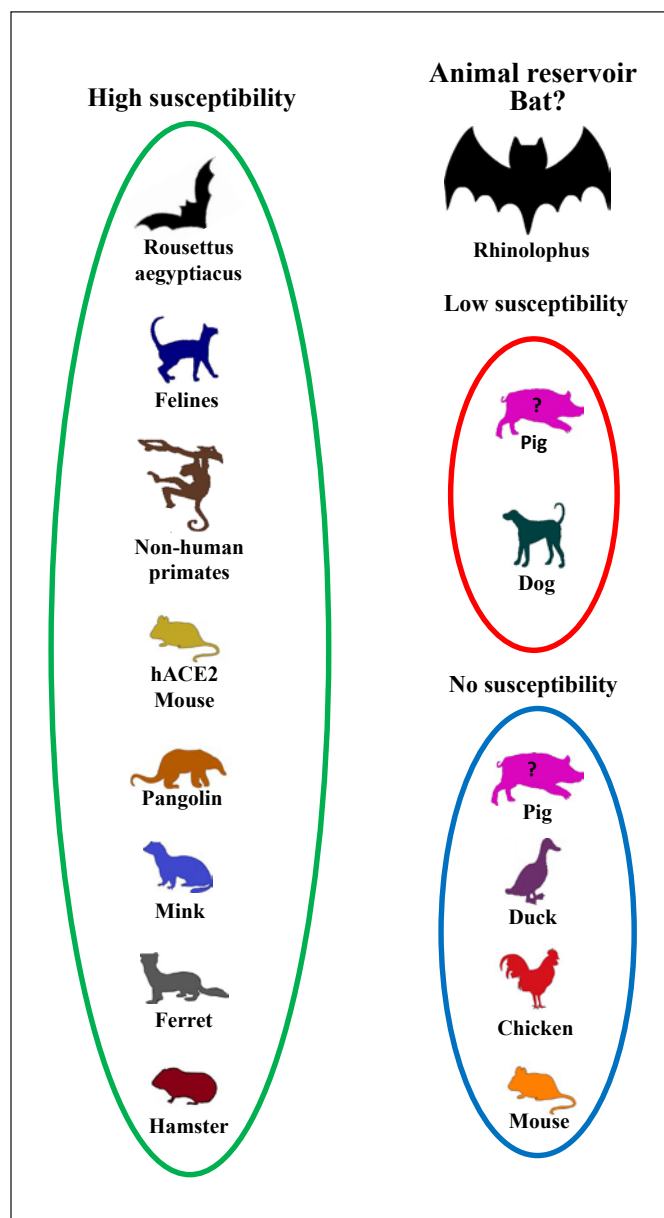

Fig. 1. Susceptibility of animal hosts to SARS-CoV-2 infection. High susceptibility: felines, NHPs, hACE2 mice, pangolins, ferrets, minks, hamsters; low susceptibility: dogs and pigs (in one experimental study [68]); no susceptibility: pigs (in one experimental study [52]) to SARS-CoV-2 infection. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; hACE2, human ACE2; NHP, nonhuman primate.

34]. The ways to overcome this problem are to adapt either the host or the virus. Mouse-adapted SARS-CoV-2 strain with binding affinity to mouse ACE2 has been obtained after sequential passaging of virus in mouse lung tissues [36]. Infection of young adult and aged BALB/c mice with

Table 1. Animal models of SARS-CoV-2

Animal species	Susceptibility (*natural/°experimental infection)	Clinical signs	Immune response	Advantages	Disadvantages	Refs.
Bat	*High/°high	No	Yes	Helpful for transmission	Wildlife animal and not easy to handle	[2, 14, 15]
Mouse	*None/°high	Yes	Yes	Useful for pathogenesis, immune response, vaccines, and therapeutics	Transgenic mice, high cost, and mild infection	[36–41]
Hamster	*None/°high	Yes	Yes	Useful for transmission, pathogenesis, immune response, and therapies	Mild infection and no severe disease	[46–50]
Ferret	*None/°high	Yes	Yes	Useful for transmission, pathogenesis, and therapies	Mild infection and no severe disease	[51–54]
Mink	*High/°not done	Yes	Yes	Useful for transmission, pathogenesis, and therapies	High cost	[29]
Monkey	*None/°high	Yes	Yes	Suitable for transmission, pathogenesis, immune response, vaccines, and therapies	Transient clinical signs and housing cost	[43, 45, 53, 55, 56, 58–65]
Dog	*Low/°low	No	Yes	Not useful	Not applicable	[54, 67]
Cat	*High/°high	Yes	Yes	Not useful	Not applicable	[66, 67]
Pig	*None/°none or low in one experimental study	No	No/low in one experimental study	Low useful	Not applicable	[52, 67]
Poultry	*None/°none	No	No	Not useful	Not applicable	[68, 69]

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

adapted SARS-CoV-2 resulted in replication in both upper and lower airways, with severe symptoms in the aged mice. The disadvantage of mouse-adapted viruses may be in the clinical features of the infection that do not recapitulate all aspects of the human disease. Another approach has been to modify the receptor-binding domain in the virus S protein to bind mouse ACE2 protein. The virus replicated in animals with a limited degree of clinical illness and mild disease symptoms, suggesting that even though ACE2 may be necessary for infection, it is not sufficient to determine the outcome of infection. Transgenic mice expressing human ACE2 (hACE2) receptor support SARS-CoV-2 infection. Different strategies have been adopted for expressing hACE2 in mice, including the use of the mouse ACE2 promoter or a heterologous gene promoter, as well as the transduction by means of adenovirus or adeno-associated virus expressing hACE2 as a transgene [31, 37–41]. Successfully studies showed mice that mimic human COVID-19 symptoms; thus, a variety of antivirals and vaccine candidates have been tested [42–45]. Thus, mice are potentially good candidates for evaluating the efficacy of antiviral drugs.

Hamster

Syrian hamsters intranasally inoculated are highly permissive to SARS-CoV-2 infection, with high levels of virus replication and histopathological evidence of disease that closely mimic those displayed by human COVID-19 patients. High levels of viral RNA were evident in the nasal mucosa, lower respiratory epithelial cells, and small intestine, which could be useful for the evaluation of therapeutic agents and vaccines. Analysis of neutralizing antibodies, detected as early as 7 days after infection, revealed protection against rechallenge with SARS-CoV-2, while naive hamsters were efficiently protected by passive immunization against high dose of SARS-CoV-2. Virus transmission to naive cohoused hamsters has been successful observed [46–48]. Recently, 2 different groups, by adopting the hamster model, have been able to show that the D614G variant in S protein results in increased virus infectivity in the upper airway of the animals and in enhanced transmissibility [49, 50]. In addition, sera from variant-infected hamsters can efficiently neutralize the virus, suggesting that SARS-CoV-2 vaccines, all of which are based on the D614 variant, will protect against the

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G614 variant. These results confirm hamsters a useful animal model in virus transmission studies.

Ferret

Ferrets are of special relevance to laboratory studies of respiratory viruses since their respiratory tract is anatomically comparable to the human one. Ferrets are susceptible to experimental infection by SARS-CoV-2, via direct contact and via the air, and are capable of replicating and transmitting the virus to other noninfected animals. Shedding of SARS-CoV-2 is observed in nasal and oropharyngeal swabs. Infectious virus was detected in the upper respiratory and gastrointestinal tract, and viral RNA was found in the saliva, urine, rectal swabs, and feces. All ferrets possessed serum with anti-SARS-CoV-2 antibody, and high titers of neutralizing antibodies were detected at day 21. Virus causes a milder respiratory syndrome in ferrets than in humans, with undetectable or mild clinical alterations. It should be noted the consistency of the results obtained from different studies [51–54]. Ferrets are a valuable model for better understanding transmission and pathogenesis of COVID-19.

Nonhuman Primate Models

The animal model of infectious diseases should reflect clinical course and pathology observed in humans to characterize viral pathogenesis and to evaluate antiviral agents and vaccines. For COVID-19, several animal species were investigated as models of human disease including the nonhuman primates (NHPs). NHPs are closely related to humans; they are invaluable models for studying emerging and re-emerging diseases. NHPs could be susceptible to SARS-CoV-2 infection, being symptoms of fever, diarrhea, and pneumonitis reported in rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and African green monkeys (*Chlorocebus aethiops*). Replication of virus at high titers was observed in both the upper and lower respiratory tracts for 7–14 days [55, 56]. Additionally, infected rhesus macaques developed humoral and cellular immune responses as well as robust protection against 28 days postinfection rechallenge, indicating full protection from reinfection [57]. In addition, as observed in older SARS-CoV-2-infected individuals, an adverse clinical outcome is associated with old rhesus macaque infected as compared to young rhesus macaque ones [53]. The use of different challenge stocks, dosages, and routes of infection in NHP models may contribute to a significant variation in the level and duration of viral replication observed in the control groups; therefore, comparative studies will need standardized protocols and challenge virus stocks. NHP models have been useful for the testing of therapeutic agents as well as of several vaccine candidates [43, 45, 58–65].

Dog and Cat

One experimental infection study showed that dogs have a low susceptibility to SARS-CoV-2 [54, 67]. No clinical signs were detected. Some animals produced antibodies against the virus, and viral RNA was detected in rectal swabs while it was absent in organs of euthanized dogs. These data suggest that dogs are not promising animal models to study SARS-CoV-2.

Cats are susceptible to experimental infection [66, 67]. Viral RNA was evident in the upper and low respiratory tracts and small intestines, and seroconversion was detected by using the ELISA test. Naive cats were shown to be susceptible to the infection if they were in close contact with infected cats shedding viral RNA in tissue and feces, representing a potential model for droplet transmission.

Pig

Domestic swine have the potential to significantly impact public health; thus, determination of the susceptibility of pigs to SARS-CoV-2 is critical. To this end, different research groups inoculated these animals intravenously, intranasally, ocularly, or orally with SARS-CoV-2. No clinical signs and no viral RNA in swabs or tissue samples have been detected, and there was no seroconversion. Naive contact animals were also seronegative, indicating that pigs could not transmit the virus. These findings suggested that swine are not susceptible to SARS-CoV-2 infection [52]. A recent study contradicts the above reported one. Indeed, of the 16 animals experimentally inoculated, 2 of them seroconverted; virus was isolated from a pig, while another one displayed mild symptoms [67]. Viral RNA was detected in oral fluids and nasal wash from 2 animals. It should be taken into account that the considerable variation observed in this study may be due to the viral isolate, infectious dose, or breed of swine. Further investigations regarding the susceptibility of these species are needed.

Poultry

Experimental infection of chicken and ducks with SARS-CoV-2 has been described. Animals did not develop clinical signs, all swabs and organ samples were negative for SARS-CoV-2 RNA, and seroconversion was not observed. Cohoused naive animals were found to be seronegative, indicating no animal-to-animal transmission [68, 69]. These finding suggested that poultry are not sus-

Table 2. Cell line, primary cell, and organoid susceptibility to SARS-CoV-2

Cell lines, primary cells, and organoids	Origins	Susceptibility	Refs.
Vero E6 cell line (African green monkey)	Renal	High viral replication	[70, 71]
Vero E6/TMPRSS2 cell line (African green monkey)	Renal	Very high viral replication	[70, 71]
Caco-2 cell line (human)	Intestinal	Robust viral replication	[70, 71]
Calu-3 cell line (human)	Pulmonary	Robust viral replication	[70, 71]
Huh-7 cell line (human)	Hepatic	Modest viral replication	[70, 71]
HEK293T cell line (human)	Renal	Modest viral replication	[71]
FRhK4 cell line (rhesus monkey)	Renal	High viral replication	[71]
LLCMK2 cell line (rhesus monkey)	Renal	High viral replication	[71]
Airway epithelial primary cells (human bronchi/bronchiolar)	Epithelial	Permissive to SARS-CoV-2 infection	[72–75]
Primary enterocytes (human)	Intestinal	Permissive to SARS-CoV-2 infection	[76]
Type I and type II primary pneumocyte (human)	Pulmonary	Permissive to SARS-CoV-2 infection	[77]
Human organoids	Bronchial	Permissive to SARS-CoV-2 infection; progeny virus	[78]
	Pulmonary	Permissive to SARS-CoV-2	[86]
	Renal	Permissive to SARS-CoV-2 infection; progeny virus	[87]
	Hepatic	Permissive to SARS-CoV-2 infection; progeny virus	[83]
	Intestinal	Permissive to SARS-CoV-2 infection; progeny virus	[81, 84, 85]

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

ceptible to the virus, and therefore, they do not represent suitable animal models for studies of SARS-CoV-2 infection.

Fruit Bat

Rousettus aegyptiacus fruit bat is genetically divergent from the predicted host reservoir *Rhinolophus horseshoe* bat. Experimental studies revealed that *R. aegyptiacus* is susceptible to SARS-CoV-2. Intranasal inoculation resulted in viral replication in the upper respiratory tract, with shedding of live virus and bat-to-bat transmission. Viral RNA was detected at a lower level in other organs, including the heart, skin, and intestine. Bats developed weak antibody responses, and clinical signs were absent [52]. The capacity of fruit bats to carry and transmit the virus makes this species a reservoir host, and therefore, it represents a useful model.

Cell-Based Approaches

Cell models of SARS-CoV-2 are essential for understanding the viral life cycle, tropism, and pathogenesis. Immortalized cell lines are widely utilized for virus isola-

tion and for screening inhibitors against SARS-CoV-2. The simian kidney epithelial derived Vero E6 cell lines, expressing ACE2 and low levels of TMPRSS2, are highly susceptible and permissive to virus replication, and they are commonly used for virus isolation [70]. The human cell lines, among which renal HEK293T, intestinal Caco-2, pulmonary Calu-3, and hepatic Huh-7, produce low titers of infectious virus and have been utilized in SARS-CoV-2 infection experiments [70, 71]. Nonhuman cell lines, such as feline kidney CRFK and rhesus macaque kidney FRhK4 and LLCMK2, are adopted to SARS-CoV-2 replication studies [71]. The limitation of immortalized cell lines is that they do not accurately mimic human physiological conditions, and resulting biological observations need to be validated in primary human cells or in animal models. Primary human airway epithelial cells have been found to display cytopathic effects 96 h after SARS-CoV-2 infection [72–74]. Other primary human cells, nasal epithelial, large and lower airway epithelial, type I and type II pneumocytes (AT1 and AT2), ciliated and secretory airway epithelial (HAE), and gut enterocytes, are permissive to SARS-CoV-2 infection [75–77]. Recently, an important finding has been obtained in human lung epithelial cells and in primary hu-

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man airway tissue where increased infectivity of the S D614G variant of SARS-CoV-2 has been demonstrated [49]. These data suggest that the D614G mutation gives rise to enhanced virus transmissibility. In addition, serum samples from D614 virus-infected hamsters can efficiently neutralize the G614 virus from infecting cells, indicating that SARS-CoV-2 vaccines, which are all based on the D614 variant of the S protein, will protect against G614 virus variants.

Organoids are self-organized 3-dimensional assemblies of cells, generated by the primary tissue or pluripotent stem cells that exhibit physiological features of an organ. Organoids derived from human cells are particularly helpful for preclinical studies, by obviating the need to extrapolate results from one species to another. Organoids are also of interest for recapitulating the physiological effects of SARS-CoV-2, for investigating virus tropism and pathogenesis and for screening SARS-CoV-2 inhibitors. SARS-CoV-2 affects several organs causing severe damage of the lung; cardiovascular, intestinal, and neurological, and endothelial systems; kidney; and liver, showing direct effects on these tissues. Recent studies showed that human bronchial, lung, kidney, liver, intestinal, and vascular organoids are all permissive to SARS-CoV-2 and may represent viral reservoir [78–84]. Antiviral effects of COVID-19 candidate therapeutic compounds have been evaluated in organoid systems [85–88]. Vascular and kidney organoids have been used to identify clinical-grade soluble ACE2 as an inhibitor of SARS-CoV-2 infection [87, 88]. Liver ductal organoids support SARS-CoV-2 replication and virus infection impaired the bile acid transporting functions of cholangiocytes, resulting in the bile acid accumulation and consequent liver damage in patients [83]. A limitation of organoid models may be the lack of relevant immune cells – e.g., macrophages, that modulate severe disease. Cell lines and organoids and currently being used in COVID-19 research are summarized in Table 2.

Conclusion

In order to answer important questions on COVID-19 including pathogenesis, transmissibility, and immune response to SARS-CoV-2, as well as comorbidities and viral coinfections, in vivo investigations adopting the best animal models are needed for validation and translation in human studies. Animal models may also serve to evaluate therapeutic countermeasures and vaccines. Current literature data indicate that although most of the animal models could mimic many features of human COVID-19, however, none of them is capable of replicating the clinical outcomes related to high mortality and morbidity of the human disease. Furthermore, it has to be considered that the innate immune response, including the defense system against viruses, diverged during evolution among the animals, which may explain the differences in the rate of infection. Additional studies will be required to refine animal models of chronic diseases to gain further insights into molecular immune pathogenesis, diagnosis and treatment of COVID-19. NHPs are closer to humans and are the most relevant animal models to test interventions before deployment to human treatment. On the other side, ferrets, mice, and hamsters developed clinical signs of the infection that are very similar to the one developed in humans. These animals might be useful in answering many questions regarding the mechanism of action of antivirals, and safety and efficacy of vaccines. Animal and in vitro/ex vivo models can be adopted and pathogenesis and interventions assessed. Finally, wild animals such as bats and/or pangolins or a yet-to-be-identified animal host could serve as reservoirs of the SARS-CoV-2 or route of transmission to humans, and surveillance should be extensively done.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors contributed to the writing of this manuscript.

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