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Animal Models, Zoonotic Reservoirs, and Cross-Species Transmission of Emerging Human-Infecting Coronaviruses

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Abstract

Over the past three decades, coronavirus (CoV) diseases have impacted humans more than any other emerging infectious disease. The recent emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 (coronavirus disease 2019), has resulted in huge economic disruptions and loss of human lives. The SARS-CoV-2 genome was found to mutate more rapidly due to sustained transmission in humans and potentially animals, resulting in variants of concern (VOCs) that threaten global human health. However, the primary difficulties are filling in the current knowledge gaps in terms of the origin and modalities of emergence for these viruses. Because many CoVs threatening human health are suspected to have a zoonotic origin, identifying the animal hosts implicated in the spillover or spillback events would be beneficial for current pandemic management and to prevent future outbreaks. In this review, we

summarize the animal models, zoonotic reservoirs, and cross-species transmission of the emerging human CoVs. Finally, we comment on potential sources of SARS-CoV-2 Omicron VOCs and the new SARS-CoV-2 recombinants currently under investigation.

1. INTRODUCTION

1.1. Prevalence and History of Coronavirus Diseases

Efforts to fight emerging infectious diseases have focused mainly on acute infectious diseases that have caused large-scale outbreaks following a spillover event. Coronaviruses (CoVs) infect a wide range of animal species, and those infecting humans typically cause a common respiratory illness that can be associated with gastroenteritis and mild respiratory infections. However, the world is currently experiencing the third emergence and spread of a novel zoonotic CoV within two decades, in this instance causing a global pandemic, in which infection can result in severe respiratory disease with comparatively higher case fatality rates (CFRs).

Seven human-infecting CoVs have been identified so far. Because four of them, namely HCoV-229E, HCoV-OC43, HCoV-NL63, and HKU1, are less virulent to humans and cause only common cold–like illnesses, they are not the focus of this review. Here we discuss in detail the three major CoVs that have significantly impacted human health. Of note, two newly identified CoVs, CCoV-HuPn-2018 and Hu-PDCoV, have recently been reported to cause human infections.

On November 16, 2002, the first outbreak of severe acute respiratory syndrome (SARS), caused by SARS-CoV, started in Guangdong Province in China (1). SARS-CoV spread to multiple countries around the world and caused approximately 750 deaths, with more than 8,000 cases reported from 26 countries before its eventual containment (2). Ten years later, a second novel CoV epidemic appeared in the Middle East, caused by the Middle East respiratory syndrome (MERS)-CoV. The disease was first observed in a 60-year-old man with acute pneumonia, who further developed subsequent renal failure before he died in Jeddah Hospital, Saudi Arabia, in 2012 (3). Following several subsequent clusters and many nosocomial cases, the virus spread to other Asian countries through many superspreading events, as well as America, Europe, and Africa (4). With the highest CoV CFR ever recorded (35–40%), MERS has resulted in 2,494 laboratory-confirmed cases and 850 deaths among 27 countries to date (5).

In December 2019, coronavirus disease 2019 (COVID-19), caused by a SARS-like virus named SARS-CoV-2 (initially called HCoV-2019), was first reported in Wuhan, Hubei Province, China (6–8). The disease has resulted in the ongoing pandemic associated with mild respiratory illness; however, older patients, the unvaccinated, and those with underlying medical issues are more likely to develop severe pneumonia resulting in death. Since the initial outbreak, several SARS-CoV-2 variants associated with increased transmissibility and/or pathogenicity have been recorded. Thus far, COVID-19 has caused the most threatening and devastating pandemic in the twenty-first century, with more than 450 million confirmed cases and 6 million deaths as of mid-March 2022 (9).

1.2. Genomic Similarities and Differences Between Emerging Human Coronaviruses

SARS-CoV, MERS-CoV, and SARS-CoV-2 belong to the genus *Betacoronavirus* (beta-CoV). They share similar genomic organization with other CoV genomes, consisting of a long and nonsegmented RNA (26–32 kb), single-strand and positive-sense. The genome contains six conserved open reading frames (ORFs), with untranslated regions at the two extremities: a 5′ methylated cap and a 3′ polyadenylated tail. The 5′ end consists of two ORFs (ORF1a and ORF1b) coding

for the replicase necessary for virus replication and transcription, and the 3′ end consists of genes encoding structural proteins such as the spike glycoprotein (S), envelope (E), membrane (M), and nucleocapsid (N) proteins.

SARS-CoV and SARS-CoV-2 belong to the same subgenus, *Sarbecovirus*, with bat-SARS-like CoVs (RmYN02 and RaTG13), and are more distantly related to MERS-CoV, which belongs to the *Merbecovirus* clade within the beta-CoV genus. Genomic comparison of SARS-CoV-2 to other related SARS-CoVs showed a nucleotide identity of 96%, 93%, 90%, 80%, and 50% to the genomes of bat-CoV-RaTG13, bat-CoV-RmYN02, Malayan pangolin-CoV, SARS-CoV, and MERS-CoV, respectively, suggesting that SARS-CoV is more distantly related to SARS-CoV-2 compared to other bat-origin SARS-related CoVs (10).

The spike sequence of CoVs is of great importance for the viruses' ability to infect host cells, and its instability may be either beneficial or fatal for virus survival. For instance, although bat-CoV-RaTG13 is currently the closest known CoV to SARS-CoV-2 at the whole-genome level, the receptor-binding domain (RBD) of pangolin-CoV appears to be more similar to that of SARS-CoV-2, with the same five key amino acid residues involved in its interaction with human angiotensin-converting enzyme 2 (ACE2) (11–13). Importantly, in the subgenus *Sarbecovirus*, only SARS-CoV-2 and the isolated bat-CoV-RmYN02 have amino acid insertions in the cleavage site at the junction of the two subunits (S1 and S2) of the spike protein (14). These data suggest different evolutionary routes of beta-CoVs, involving various distinct reservoirs and/or intermediate hosts.

1.3. Diseases and Symptoms Caused by Emerging Human-Infecting Coronaviruses

Human-infecting CoVs spread mainly through respiratory droplets from a viremic individual. The median incubation period is approximately 4–6 days for SARS, MERS, and COVID-19. The initial symptom for these infections is a fever, and cough is the second most common symptom. Patients frequently displayed the following symptoms depending on the type of virus infection: Both SARS and MERS included chills and dyspnea, but SARS also included myalgia and headache, whereas MERS included shortness of breath. COVID-19 patients suffered from fatigue, sputum production, and myalgia as the next most common symptoms. However, diarrhea was noted in only a smaller proportion in SARS (17.3%) and COVID-19 (24%) patients (15).

Investigation during the MERS outbreak in Korea during 2016 found that in 186 cases, nearly 20% had developed diarrhea, supporting gastroenteritis as a frequent symptom of CoV infection (16). Most COVID-19 patients admitted into the intensive care unit have at least one preexisting chronic disease as a background comorbidity, and hypertension accounts for more than 50% of cases, whereas diabetes was the most common comorbid condition in MERS patients (17, 18). Similar radiological findings make it difficult to differentiate these three CoV infections, which may progress to severe pneumonia in later stages.

Most SARS, MERS, and COVID-19 patients had abnormal chest tests, and histopathological examination commonly showed a diffuse alveolar destruction, and pulmonary edema with hyaline membrane formation, indicative of acute respiratory distress syndrome. Whereas bilateral ground opacities with consolidation are more frequent in COVID-19 patients, the pathological features of MERS patients included exudative alveolar damage and bronchial submucosal gland necrosis (19).

Mechanisms underlying CoV disease pathogenesis are not understood fully. In both SARS and COVID-19 patients, the disease progression is correlated with a significant increase of macrophages and neutrophils, and severe outcomes are associated with an impaired T-cell response/lymphopenia and increased ferritin levels. Inflammatory markers such as interleukin 6 (IL-6), IP-10, MCP1, MIP1A, and TNF-α are frequently reported at a high level in hospitalized

COVID-19 patients, and uncontrolled inflammation is caused by an excess release of proinflammatory cytokines such as IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGF-β and chemokines including CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10 from immune effector cells (20). $CD4^+$ T cells expressing low levels of IL-6 and GM-CSF were also reported in COVID-19 patients who did not require admittance into the intensive care unit (21).

MERS-CoV infects a broad range of immune cells, which induce a sustained production of proinflammatory cytokines and chemokines such as TNF-α, IL-6, CXCL10, CCL2, CCL3, CCL5, and IL-8. In addition to T-cell apoptosis, MERS-CoV was also reported to induce apoptosis of both lung and kidney cells through upregulation of Smad7 and fibroblast growth factor 2 expression, providing a potential explanation for its high CFR (~35%) (20). The late stages of CoV diseases share common features, including hyperinflammation associated with multi-organ damage and failure, accentuating disease progression toward a fatal outcome.

1.4. Facts on Human-Infecting Coronaviruses

An important characteristic of CoVs is the high instability of their spike protein, as evidenced by numerous lineages and variants. A lineage is a set of closely related viruses with a common ancestor, and a variant contains one or more mutations with the potential to alter viral phenotype, with implications for viral fitness and/or pathogenicity (22). For the current pandemic, different nomenclature systems [Nextstrain, Pango, World Health Organization (WHO)] have been established to facilitate the tracking of SARS-CoV-2 lineages and variants. The variants under monitoring represent SARS-CoV-2 variants with unclear evidence of phenotypic and epidemiological impacts. The variants of interest are attributed to specific genetic markers of SARS-CoV-2 that are known to affect viral fitness, immune escape, and diagnostics without causing serious, large-scale outbreaks. SARS-CoV-2 variants are considered variants of concern (VOCs) if there is evidence of increased transmissibility; increased disease severity; or a negative impact on diagnostics, treatments, or vaccines (22).

During the SARS-CoV outbreak, the mutation D480A/G within the RBD caused an antigenic drift, and the resulting SARS-CoV variant became dominant as the epidemic proceeded (23). Recently, a new bat-CoV, named NeoCoV, was discovered and identified as the closest MERS-CoV relative (24). However, compared to MERS-CoV, NeoCoV efficiently used some types of bat ACE2 and, less favorably, human ACE2 for cell entry. Following the amino acid mutation T510F within the receptor-binding motif, NeoCoV efficiently infected human ACE2 cells. This confirmed the first case of ACE2 usage of a MERS-related CoV and supported a risk of emergence of a potential biosafety threat from the subgenus *Merbecovirus*. In contrast to previous human CoVs, SARS-CoV-2 has spread rapidly to human populations and animals in close proximity, and numerous mutations in the spike protein and recombinations have been identified. Five SARS-CoV-2 VOCs, including Alpha (Pango lineage B.1.1.7), Beta (Pango lineage B.1.351), Gamma (Pango lineage P.1), Delta (Pango lineage B.1.617.2), and Omicron (Pango lineage B.1.1.529), have been recorded to circulate since the first outbreak was reported in Wuhan (7).

The SARS-CoV-2 genome has been evolving more rapidly since the emergence of the Omicron VOC. Two Delta and Omicron recombinants (XD, XF) and one recombinant from Omicron subvariants BA.1 and BA.2 (XE) have already been reported (25). Recent data further identified new Omicron variants (Pango lineage BA.4 and BA.5) containing unique additional mutations (S:L452R, S:F486V) with the potential for immune escape from vaccines based on the prototype SARS-CoV-2 from Wuhan (26). Further investigations are needed to highlight evidence regarding changes in viral properties and transmissibility and the impact on current COVID-19 countermeasures. Details relating to the molecular and epidemiological features of these VOCs are summarized in **Table 1**.

Table 1 Molecular and epidemiologic characteristics of SARS-CoV-2 variants of concern

Name		Location and date of the first case	Important mutations (molecular location)	Impact on viral properties and disease severity	Impact on diagnostic, treatment, and vaccine	Human/ animal origin	Reference
WHO	Pango lineage						
Alpha	B.1.1.7	September 2020 United Kingdom	Δ144 (NTD) N501Y (RBD) D614G (S1-CTD)	Increased ACE2 binding affinity Fitness gain	Moderate antibody escape	Human	(27, 28)
Beta	B.1.351	December 2020 South Africa	Δ243–244 (NTD) K417N (RBD) E484K (RBM) N501Y (RBM) D614G (S1-CTD)	Increased ACE2 binding	Significant mAbs escape Reduced convalescent plasma neutralization	Human	(28, 29)
Gamma	P.1	December 2020 Brazil	K417T (RBD) E484K (RBM) N501Y (RBM) D614G (S1-CTD)	Immune system evasion	Decreased vaccine efficacy	Human	(30, 31)
Delta	B.1.617.2	December 2020 India	L452R (RBM) T478K (RBM) P681R (FCS) D614G (S1-CTD)	Increased ACE2 affinity Rapid spread and disease severity	Partial resistance to vaccine-elicited antibodies and convalescent sera	Human	(30, 32)
Omicron	B.1.1.529	November 2021 South Africa	K417N (RBD) E484A (RBM) Q493R (RBM) Q498R (RBM) N501Y (RBM) D614G (S1-CTD) P681H (S1/S2)	Significant increase in infectivity and transmissibility Significant decrease in disease severity	Substantial fall (20%) in neutralization Significant decrease in vaccine efficacy Negative signal in some S-gene targeting PCRs	Mouse/human	(31, 33, 34)

Abbreviations: ACE2, angiotensin-converting enzyme 2; CTD, C-terminal domain; FCS, furin cleavage site; mAbs, monoclonal antibodies; NTD, N-terminal domain; PCR, polymerase chain reaction; RBD, receptor-binding domain; RBM, receptor-binding motif; S1/S2, junction subunit between subunit S1 and S2; WHO, World Health Organization.

2. ANIMAL MODELS FOR STUDYING EMERGING HUMAN-INFECTING CORONAVIRUSES

2.1. Common Laboratory Animals

An urgent task upon the emergence and spread of any virulent pathogen within the human or animal population is to identify an ideal animal model for testing the effectiveness of potential countermeasures. Regarding human CoV diseases, standard laboratory animals, including rodents and nonhuman primates (NHPs), have been extensively tested and/or used during preclinical investigations.

2.1.1. Nonhuman primates. The results from animal models for the study of CoVs are summarized in **Table 2**. Nonhuman primates are generally considered to be the closest organisms to humans based on phylogenetic and biological similarities. The use of NHPs to model human viral diseases, including those caused by human-infecting CoVs, is an important resource to study key aspects of disease pathogenesis and evaluate candidate vaccine and antiviral efficacy.

The CoVs known to cause disease in humans can generally infect and replicate in the respiratory tracts of NHPs, including rhesus macaque (*Macaca mulatta*), cynomolgus macaque (*Macaca fascicularis*), African green monkey (*Chlorocebus aethiops*), and common marmoset (*Callithrix jacchus*), with disease-free illness to moderate clinical symptoms (35, 36). The clinical manifestation differs depending on the type of CoV and host species. For example, in contrast to other NHPs, common marmosets have demonstrated several key features of human MERS (37). Animals exhibited severe pneumonia, pulmonary edema, hemorrhage, degeneration and necrosis of pneumocytes and bronchial epithelial cells, and infiltration of eosinophils and neutrophils. MERS-CoV antigen was identified in type I and II pneumocytes, alveolar macrophages, and bronchial epithelial cells.

The experience gained during SARS and MERS, and the number of candidate vaccines and drugs that were under development, has helped accelerate the fight against COVID-19. Following the emergence of SARS-CoV-2, various NHP species were experimentally investigated. NHPs infected through different routes with a dose range of $1 \times 10^{4-7}$ plaque-forming units (PFU) showed a high susceptibility to SARS-CoV-2 infection and generally displayed mild clinical signs. Except for acute respiratory distress syndrome developed by African green monkeys, increased body temperature, reduced activity and appetite, and changes in respiratory patterns were the major clinical findings from SARS-CoV-2-infected NHPs (35, 36). SARS-CoV-2 RNA and infectious particles were found in nasal, oral, and several other tissues, including the lungs. Consistent with this, histopathological analysis supported mainly interstitial pneumonia. However, compared to macaques, baboons developed severe lung disease with a high level of inflammation (35).

Multiple SARS-CoV-2 antibody subclasses, including those targeting the RBD, the perfusion S ectodomain, and nucleocapsid domain (N), as well as SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T-cell responses, were observed in challenged NHPs. Although the induction of innate and adaptive immune responses and the relative importance of neutralized antibodies have been observed, finding immune correlates of protection in SARS-CoV-2-infected NHPs remains a challenge. These results suggest the need for further investigation of other primate species in the wild and in captivity.

2.1.2. Mice. Previous investigations have found that wild-type mice are less permissive to SARS-CoV and MERS-CoV infection (38, 39). These results found a weak binding affinity between the homologs of murine ACE2 or dipeptidyl peptidase 4 (DPP4) and the RBDs of these viruses.

Mice were subsequently either transduced to express their human counterpart hACE2/hDPP4 or adapted to these viruses by serial passage to display a productive infection. Transgenic mice

Table 2 Summary of experimental infection of common laboratory animals (except deer mice) for establishing the SARS-CoV-2 animal model

Animal	Dose and route of exposure	Clinical signs	Pathology and death	Virus isolation	Shedding and transmission	Reference
Human	Inhalation of respiratory droplets	Fever, dry cough, myalgia, joint pain and dyspnea	DAD, hyaline membrane formation with infiltrations, thickening of alveolar wall, multi-organ failure, death	Respiratory tracts and lungs, other organs (Kidney, liver, spleen and intestine)	Direct and indirect via aerosols	(17, 20)
NHPs	$1 \times 10^{4-7}$ $TCID_{50}$ (IN, IT, IO, IOc)	Modest increase in body temperature, decreased appetite and responsiveness, acute respiratory distress syndrome	Mild to moderate transient/interstitial pneumonia, gross lung lesions with a high level of inflammation	Upper and lower respiratory tracts	Not documented	(35–37)
Transgenic mice	$\sim 7 \times 10^{5}$ $TCID_{50}$ (IN)	Lethargy, fever, body weight loss	Moderate to severe lung pathology that can be associated with neurological manifestations, death	Upper and lower respiratory tracts, intestines	Not documented	(42, 43)
Mouse-adapted	1×10^{5} $TCID_{50}$ (IN)	Mild clinical symptoms, weight loss in some aged mice	Denatured trachea, moderate to severe lung damage, death	Upper and lower respiratory tracts, feces	Not documented	(40, 44)
Deer mice	$2 \times 10^{4-5}$ $TCID_{50}$ (IN)	Subclinical, occasional ruffled fur	Mild neutrophilic infiltration in the submucosa, lung lesions with infiltrations of histiocytes and neutrophils with occasional multinucleated syncytial cells, occasional discrete foci of interstitial pneumonia	Lungs; high titers in nasal turbinates	Direct contact	(76)
Ferret	$1 \times 10^{5-6}$ $TCID_{50}$ (IN)	Subclinical, increased body temperature, reduced activity and appetite, ruffled fur, coughing, snoring	Mild to moderate lesions in nasal turbinate and lung	Nasal turbinates (high titers), oral, saliva, urine, feces	Direct contact, and indirect via aerosol	(55, 56)
Hamster	10^{3-5} PFU (IN)	Weight loss, lethargy, rapid breathing, ruffled fur, hunched posture	Moderate-to-severe lesions in nasal turbinates, trachea, lungs, spleen, intestines, lymph nodes, kidneys, adrenal glands, and reproductive organs, focal lesions in liver, gallbladder, heart, and lymph nodes, death (>10 months hamster)	Broad range of organs consistent with the systemic infection	Indirect via aerosol and direct contact	(63–66)
Rabbit	$3 \times 10^{4-5}$ (IN)	Subclinical	Mild to moderate lung lesions	Not documented	Not documented	(73, 74)
Tree shrew	$1 \times 10^{6-7}$ $TCID_{50}$ (IN, IO, IOc)	Absent	Pulmonary infiltrates, thickened alveolar septa and interstitial hemorrhage	No live virus, higher viral RNA in lungs	Not documented	(75)

Abbreviations: IN, intranasal inoculation; IO, oral inoculation; IOc, ocular inoculation; IT, intratracheal; NHP, nonhuman primate.

showed obvious clinical manifestations including lethargy, fever, sneezing, nasal discharge, and evidence of lung pathology. Disease severity and death depended on receptor expression levels. For example, SARS-CoV (Urbani strain, GenBank accession no. AY278741.1) was adapted to young BALB/c mice by serial passage in the animal lungs, and after 15 passages, 100% mortality was achieved with a clone named M15 (40). A generation of humanized exons 10–12 of the mouse DPP4 locus, followed by 30 serial passages through the lungs of knock-in mice, also provided promising results (41). A mouse-adapted MERS-CoV named MERSMA was obtained and the infection of knock-in mice with MERSMA resulted in weight loss and diffuse alveolar damage with membrane hyaline formation associated with infiltration, and the MERSMA antigen was identified in airway epithelia, pneumocytes, and macrophages (41). Nevertheless, evidence of encephalitis for SARS-CoV-infected mice indicates the virus spread to rodent brains.

Transgenic mice including HFH4-hACE2 and K18-hACE2 have also been used extensively to understand COVID-19 pathogenesis. Collectively, the infection of hACE2 mice resulted in robust replication of SARS-CoV-2 with high viral titers in upper and lower respiratory tracts, body weight loss, and histopathological lesions in lungs (42, 43). Similar to previous reports for SARS-CoV animal infection, disease severity and animal deaths were often associated with either hACE2 expression levels or the migration of SARS-CoV-2 to animal central nervous system.

Additionally, SARS-CoV-2 has also been adapted to mice after several passages to display severe disease in these animals. Mouse-adapted strains were also found to mount a severe but more stable disease following infection with SARS-CoV-2. For instance, a novel mouse-adapted SARS-CoV-2 strain, MASCp36, exhibited age- and gender-related mortality akin to lethal COVID-19 (44). While mutations including N501Y and Q493H enhanced the binding affinity to hACE2, triple mutations at N501Y/Q493H/K427N decreased the affinity and plummeted MASCp36 infectivity. These results supported the N501Y mutation as an important adaptive mutation. Interestingly, this mutation was also found in several SARS-CoV-2 VOCs (Alpha, Beta, and Gamma). However, mouse-adapted SARS-CoV-2 did not replicate in the central nervous system or impair replication in human airway cells and maintained antigenicity similar to human SARS-CoV-2 strains. Compared to previous SARS-CoV-2 VOCs, several wild-type (129, C57BL/6, BALB/c) and transgenic (K18-hACE2) mice infected with 10^4, 10^5, or 10^6 PFU of Omicron variant (B.1.1.529) exhibited less disease burden, including absence of weight loss and low viral load in the upper and lower respiratory tracts (45–47). Measurement of pulmonary function showed no increase in lung enhanced pause (Penh), with a stable ratio of peak expiratory flow (Rpef) in BALB/c mice. Additionally, evidence of lower levels of proinflammatory cytokines and chemokines suggested that Omicron VOC B.1.1.529 is not pathogenic for common laboratory mice. Overall, both transgenic and mouse-adapted SARS-CoV-2 represent useful tools and have been used broadly to develop SARS-CoV-2 countermeasures.

2.1.3. Ferrets. Ferrets represent a good model for many respiratory viral diseases, including influenza, respiratory syncytial virus, and CoV (48). Regarding human CoVs, ferrets were found to be highly susceptible to infection from SARS-CoV and SARS-CoV-2 but resistant to MERS-CoV infection (49–52). Even with high expression of hDPP4 in the bronchiolar epithelium and the lungs, ferrets remained nonsusceptible to MERS-CoV infection, complicating in vivo investigations with MERS-CoV in this animal (53).

After infection with doses ranging from 10^3 to 10^7 $TCID_{50}$ SARS-CoV, animals yielded a productive infection with obvious clinical disease, such as lethargy, fever, sneezing, nasal discharge, and evidence of lung pathology (54). However, histopathological analysis supported evidence of hepatic lipidosis and emancipation, implying that mortality was not associated with pneumonia (49).

Inoculating ferrets with SARS-CoV-2 led to an increased body temperature associated with virus replication and shedding in nasal washes, saliva, urine, and feces for up to 8 days postinfection (dpi) (55). All direct and a few indirect naïve-contact ferrets were infected by 2 dpi, implying airborne transmission, which is a fundamental characteristic of highly efficient dissemination by SARS-CoV-2. After a high (5×10^6) or medium (5×10^4) dose of SARS-CoV-2 was delivered intranasally (IN), mild multifocal bronchopneumonia in 5–15% of the ferret lungs was observed on 3 dpi in both high- and medium-dosed groups. Overall, the SARS-CoV-2 ferret model displayed mild clinical disease and relatively lower virus titers in the lungs, supporting ferrets as useful animals to understand SARS-CoV-2 pathogenesis and transmission (56).

2.1.4. Hamsters. Hamsters were also identified as nonpermissive and less susceptible to MERS-CoV and SARS-CoV infection (57). After infection with SARS-CoV, hamsters exhibited no apparent clinical symptoms (58–60). Subsequent attempts using different SARS-CoV strains, including Urbani, HKU-39849, Frankfurt 1, and a recombinant clone GD03T0013, have resulted in limited infection, suggesting that the hamster model is less useful compared to hACE2 mice and mouse-adapted SARS-CoV models for this particular pathogen (61).

However, following the emergence of SARS-CoV-2, the binding of the spike to ACE2 in Syrian hamster was first predicted by Damas et al. (62) and subsequently supported by other bioinformatics and protein structural studies (63). After inoculation with 10^5 PFU of SARS-CoV-2, hamsters displayed clinical symptoms such as weight loss, lethargy, and rapid breathing between 2 and 7 dpi (64). SARS-CoV-2 infection adversely affected the tracheas of these animals, and high viral titers were found in lungs (10^5–10^7 $TCID_{50}$/g at 2 and 4 dpi). Interestingly, potent induction of IFN-β to SARS-CoV-2 infection suggests innate immune response activation. Despite earlier detection of neutralizing antibodies from 7 dpi and following passive immunization, challenged hamsters showed no amelioration in clinical signs, raising concerns about protective immunization induced only by antibodies against SARS-CoV-2. A similar concern with SARS-CoV was raised previously.

The hamster model demonstrated net age differences in histopathology (65). Young hamsters triggered an earlier and robust influx of strong infiltration of immune cells and showed less weight loss. Both rechallenge and passive transfer of convalescent serum to naïve hamsters suppressed SARS-CoV-2 replication in the animal lungs (66).

Experimental infection of hamsters with SARS-CoV-2 VOCs (except Omicron) showed comparable results to the parental strain Wuhan-Hu-1. The infection of hamsters with Alpha, Beta, and Delta VOCs resulted in efficient infection with no significant difference in terms of viral load and pathology (67). A comparative study of the pathogenesis of Beta and Delta variants in the Syrian hamster found similar viral shedding patterns among all tested variants (68). Delta-infected hamsters showed increased levels of subgenomic RNA in the respiratory tract of animals with moderate lung disease for up to 2 weeks. However, moderate discrepancies exist in the results of SARS-CoV-2 Omicron VOC (B.1.1529) experimental infection regarding the hamster model. Findings from Halfmann et al. (45) and Abdelnabi et al. (47) supported a weak ability of Omicron to trigger a productive infection in hamsters. Six- to ten-week-old Syrian hamsters infected with 10^3 PFU of Omicron (Pango B.1.1.529) exhibited limited viral infection and mild clinical disease and pathology. Infected hamsters showed no changes in Penh, Rpef, and respiratory rate characteristics of an attenuated lung infection. Examination of lung pathology showed small foci of inflammation in the alveoli and peribronchial regions and fewer viral RNA in the alveoli.

Compared to previous results cited above, Zhang et al. (46) and Yuan et al. (69) found that Omicron can readily infect hamsters with obvious high fitness, pathogenesis, and transmissibility when administered with titers of 2×10^4 and 10^5 PFU. Collectively, infected animals showed moderate viral burden associated with mild body weight losses, clinical scores, and viral RNA

shedding in oral and fecal samples for up to 12 dpi. At 4 dpi, the Omicron variant exhibited ~10–20% higher transmissibility than the Delta variant in repeated noncontact transmission assays and outcompeted the Delta variant under immune selection pressure. Animals displayed low and temporary dysregulation of inflammatory cytokine/chemokine response and significant low antibody response against Omicron variant–specific RBD. Histopathological changes were less severe for Omicron-infected hamsters and rapidly returned to normal by 7 dpi. Taken together, these results show a dose-dependent effect on hamsters infected with the Omicron variant; when inoculated at sufficiently high doses, hamsters replicated some key aspects of human Omicron disease, including a growth advantage, low disease severity, and a significant reduction in neutralization compared to the Delta variant (25).

2.1.5. Guinea pigs, rabbits, and tree shrews. Guinea pigs (*Cavia porcellus*) infected with SARS-CoV exhibited interstitial pneumonitis with no clinical disease (70). New Zealand white rabbits were permissive to MERS-CoV infection (71). MERS-CoV RNA, as well as infectious viruses, were confirmed by polymerase chain reaction (PCR) and titration up to 4 dpi in pigs (72). However, following reinfection with MERS-CoV, rabbits displayed increased lung inflammation and complement proteins, but neutralizing antibodies were lacking (71). Compared to results in SARS-CoV, rabbits appeared to be susceptible to SARS-CoV-2 following experimental infection, and the disease was subclinical (73). Rabbits were also used to evaluate SARS-CoV-2 candidate vaccines (74). Young tree shrews (*Tupaia belangeri*) inoculated with SARS-CoV-2 shed virus at earlier time points, whereas longer-duration virus shedding was observed in adult shrews (75). Despite a close phylogenetic relationship to primates, tree shrews displayed limited susceptibility to SARS-CoV-2 infection, rendering them less useful for the evaluation of SARS-CoV-2 vaccines and drugs.

2.2. Nonconventional Animals

Effectively managing outbreaks of human CoV is a substantial challenge. The greatest part of this difficulty can be attributed to a lack of an ideal animal model for accurately and efficiently evaluating candidate vaccines and other antivirals. Given that emerging human CoVs have an array of animal hosts, various wild and domestic/farm animals have been experimentally infected with these viruses to assess their susceptibility.

2.2.1. Deer mice. Recent investigations identified deer mice (*Peromyscus maniculatus*) as susceptible to SARS-CoV-2 infection (76). Compared to laboratory mice, deer mice were identified to better model SARS-CoV-2 infection. Deer mice inoculated intranasally with 2×10^4 PFU SARS-CoV-2 exhibited robust viral replication in the upper and lower respiratory tracts and in the intestines. Viral RNA was detected up to 21 dpi in oral swabs and 14 dpi in lungs, and contact transmission occurred from infected to naïve deer mice. Although the viral RNA was also detected in animal brains, no conspicuous signs or deaths were recorded. Several genes of the innate immune response, including *IFN-γ* and *IL-21*, were highly expressed in lungs. Although lab rodents are widely known to be resistant to these CoV infections, this study showed that deer mice are highly susceptible to SARS-CoV-2 infection, calling into question the role that wild rodents can play in cross-species transmission or as potential reservoirs or intermediate hosts of SARS-CoV-2 in nature.

2.2.2. Camelids. Members of the family Camelidae, including camels, llamas, and alpacas, are susceptible to MERS-CoV infection, and disease manifestation is significantly species specific. Camelids experimentally infected with MERS-CoV showed a nasal discharge and early shedding of infectious viral particles, lasting until 8 dpi for dromedary camels (77). Pathological findings in the upper respiratory tract displayed epithelial cell necrosis associated with massive cell loss and

depletion of DPP4 cell-surface receptors, and in the lower respiratory tract, inflammation was limited to metaplasia and lymphocyte infiltration (78).

The rapid clearance of MERS-CoV from these large mammals suggests they are less suitable for MERS-CoV in vivo studies. Additionally, camelids are generally large and difficult to handle. Special containment facilities are therefore required, providing substantial challenges for in vivo investigations in these animals. However, the alpaca was used recently to develop nanobodies against SARS-CoV-2 infection. Alpaca nanobodies were identified to target the spike of the RBD of SARS-CoV-2 and were found to block virus entry (79).

2.2.3. Minks. Experimental infection of American mink (*Neovison vison*) with an IN dose of 5×10^6 PFU SARS-CoV-2 triggered robust viral replication, and infectious viruses were recovered from the upper and lower respiratory tracts (80). Infected mink shed viral RNA for up to 12 dpi and exhibited body weight loss. Histopathological lesions in lungs and respiratory airways, as well as the lethal form of the disease in natural infection with SARS-CoV-2, showed that mustelids have potential in modeling SARS-CoV-2 pathogenesis and evaluating candidate SARS-CoV-2 vaccines and therapeutics.

2.2.4. Cats and dogs. The susceptibility of various animals, including pets and other domestic animals, was suspected following instances of natural infection. After experimental inoculation with doses of $1–7 \times 10^5$ PFU of SARS-CoV-2, cats exhibited a strong viral replication and shedding through oral and rectal swabs (81, 82). Isolation of infectious SARS-CoV-2 particles in the upper and lower respiratory tracts was consistent with mild histopathological lesions. Direct and indirect cat-to-cat transmission of SARS-CoV-2 has also been confirmed. Reinfected cats were unable to transmit SARS-CoV-2 to naïve cats, suggesting strong production of specific SARS-CoV-2-neutralizing antibodies. Conversely, dogs are not permissive to SARS-CoV-2 infection (82). Although they seroconverted and produced neutralizing antibodies, dogs did not shed SARS-CoV-2 RNA, and no viable virus was found in swabs and tissue samples during these studies. These findings confirmed the absence of clinical and histopathological changes, and dogs are not deemed to be useful for SARS-CoV-2 preclinical studies.

2.2.5. Raccoon dogs. Raccoon dogs (*Nyctereutes procyonoides*) were also identified to be susceptible to SARS-CoV-2 infection, with a long viral RNA shedding period (up to 16 dpi) in nasal and rectal swabs (83). Surprisingly, with no infectious virus recovered from the lower respiratory tract, raccoon dogs could successfully transmit SARS-CoV-2 virus to naïve animals under farm-like conditions.

2.2.6. White-tailed deer. Primary computational investigations predicted the white-tailed deer as susceptible to SARS-CoV-2 infection (62). After IN inoculation with 2–5 mL of viral suspension containing $10^{6–6.3}$ $TCID_{50}$ of SARS-CoV-2, white-tailed deer displayed productive infection without significant lung disease (84). However, consistent with a systemic infection with infectious viral shedding through the upper respiratory and enteric tracts, infected animals efficiently transmitted SARS-CoV-2 to naïve deer. Interestingly, one experimental study in white-tailed deer discovered in vivo competition of SARS-CoV-2 isolates and vertical transmission from the doe to the fetus. These studies demonstrated that adult white-tailed deer are permissible to SARS-CoV-2 infection and may be an important animal for constant epidemiological surveillance to prevent transmission (85).

2.2.7. Bats. Bats are generally susceptible to infection from many zoonotic viruses that spill over to humans and rarely show overt clinical manifestations even when persistently infected with highly virulent pathogens. Experimental infection of Egyptian fruit bats (*Rousettus aegyptiacus*)

and American big brown bats (*Eptesicus fuscus*) with 10^5 $TCID_{50}$ SARS-CoV-2 through IN and intraoral routes showed divergent results (86, 87). Whereas American big brown bats appeared to be completely nonpermissive to SARS-CoV-2 infection, Egyptian fruit bats displayed transient infection. SARS-CoV-2 RNA was detected in oral and fecal samples and live virus recovered from only the trachea ($10^{2–2.5}$ PFU) and the nasal epithelium ($10^{1.75}$ PFU) at 4 dpi. With no obvious disease symptoms and minimal histological changes, inoculated fruit bats transmitted SARS-CoV-2 to one out of three naïve co-housed fruit bats. These results support a moderate susceptibility of fruit bats and a resistance of insectivore bats to SARS-CoV-2 infection. Also, because bats are primary reservoirs of various zoonotic CoVs, the high diversity of key residues in the receptor-binding motif is a critical factor regarding bat susceptibility to a given CoV species, while high diversity within ACE2 protein sequences, especially in the region that interacts with the spike RBD, is another explanatory factor regarding these divergent results.

3. ZOONOTIC RESERVOIRS OF EMERGING CORONAVIRUSES

3.1. SARS-CoV

SARS-CoV is the first zoonotic CoV to cause severe respiratory disease in humans. Although bats are known to be a putative reservoir host, various wild and domestic animals were further naturally or experimentally infected with SARS-CoV, and the resulting disease was generally absent or mild.

3.1.1. Putative reservoir in horseshoe bats. In 2005, serological and molecular studies identified several SARS-like CoVs in different species of horseshoe bats. A high prevalence of antibodies and viral nucleic acid was observed in various bat species, especially in the Chinese horseshoe bat, *Rhinolophus sinicus* (88, 89). Molecular and phylogenetic studies supported bats, particularly the genus *Rhinolophus*, as the potential reservoir of SARS-CoV, and sequence analysis showed that bat-origin SARS-like CoVs shared 88–92% sequence identity with human SARS-CoV and civet SARS-like CoVs, with the most variable region found at the 5′ end of the *S* gene (90).

3.1.2. Intermediate hosts. SARS-CoV is the first human CoV to have caused an epidemic of significant public health concern in recent history. Following its emergence in Guangdong Province, initial investigations suggested an animal origin. Early epidemiological studies on traders from several animal markets in Guangzhou, Guangdong Province, in 2003, as well as animal food handlers, found a high prevalence of SARS-CoV IgG antibodies compared to in vegetable traders and control groups (91, 92). Thereafter, Guan et al. (93) performed a SARS-CoV nucleic acid test on nasal and fecal swabs of 25 individuals from different animal species gathered in Dongmen Market, Shenzhen, China, and identified all the masked palm civets and one raccoon dog as positive for SARS-CoV RNA. However, further data suggested palm civets as amplifying or intermediate hosts rather than the SARS-CoV natural reservoir, as sequence analysis depicted a nucleic acid identity of 99.8% of SARS-CoVs isolated from palm civets, and most of the investigations on farmed and trapped masked palm civets (wildlife) were negative for SARS-CoV (90). In addition to palm civets and a raccoon dog, SARS-CoV was detected in ferret badgers, red foxes, domestic cats, and rice field rats, suggesting these animals' potential role as either intermediate hosts or mechanical carriers of SARS-CoV, as the virus could be transmitted from humans to these animals (93, 94).

3.2. MERS-CoV

Similar to SARS-CoV, bats have been suspected to be the reservoir host of MERS-CoV. However, compared to SARS-CoV, MERS-CoV has affected livestock, and dromedary camels were found to be a MERS-CoV reservoir or intermediate/amplifying host.

3.2.1. Putative reservoir in bats. Following the first MERS outbreak in the Middle East, many research teams raised suspicions of a bat origin for MERS-CoV. Using real-time PCR for RNA detection, Annan et al. (95) performed a screening study on 4,758 bats from 10 different species from Ghana and 272 *Pipistrellus* bats from 4 European countries, and identified beta-CoVs closely related to MERS-CoV (EMC/2012) in two bat genera (*Nycteris* and *Pipistrellus*). Another bat-CoV, named PML/2011, which is more closely related to MERS-CoV, was identified in one pellet sample of *Neoromicia* cf. *zuluensis* from South Africa (96). Amino acid analysis showed that PML/2011 differed from MERS-CoV by only one amino acid in the translated 816 nucleotides of the RNA-dependent RNA-polymerase gene (*RdRp*), suggesting PML/2011 as the closest related virus to MERS-CoV.

During the MERS outbreak, Memish et al. (97) detected a beta-CoV from a fecal pellet of a bat (*Taphozous perforatus*) with 100% shared nucleotide identity to MERS-CoV (β-CoV 2c EMC/2012) cloned from an index case patient in Bisha, Saudi Arabia. Interestingly, experimental studies investigating bats' potential role as a MERS-CoV reservoir showed evidence of infection, and bats shed the virus without any clinical signs of disease (98). Subsequently, several bat MERS-like CoVs (distantly related to MERS) were identified in America and Asia, and current data on the bat origin of MERS-CoV suggest insectivorous bats, especially the genera *Neoromicia* and *Pipistrellus*, as likely MERS-CoV reservoir groups. The isolation of many CoVs relatively distant to MERS-CoV in diverse bat taxa, with high similarity in some genomic regions, and the high susceptibility of fruit bats to MERS-CoV infection, suggest investigations should be expanded to other bat taxa.

3.2.2. Dromedary camels as the main reservoir and intermediate host. In contrast to results in bats, extensive data support direct transmission of MERS-CoV from camels to humans. Evidence implicating camels in interspecies transmission came from seroprevalence and molecular studies identifying MERS-CoV-specific antibodies as well as viral RNA in camel samples and products (milk and meat) through a wide range of Middle Eastern and North African countries (99, 100). Dromedary camel owners/farmers were also found to be infected, and a nearly full genome of MERS-CoV with a very close phylogenetic relation to human MERS-CoV was obtained from one camel (101).

Multiple retrospective studies investigating the time frame of MERS-CoV introduction to dromedary camel populations found that MERS-CoV had been circulating in camels in the Middle East and Africa for decades before the first human MERS outbreak in Saudi Arabia (99, 100, 102). These data implicated camels as the reservoirs and carriers of MERS-CoV and suggested the ability of these large mammals to maintain MERS-CoV circulation before its first human outbreak and the risk for future emergence of a MERS-related CoV. In such cases, characterizing the different MERS-CoV strains that were circulating in camels before the outbreak and comparing them to the epidemic strains, but also comparing MERS-CoV epidemic strains in different geographical locations, could help to increase understanding of MERS-CoV evolution from camel to human and vice versa. However, the difference in risk profile and the susceptibility of different populations to MERS-CoV infection (different populations with different habits and background genetics) cannot be excluded.

A study conducted in multiple African countries on other domestic mammals in contact with infected camels showed the presence of neutralizing antibodies in the sera of sheep and one goat, as well as MERS-CoV RNA in swabs from sheep, goats, cows, and donkeys (103). Though bats are considered the primary origin of MERS-CoV, direct evidence of the virus's evolution and transmission patterns in dromedary camels showed that these animals represent a main intermediate group, and that MERS-CoV surveillance should also include other domestic animals in close proximity to these camels.

3.2.3. Llama and alpaca as potential hosts for MERS-CoV. MERS-CoV antibodies were also found in alpacas and llamas, suggesting the susceptibility of Camelidae species and the potential role they might play as amplifying hosts (104). Similar to dromedary camels, these two species demonstrated a strong susceptibility to MERS-CoV infection, but they can be more easily handled in the laboratory and can serve as animal models for studying MERS-CoV in vivo (72, 105). Similar to in camels, seroconversion was reported for all of these infected animals, with a possibility of MERS-CoV reinfection (106).

3.3. SARS-CoV-2

Compared to SARS-CoV and MERS-CoV, SARS-CoV-2 has infected more animal species due to its high transmission rate. Although bats and pangolins were initially suspected to be reservoir and intermediate hosts of SARS-CoV-2, natural infection of farm, domestic, and wild animals by SARS-CoV-2 is indicative of its expanded host range, suggesting that many animal species could potentially play a role as reservoir or intermediate hosts for SARS-CoV-2 adaption to human populations.

3.3.1. Putative reservoir in bats and pangolins. Phylogenetic analysis showed that SARS-CoV-2 clustered (~88% identity) with two bat-derived SARS-CoV-2-like CoVs, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, Eastern China (107). Bat-CoV RaTG13 detected in *R. sinicus* sampled in Yunnan Province currently represents the closest CoV to SARS-CoV-2 on the whole-genome level (108). In comparing genes individually, although RaTG13 is the closest relative of SARS-CoV-2, with an overall genome sequence identity of 96.2%, the RBD is highly divergent between the two viruses. The main differences are three short insertions in the N terminus of the RBD, with changes in four out of five key amino acid residues (108).

Virome investigations in pangolins have also identified this animal as a potential intermediate host of SARS-CoV-2. Pangolins were trafficked illegally in a wide range of geographical areas from Africa to Southeast Asia and served as a food source, with their scales being used in traditional medicine. Guangxi and Guangzhou customs officers obtained tissue samples from pangolins during anti-smuggling operations, and the sequencing results identified two sublineages related to SARS-CoV-2, with a sequence similarity ranging from 85.5% to 92.4% (109). Compared to RaTG13, SARS-CoV-2-related CoV from Guangdong pangolins (*Manis javanica*) was identified to have the highest sequence similarity to SARS-CoV-2 in RBD with 97.4% of amino acid similarity. Moreover, five key amino acid residues involved in the interaction with human ACE2 were completely consistent between pangolin-CoV and SARS-CoV-2, suggesting the pangolin as a possible reservoir or intermediate host of SARS-CoV-2, requiring further in-depth studies.

Recent data support the circulation of several SARS-CoV-2-like CoVs in bats and pangolins in mainland Southeast Asia (51, 110–112). The isolate RacCS203 was identified from *Rhinolophus acuminatus* in Thailand and is the closest discovered CoV to RmYN02 (93.7%) (113). Also, antibodies neutralizing SARS-CoV-2 were recovered from the same *Rhinolophus* bat colony and in a pangolin at a wildlife checkpoint. RpYN06 is another SARS-CoV-2-related CoV isolated in Yunnan Province from *Rhinolophus pusillus* (110). This CoV shares 96.10% and 94.48% sequence identity with RaTG13 and SARS-CoV-2 at the whole-genome level and 97.19% and 97.18% with SARS-CoV-2 and RmYN02 at the *ORF1ab* gene. RpYN06 exhibited 76.33% nucleotide identity to the SARS-CoV-2 *S* gene and was identified to be the closest isolate to SARS-CoV-2 at the *RdRp* (98.36%) and *ORF7a* (96.72%) genes. Another SARS-CoV-2-like virus (PrC31), isolated from *Rhinolophus blythi* in Yunnan Province, was found to contain the closest *ORF8* (98.1%) and *ORF1a* (96.6%) genes to SARS-CoV-2 but shares the most common ancestor with SARS-CoV (111). A

recent investigation (112) also identified numerous alpha- and beta-CoVs circulating in various *Rhinolophus* species in Northern Laos. Among these viruses, three sarbecoviruses, named BANAL-52, BANAL-53, and BANAL-236, were found to harbor RBDs that differed from those of SARS-CoV-2 by only one or two residues and bonded efficiently to hACE2. However, none of these SARS-CoV-2-related CoVs contain the furin cleavage site observed in SARS-CoV-2, and the nucleotide identity between these CoVs and SARS-CoV-2 did not exceed 76.33% at the *S* gene.

3.3.2. Other animal species susceptible to SARS-CoV-2 infection. Because the question of the original host or animal reservoir of SARS-CoV-2 is unresolved, identifying other animals that are highly susceptible to this virus infection is an important task for pandemic control and prevention. Given their close interaction with humans, domestic animals and pets frequently transmit or receive pathogens to and from humans. Cats and dogs were among the first companion animals affected by SARS-CoV-2 in Asia, Europe, and America (114). Big cats in zoos, including cougars, leopards, lions, and tigers, have been confirmed positive for SARS-CoV-2 infection (115). Aside from domestic cats, which were found to be clearly susceptible to the SARS-CoV-2 infection, other cat species were not found to exhibit strong viral replication (114).

Based on natural and experimental infection, mustelids represent one of the animal species that is highly susceptible to SARS-CoV-2 infection. Minks remain the only animal group to experience large-scale outbreaks in Europe, Canada, and the United States, as well as a spillback of mink-SARS-CoV-2 variants to humans (114, 116, 117). Initial outbreaks in the Netherlands and Denmark from April to November 2020 showed a systemic infection of SARS-CoV-2, severe respiratory distress due to lung failure, and increased mortality in mink populations. The genomic analysis of the *S* gene of mink SARS-CoV-2 isolates depicted various substitutions in the S protein. Different investigations found several new SARS-CoV-2 variants in minks with mutations that could reduce the antibody neutralization effect (Y453F) and circulating simultaneously in humans and farmed minks (−ORF8+N501T) (118, 119). This suggests a selection pressure during viral adaptation to the mink population, and these animals are becoming a constant source of new SARS-CoV-2 variants, with a potential to spill back to humans. The finding that deer mice and bushy-tailed woodrats support efficient SARS-CoV-2 replication compared to previous human CoVs suggests the need for enhanced screening in wildlife populations.

4. CROSS-SPECIES TRANSMISSION OF EMERGING CORONAVIRUSES

Index cases of human CoV outbreaks from SARS to COVID-19 were suspected to have direct or indirect contact history with a wild (civet, pangolin) or domesticated (dromedary camel) animal. This suggests a zoonotic source of these human CoVs, and the question of whether they jumped directly from their original hosts to humans or via intermediate animals remains unsolved.

Host switching is not always successful and requires several variables. A viral pathogen's ability to cross the species barrier is determined by the nature and intensity of the exposure between the reservoir host and recipient host, the viral determinants to transmission, and virus–host interaction, as well as other additional factors that affect susceptibility to infection (120).

4.1. Major Determinants of Tissue and Host Tropism for Emerging Human-Infecting Coronavirus

From the virus side, structural proteins are essential components for infection and represent the major determinants of host and tissue tropism. The S protein is the CoV component that binds host cell receptors. SARS-CoV, HCoV-NL63, and SARS-CoV-2 use ACE2 for cell entry, and MERS-CoV uses DPP4 as the receptor for host cell infection (10). Although other cellular proteins, such as C-type lectin CD209L and DC-SIGN, can play a secondary role in

SARS-CoV/SARS-CoV-2 virion attachment, ACE2 remains the key functional receptor for the entry of these viruses into the host cell.

Interaction between the RBD and host cell receptors impacts the virus tropism and provides clues on which animals may be susceptible to each CoV infection. Therefore, animal orthologs of ACE2 and DDP4 are also key components that may explain their permissiveness to CoV infection. *ACE2/DDP4* genes seem to be conserved among mammals, suggesting a possible susceptibility of various animal species to SARS-CoV/SARS-CoV-2 and MERS-CoV infection (62).

In addition to type 2 alveolar cells, ACE2 is expressed in several tissues in humans, including the testis, heart, intestines, pancreas, kidney, and corneal epithelial cells. Spatial and cellular localization found a low expression of DDP4 in the nasal cavity with increased expression in mononuclear leukocytes and serous cells of submucosal glands, in type I and II alveolar cells in alveolar macrophages, and in the vascular endothelia and pleural mesothelia (121).

From NHPs to small mammals, several domestic and wild animals are susceptible to the CoVs that commonly infect humans. Though SARS-CoV, MERS-CoV, and SARS-CoV-2-related CoVs were identified mainly in bats, their S proteins were found to be highly divergent from those of human CoVs. Inversely, the molecular basis of bat susceptibility to human CoVs is not well-addressed. In long-term infection with MERS-CoV in vitro, bat cells repeatedly selected viral variants that contained mutations in the viral ORF5 (122). Using virus–host receptor binding and infection assay, a study involving 46 ACE2 orthologs from diverse bat species identified that many species may not be potential hosts for SARS-CoV and SARS-CoV-2 (123). To evaluate critical residues in bat ACE2 that affect viral entry, the authors found a range of phenotypes for efficient RBD binding and viral entry. Interestingly, the SARS-CoV-2 RBD could bind to bat ACE2 receptors from *Rhinolophus macrotis* with a lower affinity compared to hACE2 (124). The residue Y41H found in numerous bat species attenuated the binding capacity of bat ACE2.

Nonetheless, several small laboratory mammals seem to be naturally resistant to human CoV infection, suggesting a weak binding affinity between their orthologs (ACE2/DDP4) and the RBD of these viruses. Attempts to understand mouse susceptibility to CoV infection found that three substitutions (D30N, Y83F, and K353H) differ between mouse/rat ortholog of ACE2 and hACE2 (125, 126). High expressions of ACE2 in rodents were found in the ileum, supporting the poor ability of common laboratory mice to display productive infection (127). Similar observations found rhesus macaques, but not hamsters, ferrets, or mice, to be susceptible to MERS-CoV infection, and the modeling of the binding energy between MERS-CoV RBD and DDP4 of human (susceptible) and hamster (resistant) showed that five amino acid residues were involved in the RBD–DDP4 interaction (57).

Beyond bats, the S protein of two pangolin CoVs, GX/P2V/2017 and GD/1/2019, was found to efficiently bind hACE2 with a broader host range compared to SARS-CoV-2 (13). Although pangolin CoVs possess the same molecular binding modes as SARS-CoV-2, the Q498H substitution expanded the binding affinity of these viruses to mouse, rat, and European hedgehog homologs. Similar studies for MERS supported Camelidae (dromedary and Bactrian camels) as potential intermediate hosts (128). The expression of DPP4 from various livestock species on BHK cells suggested a broad range of livestock animal groups as potential amplifying hosts of MERS-CoV.

4.2. Interspecies Transmission of Emerging Human-Infecting Coronaviruses: Implication of Bats and Other Animal Species

Bats are the second most diverse animal group and play an important role in ecosystem balance. They are also widely expected to be the evolutionary origin of many emerging viruses, for example, lyssaviruses, filoviruses, henipaviruses, and CoVs. The emergence of the three highly pathogenic human-infecting beta-CoVs and other CoVs that have caused important animal losses, including

swine acute diarrhea syndrome CoV, and associated with the identification of diverse bat-CoVs closely related to these human CoVs, underpinned bats as the primary source of the progenitors of emerging CoVs (129).

The largest diversity of CoVs has been isolated from bats. Alpha-CoVs and beta-CoVs in particular have been detected in mammals. A survey of CoV diversity in approximately 20,000 animals (12,333 bats, 3,387 rodents, and 3,470 NHPs) in Latin America, Africa, and Asia found host ecology to be the primary driving factor of bat-CoVs (130). The study also showed a strong correlation between viral richness and regions with the highest bat-CoV diversity and some particular associations between viral subclades and bat families, suggesting a nonrandom distribution of bat CoVs, influenced mostly by the complexity of bat biogeography. The results further supported that the proportion of host-switching events was much higher in Africa and Asia, pointing out a higher risk of spillover events in these regions where bat diversity is important. In the twenty-first century, Asia remains the continent where the majority of novel CoVs posing a threat to both human and animal health have emerged, and the conditions of their emergence have not been explored fully. Using a Bayesian statistical framework and a large sequence data set from bat CoVs, Latinne et al. (131) evaluated the macroevolution, cross-species transmission, and dispersal of bat-CoVs in China. More frequent host switching between more distantly related host taxa was noted in alpha- compared to beta-CoVs, and interfamily and intergenus hopping were found to be more common in Rhinolophidae and the genus *Rhinolophus*. The families Rhinolophidae and Hipposideridae showed a similar pattern in terms of beta-CoV diversity, with frequent interfamily host switching being more common between these two bat families. The authors further found Southwestern and Southern China to be the two hot spots of CoV diversity. The high biodiversity in these regions associated with several bat species implies that along with host taxa, geographic regions have consequently contributed to CoV diversification, and the results also suggested *Rhinolophus* spp. as a key target for future longitudinal surveillance programs, but also the genera *Hipposideros* and *Aselliscus*, due to their potential capacity to host several beta-CoVs.

Many other factors implicate bats as the main reservoir hosts of CoVs, including their large population size and species diversity, their broad geographical distribution and ability for long-distance migration, their habit of gathering in large groups with some aggressive species, and their high sensitivity to nutritional and environmental stresses. Several intracellular mechanisms can either enhance or restrict cell infection by viruses. Human infection by beta-CoVs is associated with increased proinflammatory cytokines. Excessive inflammatory response provokes tissue damage and complicates disease outcome (20). However, bats have a special set of antiviral immune responses that can control inflammatory immune responses while stably maintaining the growth of different viruses. The dampening activation of the NLRP3 inflammasome and robust expression of IFN-α, IFN-β, and other IFNs were identified to allow bat cell lines persistently infected with viruses (122, 132, 133). However, this resistance to highly pathogenic viruses seems to be a species-specific rather than a general feature of bats (134).

Recognizing different temporal and spatial factors that directly or indirectly diminish bat immune response is important to predict episodic virus shedding from persistently infected individuals and consequently to adapt prevention measures to reduce the risk of potential virus spillover to humans or livestock. Nutritional stress, habitat loss or disruption, and reproductive strategies including mating and pregnancy all coincide with an increased transmission efficiency of bat-borne viruses within the bat population and are associated with spillover events (135). Immunosuppression during pregnancy is common in mammals. Pregnancy correlates with high seroprevalence and seasonal spillover of Hendra, Nipah, and filoviruses (136–138). Seasonal fluctuations and natural disasters (i.e., cyclones) are other factors identified to compromise bat nutritional state and were correlated to interspecies transmission and increased antibody

prevalence of Hendra virus (135). Moreover, access to food represents a key factor triggering bat population migration, particularly in tropical regions. In these hot and humid environments, bats are also stressed during wet seasons, and many species are known to give birth just after these seasons. This time interval during and a few months after rainy seasons may represent a crucial period of potential CoV switching between closely and more distantly related bat species. Given the rapid growth of the resident human population and the increasing need for new lands, bat habitat loss or disruption is another factor contributing to the displacement of bat populations.

In addition to that of bats, understanding the potential role of other animal species in CoV disease transmission is valuable. However, due to its high transmission efficiency and better adaptation to humans, SARS-CoV-2 has affected more diverse animal species close to humans, including pets, farm animals (cattle, mustelids), and wild animals in zoos or in captivity (81). The fact that most of the caretakers of these animals have been reported positive for SARS-CoV-2 infection confirms potential human-to-animal transmission.

Natural transmission of MERS-CoV from animal (dromedary camel) to human confirmed camels as a key intermediate host. Other camelids, such as llamas and alpacas, have demonstrated increased susceptibility to MERS-CoV infection and exhibited moderate disease symptoms. Companion animals, such as donkeys and horses, and various livestock species, including cattle, goat, pig, and sheep, were all identified to harbor either MERS-CoV-specific antibodies or RNA, suggesting their susceptibility to MERS-CoV infection (103). In the Middle East and Africa, traditional breeding of livestock, pets, and other domesticated wild animals is common, and their close contact with camels contributed to MERS-CoV jumping to some of these animals, supporting potential MERS-CoV spillover events between distantly related mammalian taxonomic groups. Based on this evidence, MERS-CoV or a closely related CoV is likely to appear in the future, and precautions should be taken.

During the first months of the COVID-19 outbreak, scientists put forward several theories regarding cross-species transmission. Computational reports have tentatively suspected snakes and turtles in the SARS-CoV-2 evolutionary pathway (139). However, from a biological perspective, that was shown to be highly unlikely and speculative. Although snakes are a potential predator for bats, and both rodents and other wild animals share caves, recent data on their ortholog ACE2 showed that nearly half of the key residues involved in binding to SARS-CoV-2 RBD were different from those on hACE2 (140). Based on genomic CpG ratios and high zinc finger antiviral protein (ZAP) expression, stray dogs were also a suggested source of SARS-CoV-2. In association with other proteins, ZAP is known to exhibit tissue-specific expression and degrade various viral RNA genomes, especially those with an increase in CpG dinucleotides. The canine CoV was reported to have the lowest CpG (38.17%). SARS-CoV-2 was shown to have with RaTG13 the most extreme CpG deficiency in the genus beta-CoV. Xia (141) therefore proposed a possible canine origin of the ancestor of SARS-CoV-2 and focused on the interaction between host defense and the virus genome and the selective pressure host tissue exerted on viral genome composition (141). However, similar CpG ratios for many other viruses, including CoV, and the complexity of the evolutionary routes between SARS-CoV-2 and canine CoV suggest that the involvement of stray dogs as a possible intermediate host is also speculative. Therefore, more studies in bats, pangolins, and other susceptible animals are necessary to understand the transmission pathway responsible for COVID-19.

4.3. Recombination: The Main Evolutionary Force Driving CoV Spillover Threats

Genetic recombination is a fundamental driving force in evolution. It increases RNA virus variability, enhances viral fitness, and accelerates viral adaptation to new hosts or alters the tissue

tropism following drastic changes in viral phenotype. Compared to other single-stranded RNA viruses, CoVs have a marked ability of homologous RNA recombination during virus replication, a process by which different strains or closely related CoVs exchange genetic material in the context of coinfection. These small genomic subregions have independent origins and can be detected by sampling sufficient suspected animal reservoirs. For example, during the SARS epidemic, most human SARS-CoV strains harbored a signature 29-nucleotide deletion in *ORF8*, in contrast to civet SARS-CoV (142). Two SARS-related (SARSr)-CoVs, SARSr-Rf-Bat-CoV YNLF_31C and YNLF_34C, from greater horseshoe bats (*Rhinolophus ferrumequinum*), which share 93% nucleotide identities with human/civet SARSr-CoV genomes, possessed high amino acid identities (80.4–81.3%) in their *ORF8* (143, 144). Between SARSr-Rf-Bat-CoVs and SARSr-Rs-Bat-CoVs (a bat-CoV detected in Chinese horseshoe bats), potential recombination events were identified around *ORF8*, suggesting civet SARS-CoV *ORF8* may have originated from greater horseshoe bat SARSr-CoV through recombination. Furthermore, although bat SARS-like CoVs SHC014 and Rs3367 have a high amino acid sequence identity to SARS-CoV RBD, several recombination breakpoints were identified, including the *ORF1b* (20,827 nucleotides), *M* (26,553 nucleotides), and *N* (28,685 nucleotides) genes.

MERS-CoV also infects various animal species, and several distinct lineages have been detected in the same or different hosts. Phylogenetic and bioinformatics analyses found 28 potential recombinant sequences classified into 7 recombinant types, and similar to that of SARS-CoV, MERS-CoV S protein was under strong positive selection during host switching (145). Six of nine positive selection sites were detected in the RBD, supporting an adaptive evolutionary pressure altering host tropism. Interestingly, in one MERS-CoV clade, five possible human-to-camel transmission events and one camel-to-human transmission event were found, supporting an interface of frequent back-and-forth transmission events between humans and dromedary camels.

Though camels are considered the main reservoir of MERS-CoV, strong evidence supports bats as the evolutionary source of MERS-CoV ancestry. MERS-CoV and many other bat-CoVs in group C beta-CoVs are identified in various bat species in the Vespertilionidae family. The S proteins of MERS-CoV and HKU4 are extremely similar, and both can use hDDP4 for virus entry (146). The remarkable similarity between bat-CoV PML/2011 and MERS-CoV in the *RdRp* region suggests a possible transmission of MERS-CoV ancestry from the Vespertilionidae family either to dromedary camels or directly to humans via a spillover model, although a circulation model cannot be excluded (120, 147). According to the circulation model, and as supported by serological evidence, MERS-CoV or closely related viruses were circulating in dromedary camels and probably in people in close contact with these animals for more than 20 years before the first recorded MERS-CoV outbreak in the Middle East. Camels' capacity to be infected by distinct MERS-like CoVs without any apparent clinical symptoms supports this idea. This is similar to several other CoVs known to undergo quasispecies evolutionary processes until the virus evades the immune system and establishes productive infection under strong positive selection. Also, the adaptation of CoVs to their animal hosts should not exclude the risk of possible emergence of new lineages; the ongoing emergence of SARS-CoV-2 VOCs is a perfect example of this.

Early data showed that RaTG13 is the CoV most closely related to SARS-CoV-2, whereas the pangolin-CoV receptor-binding motif is the closest to that of SARS-CoV-2. Many authors thus suggested that SARS-CoV-2 may have originated through recombination from bats in the genus *Rhinolophus* and Malayan pangolin. The analysis of recombination events in the history of SARS-CoV-2 found the variable loop region of the S protein to be the closest to the one of pangolin-CoV named Pangolin Guangdong 2019 (148). Based on the variable region of the S protein, two scenarios were proposed for RBD acquisition. The first scenario suggested an initial lineage split leading to SARS-CoV-2 and RaTG13, followed by a recombination event

between the SARS-CoV-2 lineage and Pangolin Guangdong 2019 that resulted in the acquisition of a new RBD. The second scenario supported the acquisition of a new RBD from recombination between ancestral lineages of SARS-CoV-2 and RaTG13. However, compared to that for palm civets during the SARS-CoV outbreak, extensive data highlighting the pangolin's suspected role as a potential source of SARS-CoV-2 failed to detect any CoV (149). These results suggest that the pangolin-CoV closely related to SARS-CoV-2 could reflect exposure to infected humans or other animals in the wildlife trade network.

Within the subgenus *Sarbecovirus*, only SARS-CoV-2 features the insertion of polybasic sites (PRRAR) in junction S1/S2 of the S protein. Attempts to trace the origin of the furin cleavage site were based mainly on the closest relatives to SARS-CoV-2, including RaTG13 and pangolin-CoV (150). In addition, the newly isolated bat-CoV RmYNO2 with a natural insertion (PAA) has improved insights on how beta-CoVs can recombine in nature (12). The *S* gene is one of the most divergent regions in the CoV genome, and recombination between different lineages is common (150). Highlighting evolutionary forces that have resulted in furin cleavage acquisition can help to elucidate the SARS-CoV-2 evolutionary route and facilitate the tracing of its reservoir and intermediate host.

The co-circulation of numerous SARS-CoV-2 variants is likely to increase the occurrence of coinfections and recombinations, with a high risk of the emergence of more complex lineages. Several SARS-CoV-2 recombinant lineages with the potential to alter viral properties or impact transmissibility and the vaccine landscape have been identified recently (151, 152). The emergence of Omicron VOCs remains an enigma despite some investigations implicating a possible mouse origin (33). Of note, although the Delta variant was the dominant lineage circulating before the official announcement of Omicron variants, other variants of interest or under monitoring were also circulating at lower levels. Our phylogeographic analysis from old and new SARS-CoV-2 variants based on GISAID data showed distinct and more complex evolutionary roots for Omicron and the new recombinant lineages under investigation (XD, XE, XF, and unassigned lineages) (**Figure 1**; **Supplemental Tables 1 and 2**). Omicron variants formed a clade distinct from that of the previous SARS-CoV-2 variants. When comparing the sample collection dates, Omicron variants likely have been circulating in humans and/or its potential animal source since mid-2021 (Congo_August_2021). Our analysis also showed that these new SARS-CoV-2 recombinants evolved differently from previous SARS-CoV-2 variants and might not have shared the same host of origin. This suggests that the animal origin for Omicron variants and similarly for SARS-CoV-2 recombinants requires further investigation. These data undermine recombination as a key process of CoV evolution, and understanding key underlying factors is important for current pandemic management and prevention.

Supplemental Material >

5. DISCUSSION

Elucidating the reservoir and intermediate host species for emerging human-infecting CoVs is crucial to be aware of and to predict subsequent outbreaks. The identification of civets, raccoon dogs, and especially dromedary camels as intermediate/amplifying or reservoir hosts of SARS-CoV and MERS-CoV was followed by consequent control measures that have significantly impacted public health policy. The direct implication of camels and minks in beta-CoV transmission to humans constitutes a clear picture of beta-CoV transmission from animal reservoir/intermediate hosts to humans. MERS-CoV was well-adapted to camels, and humans were likely a dead-end host (**Figure 2**). Thus, rigorous application of World Organisation for Animal Health measures, such as MERS-CoV surveillance in dromedary camels via PCR and/or isolation of the virus, resulted in disease containment and prevented virus spread from these dromedary camels.

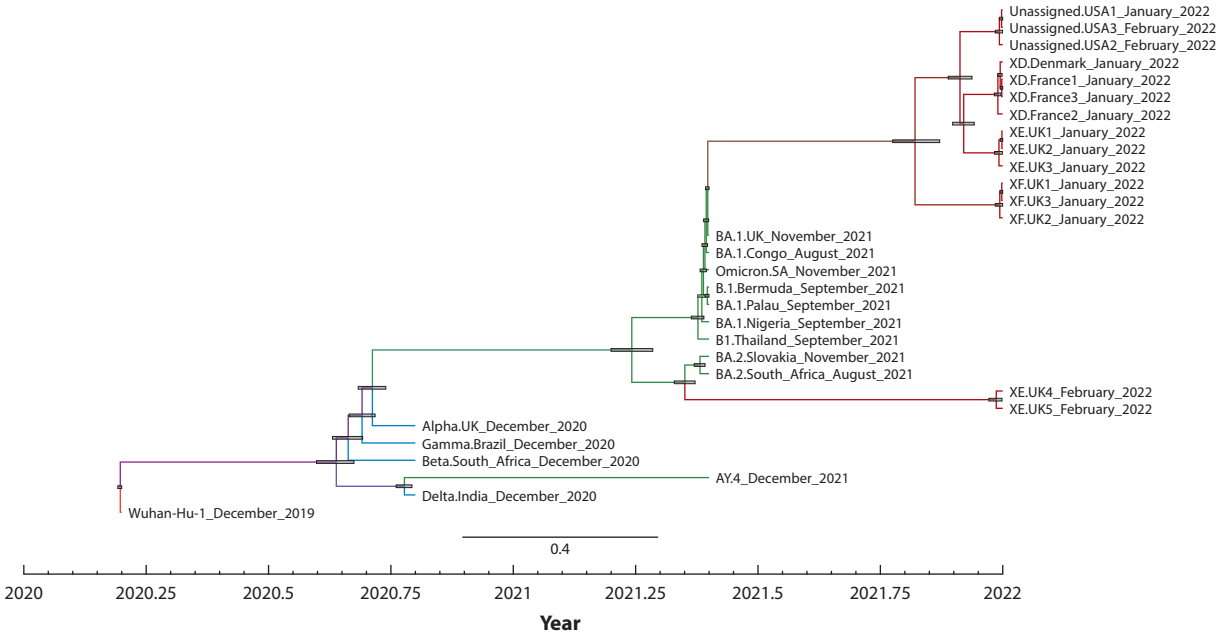

Figure 1

Maximum clade credibility phylogenies of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) variants. Node bars represent the most probable isolation dates of emergence of SARS-CoV-2 variants from ancestor lineages. AY.4 is a SARS-CoV-2 Delta variant isolated in Bulgaria. Details regarding SARS-CoV-2 sequences used for this phylogenetic analysis can be found in **Supplemental Table 1** and the program parameters used to generate the phylogenetic tree in **Supplemental Table 2** (153).

CoV adaptation to a new host often requires at minimum mutations at key residues in the RBD. Compared to previous SARS-CoV-2 VOCs, the genome of Omicron VOCs appeared to evolve more rapidly as a result of the ongoing emergence of new mutated or recombinant variants. The real mechanisms explaining the emergence of these SARS-CoV-2 lineages require more investigation (154). Moreover, the spread to humans of mink-adapted SARS-CoV-2 presents an alarming concern. With a wide range of susceptible animal hosts, the possibility that newly adapted SARS-CoV-2 variants from an animal rather than mink can spill back to humans cannot be rejected. Although appropriate host culling and safe carcass deposit can be applied to farm and domestic animals, wildlife culling has been associated mainly with a huge impact on ecosystems, with limited success in terms of animal disease control (155, 156). Also, compared to several other highly pathogenic viruses, such as Hendra, Nipah, Ebola, and Marburg virus, knowledge is lacking regarding human beta-CoV spillover events in terms of time frame and location, as well as factors enhancing such phenomena in nature. Thus, animal surveillance for highly impactful viruses, including CoVs, represents the best option to predict and/or prevent future large-scale outbreaks. Additionally, human interventions should occur on an ecological basis by encouraging the protection of wildlife in their ecological niches. Additionally, predictive modeling of the RBD-receptor interactions is highly encouraged to identify animal species at high risk of infection.

Supplemental Material >

Following the emergence of any viral disease with a high public health concern, finding a nearly perfect animal model is crucial to understand disease pathogenesis and evaluate potential countermeasures. Identifying a good CoV animal model was a challenge during the SARS and MERS outbreaks but seems to have gone better for COVID-19, owing to enormous efforts to screen more diverse animal species and generate new transgenic mice and mouse-adapted

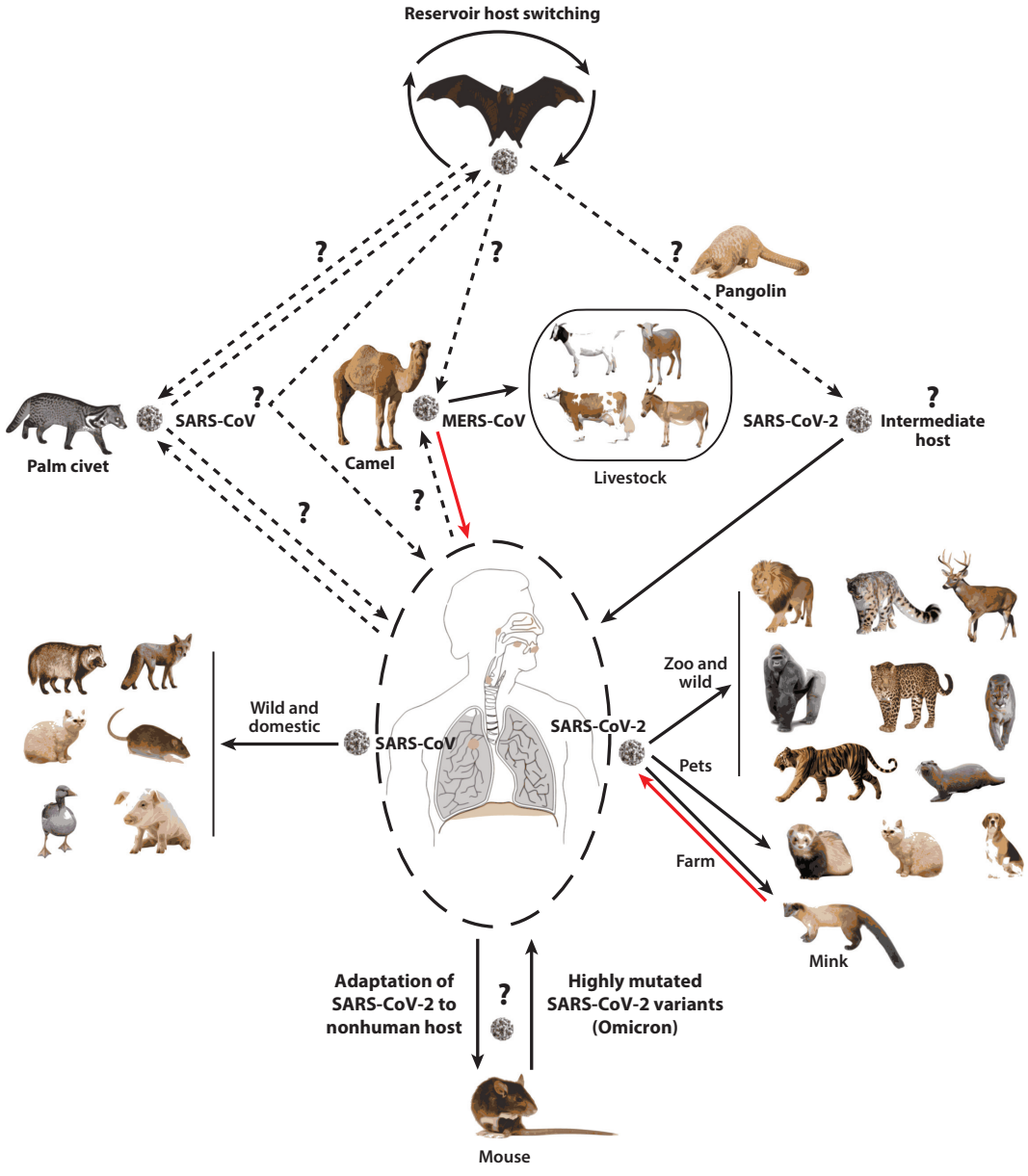

Figure 2

Zoonotic circulation of severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV-2 and the emergence of Omicron variants of concern. Arrows with solid lines indicate evidence of cross-species transmission at the human–animal interface, and red arrows highlight major reservoir (camel) or efficient amplifying host (mink).

SARS-CoV-2. However, given the cost of transgenic mice and the high-safety containment facilities required for NHP use in vivo, new approaches or animal species highly susceptible to SARS-CoV-2 infection are still needed. Although CRISPR genome-editing technology has been used to develop a new animal model for SARS-CoV-2, it remains underused and can be explored

further to increase the susceptibility of small laboratory mammals to SARS-CoV-2 infection. This technique may be more accurate by inducing stable mutations in the host genome compared to inappropriate expression of hACE2 in mice, often associated with neurological disease.

Moreover, the adaptation of a virus to a new recipient is known to generate at least one adaptive mutation that drastically changes virus behavior compared to the wild-type strain. This has direct implications for disease modeling as well as drug and vaccine evaluation. Also, 3D visualization of SARS-CoV-2 was used recently in the ferret model, facilitating the description of SARS-CoV-2 infection and suggesting some proximity of infection foci (52). This technique can be explored further to better decipher SARS-CoV-2 pathogenesis. Additionally, evaluating the susceptibility of a broad range of terrestrial and marine animals to SARS-CoV-2 infection is valuable. For example, baboons should be investigated more, as primary data show promising results for modeling SARS-CoV-2 pathogenesis. For other human-infecting CoVs not yet known to cause severe widespread outbreaks (HCoV-229E, HCoV-OC43, HCoV-NL63, and HKU1), investigations were based mainly in vitro and were not taken into account in this review.

The previous emergences of SARS-CoV and MERS-CoV, associated with the ongoing COVID-19 pandemic caused by SARS-CoV-2, have shown beta-CoVs to be a potential risk group for emergence and represent a global threat to the human population. None of the vaccines developed for these viruses, whether approved or not, can prevent infection, and the generation of new human and animal SARS-CoV-2 variants complicates efforts to develop new countermeasures. Therefore, identifying these virus reservoirs and other susceptible animals, and understanding factors driving cross-species transmission, can allow public health officials to better appreciate their emergence and help to predict and/or prevent future potential outbreaks. We have presented our knowledge on virulent human-infecting CoVs of zoonotic origin and their interspecies transmission. We note that the complexity of the evolutionary root of these CoVs in wildlife before jumping to humans is a major enigma and that humans are the main driver of CoV epidemics/pandemics.

The consensus that bats represent the principal reservoir hosts for emerging human and animal CoVs means that further investigations into these animal populations are well warranted. Compared to those of filoviruses and henipaviruses, CoV reservoir host population dynamics and ecology require in-depth investigation. Reservoir host ecology and ecophysiology have direct implications for their temporal and spatial dynamics and can elucidate how to safely interact with these animals to reduce the risk of viruses spilling over to humans and livestock. The SARS-CoV-2 mink outbreaks and the recent emergence of Omicron variants, and the difficulty of identifying its potential animal source, have displayed how challenging it is to control outbreaks of such highly transmissible and pathogenic viruses. Therefore, a proactive virus surveillance program both in wildlife and on farms is necessary to provide knowledge for early warning and to better prevent and efficiently manage future potential CoV outbreaks.

SUMMARY POINTS

1. Coronaviruses (CoVs) can cause large-scale outbreaks or pandemics of severe respiratory illness and lethal gastroenteritis in humans and animals, respectively.
2. The rapid evolution of the SARS-CoV-2 genome and the ongoing emergence of new variants with the potential for immune or neutralizing antibody escape increase the current pandemic threat of these viruses.
3. An ideal or lethal animal model for studying experimental CoV infections is still lacking.

4. The spillback of mink SARS-CoV-2 variants to humans and the suspicion of mouse origin for Omicron variants of concern implicate animals in the evolution and adaption of SARS-CoV-2 to nonhuman hosts.
5. Most of the viruses closely related to emerging human CoVs were isolated from bats.
6. Current efforts to fight CoV diseases are based mainly on the development and manufacturing of vaccines and antiviral drugs.
7. A significant knowledge gap remains regarding the origin of human CoVs.
8. Integrated surveillance of farms and domestic and wild animals is disproportionate and practically nonexistent in many areas with high bat diversity.

FUTURE ISSUES

1. Identify immune correlates for protection to efficiently design new vaccines and antiviral drugs.
2. Understand factors underlying the ambiguous origin of SARS-CoV-2 and the rapid evolution of its genome.
3. Understand host differences in infectivity, immune response, and pathogenesis, especially in bats and other animal species that are known to harbor CoVs.
4. Establish sentinel surveillance systems for CoV screening in the animal trade system, from farms to markets where live animals or their products are sold.
5. Periodically screen wildlife and domestic animals for CoV infection, especially bats, dromedary camels, and animals in close proximity.
6. Communicate efficiently with local populations to increase awareness about the risk of clandestine interactions with wildlife.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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