Zoonotic Coronavirus Literature Review

United States Department of Agriculture

Animal and Plant Health Inspection Service

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Acronym Definitions

3CLpro	3C-like protease
ACE2	angiotensin-converting enzyme 2
ADAR	adenosine deaminases acting on RNAs
ADE	antibody-dependent enhancement
APHIS	Animal and Plant Health Inspection Service
APOBEC	apolipoprotein B mRNA editing enzyme, catalytic polypeptides
ARDS	acute respiratory distress syndrome
ARP	American Rescue Plan Act
ASGR1	asialoglycoprotein receptor 1
AURKB	aurora kinase B
BAL	bronchoalveolar lavage
BLAST	Basic Local Alignment Search Tool
Bov-CoV	bovine coronavirus
BPL	β-propiolactone
BSL-2	biosafety level 2
BSL-3	biosafety level 3
CCL2	chemokine ligand 2
CD147	cluster of differentiation 147
CDC	Centers for Disease Control and Prevention
COBALT	Constraint-based Multiple Alignment Tool
CPE	cytopathic effect
CRISPR	clustered regularly interspaced short palindromic repeats
CT	computed tomography
cVNT	conventional virus neutralization test
CXCL10	C-X-C motif chemokine ligand 10
DC-SIGN	dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DPI	days post infection
DPP4	dipeptidyl peptidase-4
EGCG	epigallocatechin gallate
ELISA	enzyme-linked immunosorbent assays
G-CSF	granulocyte colony stimulating factor
GRAMM-X	Global Range Molecular Matching X
hACE2	human angiotensin I converting enzyme 2
HADDOCK	High Ambiguity Driven protein-protein DOCKing
HIV	human immunodeficiency virus
HPAI	highly pathogenic avian influenza
I-TASSER	Iterative Threading ASSEmbly Refinement
ID	Innovation Diagnostics
IFN	Interferon
IFN-α	interferon alpha

Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
IL28RA/IL10Rβ	Interleukin 28 receptor alpha/beta
IP-10	interferon gamma-induced protein 10
IRF3	interferon regulatory factor 3
ISG	interferon stimulated genes
JAK	janus kinase
LIPS	luciferase immunoprecipitation system
L-SIGN	liver/lymph node-specific intracellular adhesion molecules-3-grabbing non-integrin
mAb	monoclonal antibody
MAFFT	Multiple Alignment using Fast Fourier Transform
MAVS	mitochondrial antiviral signaling
MCP-1	monocyte chemoattractant protein 1
MEGA	Molecular Evolutionary Genetics Analysis
MERS-CoV	Middle East respiratory syndrome coronavirus
MERSr	MERS-related
MCP-1	monocyte chemoattractant protein 1
mg/kg	milligrams per kilogram
MHV	murine hepatitis virus
MIA	microsphere immunoassay
miSARS-CoV	mink-associated coronavirus 2
MLV	murine leukemia virus
MNA	microneutralization assay
MUSCLE	Multiple Sequence Comparison by Log- Expectation
NAHRS	National Animal Health Reporting System
NET	neutrophil extracellular trap
NFκB	nuclear factor kappa-light-chain enhancer
NHP	non-human primate
NK	natural killer
NRP1	neuropilin 1
PISA	Proteins, Interfaces, Structures and Assemblies
PPE	personal protective equipment
ppNT	pseudoparticle neutralization test
PRNT	plaque reduction neutralization test
RBD	receptor binding domain
RdRp	RNA-dependent RNA polymerase
RSCU	relative synonymous codon usage
RT-PCR	reverse transcription-polymerase chain reaction
S	spike protein
SARS-CoV-1	severe acute respiratory syndrome coronavirus 1
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SARSr	SARS-related

SCID	severe combined immunodeficient
sVNT	surrogate virus neutralization test
TGEV	transmissible gastroenteritis virus
TGFβ	transforming growth factor beta
TIM1	T-cell immunoglobulin and mucin domain 1
TLR-7	toll-like receptor-7
TLR-8	toll-like receptor-8
TMPRSS	transmembrane protease, serine
TMPRSS2	transmembrane protease, serine 2
TNF	tumor necrosis factor
TNFα	tumor necrosis factor alpha
TRACE	Tracking Resistance and Coronavirus Evolution
UAE	United Arab Emirates
USDA	United States Department of Agriculture
VSV	vesicular stomatitis virus
WOAH	World Organisation for Animal Health

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Executive Summary

This report presents the results of a comprehensive literature review regarding research and public health progress on emerging coronaviruses—specifically severe acute respiratory syndrome coronavirus 1 and 2 (SARS-CoV-1 and SARS-CoV-2) and Middle East respiratory syndrome coronavirus (MERS-CoV)—in various animal species. The report is intended as a tool to supplement future in-depth gap analyses to identify areas for research funding with maximum potential impact on animal health.

Understanding Emerging Coronaviruses in Animals

Epidemiology

SARS-CoV-1, SARS-CoV-2, and MERS-CoV most commonly spread via airborne droplets, and their airborne travel distance depends heavily on environmental conditions that affect virus stability in droplets (e.g., colder temperatures, high or low relative humidity). Virus stabilizing conditions can therefore partially dictate disease spread or lack thereof at different population densities. While these three coronaviruses can survive on some solid surfaces (i.e., fomites), transmission via fomites is poorly understood. Moreover, little is known about the transmissibility of these emerging coronaviruses in feces (including fecal-contaminated water) and animal products. Although coronaviruses and other RNA viruses have been isolated from these potential sources, their concentrations may be insufficient to infect additional hosts.

Many emerging coronaviruses can be traced to Chiropterans as both origin and primary reservoir species. SARS-related (SARSr), MERS-related (MERSr), and other related coronaviruses have been detected in diverse Chiropteran species across multiple continents. Unique aspects of Chiropteran immunity enable coronavirus coinfections and frequent recombination events that can result in novel viruses with broader species tropisms, ultimately spreading these viruses to additional potential reservoirs living proximally to Chiropteran colonies. Such reservoir species include dromedary camels, palm civets, raccoon dogs, and some mustelid species. Significant proportions of dromedary camel populations have been infected with MERS-CoV in both the Middle East and Africa. Based on surveillance studies of live animal markets, palm civets and raccoon dogs were identified as SARS-CoV-1 reservoirs. Although no definitive reservoir has been identified for SARS-CoV-2, palm civets, racoon dogs, and certain mustelid species, especially American mink, represent potential current and future reservoirs for this virus.

Identification of additional reservoir species and tracing of close interspecies contacts can help inform emerging coronavirus surveillance and disease control to proactively prevent viral spread to additional animal species. Novel approaches using computational methods have rapidly predicted species susceptible to SARS-CoV-2 infection based on models of spike protein-angiotensin converting enzyme (ACE2) protein interactions. However, some of these predicted species cannot be infected with SARS-CoV-2 and sometimes SARS-CoV-1 and MERS-CoV, as evidenced by in vitro and in vivo infection experiments, studies of host receptor expression, and detection of natural infections. Ultimately, even species that can be infected experimentally

need to be exposed to these viruses either by living in close proximity to or preying upon other infected species to naturally contract these infectious diseases. The susceptibility of select species and groups of species detailed in this literature review are briefly summarized below.

Based on computational studies, multiple mustelid species appear moderately to highly susceptible to SARS-CoV-2. These findings have been corroborated with experimental infection and surveillance studies, particularly in ferrets and American mink. Therefore, mustelids preying on Chiropterans or residing near Chiropteran roosts are at high risk for contracting SARS-CoV-2. Farmed mustelids and those traded at live animal markets are also at particularly high risk for SARS-CoV-2 infection from humans as well as other infected species housed in close proximity.

Computational modeling predicts moderate to high susceptibility to SARS-CoV-2 for a diverse set of non-human primates (NHPs). New World monkeys and great apes appear to have similar susceptibility as humans to SARS-CoV-2, while Old World monkeys, lemurs, and lorises are moderately susceptible, as evidenced by a combination of computational modeling, in vitro and in vivo experimental infections, and detection of natural infections. Some NHP models have also been successfully infected with SARS-CoV-1 in laboratory settings. NHPs living near or opportunistically preying upon Chiropterans (e.g., New World Cebidae monkeys and Old World *Cercopithecus* monkeys) may be especially at risk for coronavirus spillover events.

Various computational studies have predicted that domesticated dogs and cats have variable susceptibility to SARS-CoV-2, and in vitro studies indicate that dogs may be susceptible to SARS-CoV-1. Natural infections of SARS-CoV-1 have not been detected in dogs and cats, but numerous surveillance studies across the globe have detected active infection or antibodies to SARS-CoV-2 in both animals. Some of these cases were traced to infected owners, and other cases identified likely interspecies transmission events from American mink farms. The collective data suggest that dogs and cats are generally susceptible to SARS-CoV-2 infection.

Computational studies have also identified variable SARS-CoV-2 susceptibilities in farmed ungulates. Different SARS-CoV-2 in vitro infection confirmatory studies were also inconsistent with one another, except for white-tailed deer, which were susceptible to in vitro infection in multiple experimental contexts. Due to the animals' overall size, few in vivo experimental infection studies have been performed. Natural SARS-CoV-2 infections have been detected in white-tailed deer across the United States, and individual studies have reported very low rates of SARS-CoV-2 in goats, pigs, and sheep. MERS-CoV infections present an overall larger threat than SARS-CoV infections in farmed ungulates, with numerous reports of infections in dromedary and Bactrian camels, cattle, goats, sheep, and alpacas, as well as a small number of infections in donkeys and horses. Overall, multiple farmed ungulate species are at risk for spillover infections with emerging coronaviruses, especially those with regular contact with humans, Chiropterans, and other susceptible farmed ungulate species.

Molecular Biology and Virology

Identifying molecular similarities and differences observed between SARS-CoV-1, SARS-CoV-2, and MERS-CoV can help improve overall understanding of the factors that can enhance or

depress infectivity and pathogenesis in animals. SARS-CoV-1, SARS-CoV-2, and MERS-CoV have similar host cell entry mechanisms based on their spike proteins. These spike (S) proteins engage with host cell surface receptors, and with the assistance of cell surface proteases, promote fusion at the plasma membrane for cellular entry. SARS-CoVs engage with different host cell receptors and proteases than MERS-CoV, and host receptor binding domains differ across all three coronaviruses. Although SARS-CoV-1 and SARS-CoV-2 interact with the same host receptor, their S protein amino acid sequences are distinct. These variations in S protein sequence affect overall viral transmission and host range.

The host ACE2 receptor, a key component for SARS-CoV-1 and SARS-CoV-2 entry, is conserved across a variety of animal species. Host susceptibility is impacted by the presence or absence of amino acids that are key for binding to the ACE2-receptor binding domain (RBD). Researchers have used these key ACE2 residues to identify species susceptible to SARS-COV-2. Animal susceptibility to SARS-CoV-2 is also impacted by the expression of different ACE2 isoforms, some of which do not support SARS-CoV-2 binding.

SARS-CoV-1, SARS-CoV-2, and MERS-CoV likely originated from recombination events in Chiropterans. With the global spread of SARS-CoV-2, recombination events with MERS-CoV may occur due to (1) co-circulation of viruses in the same regions and species, (2) co-infection of type II alveolar cells, (3) high overall recombination rates, and (4) high sequence homology.

Immunology

Immunity to MERS-CoV-1, SARS-CoV-1, and SARS-CoV-2 induced after viral infection involves both the innate and adaptive immune systems. Protective immunity (i.e., immunological memory) against subsequent reinfections involves the production of neutralizing antibodies in addition to the generation of antigen specific $CD8^{+}$T cells. Many detailed studies have characterized the immune response to these coronaviruses in both humans and in animal species such as ferrets, hamsters, marmosets and other NHPs that are susceptible to infection. The robustness of these immune responses varies based on viral strain and host species, but much has been learned through cross-species comparisons about the role of different immunological factors.

Studies of SARS-CoV-1, MERS-CoV-1, and SARS-CoV-2 have demonstrated that these betacoronaviruses can evade the innate immune system by inhibiting interferon (IFN) responses. This dysregulation of IFN responses can result in more severe disease. In addition, inflammatory cell infiltration by macrophages, neutrophils, and activated T cells, as well as enhanced release of cytokines (i.e., cytokine storm), often result in acute respiratory distress syndrome (ARDS) which is observed in humans and some animal species to a lesser degree. Studies in animal models are needed to better understand the roles of the IFN and innate immune responses to SARS-CoV-2, and the various proteins used by coronaviruses to evade this response, in order to develop therapeutics for treatment of disease.

Pathogenesis

Pathogenesis data on coronaviruses primarily comes from species used for research on mechanisms and treatment of SARS-CoV-2 infection, such as laboratory hamsters, mice, and NHP. Findings from experimental species provide more detail about clinical symptoms, viral loads, histopathology, and genetic and epigenetic factors, compared to data from other species for which data come primarily from population surveillance. Overall, hamsters, NHPs, and ferrets are readily infected by SARS-CoV-2 and SARS-CoV-1, while companion animals and mice show comparatively less susceptibility to infection. In addition, these species show varying degrees of susceptibility to MERS-CoV infection, with dromedary camels being the primary reservoir for the virus.

Pathogenesis data on coronaviruses in hamsters are primarily generated in Golden Syrian hamsters used as a model for human disease. Multiple studies suggest that Golden Syrian hamsters' susceptibility to SARS-CoV-2 infection depends on both sex and SARS-CoV-2 variant. For Golden Syrian hamsters inoculated with SARS-CoV-1, high viral titers were detected in the upper and lower respiratory tracts, but hamsters remained asymptomatic. In contrast, Roborovskii hamsters develop severe or fatal disease from SARS-CoV-2 inoculation and are substantially more susceptible to infection than Golden Syrian hamsters. However, MERS-CoV cannot effectively replicate in Golden Syrian hamsters: animals show a lack of clinical symptoms, viral replication, histopathological lesions in the lungs, cytokine upregulation, and seroconversion of antibodies.

Laboratory mice inoculated with SARS-CoV-2 (WA1/2020) do not show significant signs of infection because of insufficient binding affinity between the SARS-CoV-2 S protein and murine ACE2 receptors. However, multiple variants containing an N501Y substitution in the S protein have infectious potential in mice. When inoculated with SARS-CoV-1, BALB/c mice showed age-dependent signs of infection. In addition, older mice showed a higher number of differentially regulated host cellular genes than their younger counterparts.

Multiple studies of rhesus macaques, cynomolgus macaques, and African green monkeys inoculated with SARS-CoV-2 report changes in respiratory pattern, increased body temperature, reduced appetite, hunched posture, pale appearance, and dehydration. Compared to rhesus macaques, African green monkeys and cynomolgus macaques showed more severe pulmonary lesions in lung tissue. In addition, compared to rhesus macaques inoculated with SARS-CoV-1, which showed no clinical symptoms of illness, cynomolgus macaques inoculated with SARS-CoV-1 showed similar symptoms to SARS-CoV-2 infection and more severe histological findings. When inoculated with MERS-CoV, rhesus macaques exhibited transient clinical symptoms; in contrast, marmosets displayed more severe clinical symptoms than rhesus macaques, with increased antigen detection and severe histopathological changes in lung tissues.

Similarly to mice, ferrets have shown age-dependent pathogenic characteristics of SARS-CoV-2 infection, with older ferrets containing higher viral titers from nasal turbinates compared to younger ferrets. In addition, older ferrets had more severe histopathological changes in lung tissue compared to younger ferrets. Interestingly, ferrets rechallenged with SARS-CoV-2

showed more severe clinical symptoms that were not observed during the initial challenge. In contrast, multiple studies of SARS-CoV-2 in mink populations report mixed findings, with some reporting positive cases associated with clinical symptoms and others reporting no viral detection. In addition, reports from mink that died on farms reveal histopathological changes in lung tissues. For sheep and swine farms, no evidence currently indicates susceptibility to SARS-CoV-2 infection.

Companion animals, such as dogs and cats, show varying susceptibility to SARS-CoV-2 infection. Cats inoculated with SARS-CoV-2 are typically asymptomatic, but readily shed virus orally, nasally, and rectally. However, some variants of SARS-CoV-2 have caused symptoms in cats, with more severe histopathological changes in lung tissue. Similarly, cats inoculated with SARS-CoV-1 do not show any clinical symptoms, but only shed virus from the pharynx. Dogs inoculated with or exposed to SARS-CoV-2 have shown clinical presentations ranging from asymptomatic to increases in body temperature, decreases in weight, and respiratory symptoms. However, multiple studies provide evidence that dogs are less susceptible to overall SARS-CoV-2 infection than cats and do not shed any virus.

Dromedary camels are commonly known as a primary reservoir for the MERS-CoV virus. MERS-CoV infection in camelids is characterized by minor clinical symptoms composed of mild to moderate nasal discharge. MERS-CoV is primarily shed in these nasal secretions, but has not been detected in urine, whole blood, or serum. Primary histopathological lesions are limited to the upper respiratory tract and are associated with ciliocytophthoria (i.e., ciliary loss) and depletion of dipeptidyl peptidase 4 (DPP4). Identifying mechanisms in which cilia presence and function are lost may be a key focus for future investigations in upper respiratory infections.

Controlling Emerging Coronaviruses in Animals

Surveillance

Asymptomatic and pre-symptomatic cases of SARS-CoV-1, SARS-CoV-2, and MERS-CoV in animals pose a risk of undetected intraspecies and interspecies infection spread. When not properly controlled, even detected symptomatic infections of wildlife, farmed, captive, and companion animals can spread due to close proximity to other infected animals, which may stem from changes in land use, wildlife trade, climate change, and domestic species introductions (e.g., establishment of farms near wildlife populations). Sufficient infection rates within a species increases the likelihood of spillover events.

Emerging coronavirus infections in animals can be detected through clinical evaluations and monitoring as well as diagnostic tests (e.g., viral nucleic acid- and serology-based assays). Detection of viral nucleic acids provides active infection data, while serology assays provide historic data of past infections. Serology and nucleic acid assays are performed using blood and other biological samples (e.g., oral, nasal, respiratory, anal, and fecal swabs), respectively. To reduce the cost of surveillance, nucleic acid assays can also be performed on pooled biological samples and environmental samples (e.g., air, water, fomite surfaces).

Surveillance strategies should be tailored to available resources and information to answer specific questions regarding pathogen spread. Passive surveillance strategies rely on mandatory or voluntary broad case reports of many different pathogens, while active surveillance strategies are more targeted to surveil for a specific pathogen in a specific species in a particular area. Syndromic surveillance can detect only pathogens that result in symptomatic infection and will therefore overlook asymptomatic cases. Laboratory-based surveillance is more resource-intensive but can detect both symptomatic and asymptomatic cases. Sentinel and targeted active surveillance strategies can also reduce required resources compared to whole population surveillance.

Diagnostics

Current coronavirus diagnostics detect either viral nucleic acids or serum antibodies. Viral RNA detection methods are used to identify active infections, while serologic methods detect past infections. Importantly, certain detection methods may be more or less feasible, depending on the type of animal. Further optimization of diagnostic methods can enable surveillance of additional animal species.

Metagenomics techniques are used to broadly identify pathogenic nucleic acids. While these techniques may be appropriate for broad pathogen surveillance, they are unsuitable for highly-scaled surveillance of specific pathogens or groups of pathogens. RT-PCR amplification of conserved regions followed by next-generation sequencing (i.e., targeted sequencing) is used to detect various related betacoronaviruses. Targeted sequencing strategies for emerging coronaviruses most often amplify conserved RNA-dependent RNA polymerase (RdRp), while other strategies amplify RBD or spike. These targeted sequencing strategies have been used successfully in Chiropteran species, dromedary camels, Sunda pangolins, and domestic cats.

RT-PCR for rapid and relatively inexpensive detection of emerging coronaviruses uses virus-specific primers for one or more target regions. To achieve virus-specific detection, some amplifications involve nested, heminested, or multiple sequence targets. SARS-CoV-1 RT-PCR detection strategies have been deployed in masked palm civets, while MERS-CoV RT-PCR detection strategies have been used in various species, including camelids, farmed ungulates, and Chiropterans. SARS-CoV-2 RT-PCR strategies have detected SARS-CoV-2 in domestic dogs and cats and white-tailed deer as well as other feline and small carnivore species.

Serological methods used to detect emerging coronaviruses include assays that detect the presence of antigen-binding antibodies as well as those that detect neutralizing antibodies. Importantly, antibody binding does not always correlate with virus neutralization and immunity. In addition, highly specific antibody assays are needed because antibodies specific to one coronavirus may cross-react with other coronaviruses.

Enzyme-linked immunosorbent assays (ELISAs), protein microarrays, microsphere immunoassays (MIAs), and luciferase immunoprecipitation system (LIPS) protocols have already been developed and used to detect antibodies to emerging coronaviruses in animals. ELISA methods have detected MERS-CoV antibodies in camelids; SARS-CoV-1 antibodies in palm civets; and SARS-CoV-2 antibodies in domestic dogs and cats, some farmed ungulate species,

some mustelids, white-tailed deer, and some small carnivores. Protein microarrays enable multiplexing for multiple antigens, enabling design of antibody assays with high specificity. Protein microarrays have detected SARS-CoV-2 antibodies in cats and in one beech marten. MIAs can also use multiplexing with a shorter preparation time. MIAs have detected SARS-CoV-2 antibodies in domestic cats, dogs, and rabbits. LIPS is a relatively new method for antibody detection has only been used to detect SARS-CoV-2 antibodies in cats.

Live virus neutralization assays (e.g., plaque reduction neutralization tests (PRNTs) and microneutralization assays (MNAs)), and pseudoparticle neutralization tests (ppNTs) have been used to detect coronavirus neutralizing antibodies in animals. Live virus neutralization assays require more rigorous biosafety regulations and compliance, while ppNTs and sVNTs are more accessible to laboratories without biosafety level 3 (BSL-3) certifications. At least one neutralizing assay method has successfully detected MERS-CoV antibodies in dromedary camels, alpacas, goats, cattle, and donkeys; SARS-CoV-1 antibodies in pigs, palm civets, raccoon dogs, and Chinese ferret-badgers; and SARS-CoV-2 antibodies in white-tailed deer, domestic cats and dogs, and some mustelid and feline species.

Vaccines

The development of vaccines to protect susceptible host species from infections with coronaviruses has been at the forefront of efforts to control the spread of SARS-CoV-1, MERS-CoV-1 and more recently SARS-CoV-2. Different inactivated and attenuated vaccine formulations as well as recombinant protein, mRNA, and DNA vaccines have been developed and approved for use. The S protein on the surface of coronaviruses is particularly immunogenic and therefore has been used in many of these vaccine formulations.

Most vaccines have been approved only for use in humans, and very few formulations have been approved for use as veterinary vaccines in susceptible species such as mink, cats, and captive animals residing in zoos. However, vaccine studies typically involve small animal models as a first step to evaluate immunogenicity and antibody response; further testing and efficacy studies have relied on the use of NHPs such as rhesus macaques whose immune systems are closely related to those of humans and may therefore shed light on correlates of protection. Therefore, although these vaccines may not be officially approved for use in animals, they may still be effective and safe for use in some small animal models and NHPs.

A major challenge in developing vaccines against coronaviruses has been the lack of durability of the vaccine response, as evidenced by a decrease of neutralizing titers and T cell responses. Continued development of vaccine formulations that offer more prolonged protection is ongoing. In addition, emerging coronaviruses are especially prone to genetic evolution that enables adaptation to new hosts and evasion of the host immune response. The mutated viruses can often escape the immunological response generated by current vaccines, necessitating the continuous development of new vaccine formulations. Current vaccine development efforts thus focus on generating vaccines with broader protection against multiple variants.

Animal protection strategies against coronaviruses will require vaccines that can protect various species, including companion, farmed, captive, and wild animals from infection. Several of the FDA-approved and emergency use vaccines for humans are not useful for large scale immunization of animal species due to their expense and difficult administration. Specific veterinary vaccine formulations that can be rapidly disseminated during an outbreak are also being formulated and tested. One such vaccine is an S protein vaccine developed by Zoetis that has been supplied for use in animals in zoos and mink farms.

Therapeutics

Laboratory animal models used in therapeutic efficacy studies for SARS-CoV-2, SARS-CoV-1, and MERS-CoV in humans provide insight on how mice, ferrets, rhesus macaques, hamsters, marmosets, and other similar wildlife and companion animals could be treated to reduce severity and transmission of these viruses. Drugs assessed in this literature review include antivirals that target RdRp, reverse transcriptase inhibitors, protease inhibitors, antibiotics, cas proteins, microbicides, antidepressants, polyphenols, aldehyde dehydrogenase inhibitors, non-structural protein targets, fatty acid synthesis, statins, and anti-inflammatory and anti-parasitic drugs.

Antivirals targeting RdRp include remdesivir, molnupiravir, GS-441524, GS-621762, galidesivir, and favipiravir. Some of these RdRp antivirals have demonstrated efficacy against SARS-CoV-2 in rhesus macaques, mice, ferrets, and Golden Syrian hamsters. Reverse transcriptase inhibitors, specifically emtricitabine-tenofovir, are effective against SARS-CoV-2 in ferrets, while other protease inhibitors—ensitrelvir and nirmatrelvir—were effective against this virus in mice and Golden Syrian hamsters. Prophylactic and therapeutic antibodies have shown efficacy in marmosets for MERS-CoV and hamsters for SARS-CoV-2. Drugs that target essential cellular processes and components of SARS-CoV-2, such as fatty acid synthesis, DNA replication, and cell membrane structure are effective in treating SARS-CoV-2 in rodents.

Drugs that are effective in limiting the inflammatory response to coronaviruses include baricitinib, loratadine, glucocorticoids, 1% astronomer sodium, and fluoxetine. Some of these anti-inflammatory drugs have successfully reduced SARS-CoV-2 inflammatory responses in rhesus macaques and some rodents. Notably, antimalarial drugs hydroxychloroquine (HCQ) and chloroquine were ineffective against SARS-CoV-2 in hamsters and rhesus macaques despite positive in vitro results.

Biosecurity

Humans are a primary reservoir of coronaviruses, with the potential for zoonosis and subsequent reverse zoonosis. In addition, many species-specific coronaviruses cause respiratory disease and are transmitted through droplets and/or aerosol, similar to SARS-CoV-1, MERS-CoV, and SARS-CoV-2. Therefore, human infection prevention, not only animal biosecurity measures for species-specific coronaviruses, should be considered as relevant strategies for SARS-CoV-1, MERS-CoV, and SARS-CoV-2 animal biosecurity.

Vaccines are one of the most effective methods for controlling the spread of infectious diseases from coronaviruses. However, because vaccines are currently approved only for use in humans and have been tested on a limited number of species, other biosecurity measures should also be considered, such as surveillance strategies. Surveillance of coronaviruses within animal populations is necessary for early viral detection and diagnosis, which can facilitate implementation of other biosecurity measures, such as quarantine of infected animals. Quarantining animals can reduce contact between animals and humans or other animals and should be highly prioritized to substantially reduce coronavirus exposures. Both the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) provide guidance for wildlife researchers and farmers on reducing animal contact. In addition, USDA APHIS provides guidance on proper disinfection, decontamination, personal protective equipment (PPE) usage, and carcass disposal, all of which can significantly reduce viral spread.

Other biosecurity measures for consideration include modifying land use and policy on live markets and trade. Land use changes (e.g., logging, mining) can alter the movement of wildlife and create new habitats for species, allowing contact between previously isolated species. Reducing land use changes can help keep ecosystems with high species diversity intact, leading to enhanced wildlife immune function and prevention of high viral prevalence and transmission. In addition, policy changes for live animal markets can reduce viral transmission between animals that are otherwise isolated from each other, and thus reduce the emergence of novel recombinant viruses. Depopulation/culling of coronavirus-positive animals is not recommended as a biosecurity measure because this method raises ethical concerns for both animal rights and welfare.

Report

Introduction

On March 11, 2021, President Joseph R. Biden, Jr. signed the American Rescue Plan Act of 2021 (ARP), a $1.9 trillion COVID-19 stimulus plan, into law. Under the Act, the U.S. Department of Agriculture (USDA) was provided $300 million to conduct monitoring and surveillance of susceptible animal species for incidence of SARS-CoV-2 and designated the Animal and Plant Health Inspection Service (APHIS) as the lead Agency in this charge.

In response, APHIS developed a Strategic Framework focused on actions to prevent, detect, investigate, and respond to new and emerging zoonotic disease threats including SARS-CoV-2. A key component of APHIS' effort is acknowledging known gaps in its current One Health infrastructure and identifying specific actions to address them.

To meet the charge set by Congress, APHIS must understand how SARS-CoV-2 moves between people and animals as well as how to take a One Health approach to the problems the global SARS-CoV-2 pandemic has highlighted. This understanding includes a need to learn more about the virus, which animals it affects, and how it is spreading to new locations or species, as well as other potential emerging coronaviruses that could pose a threat to both people and animals.

As a first step in this work, APHIS commissioned a research report on emerging zoonotic coronaviruses based on scientific literature, other publications, and outreach to researchers. The scope of the report extended across any research deemed vital in the fields of biology and disease control, including surveillance, virology, diagnostics, pathogenesis, immunology, vaccinology, and epidemiology, concerning SARS-CoV-2 and other potential emerging coronaviruses that could pose a threat to both people and animals. This report is a first step toward a gap analysis that will determine where APHIS can optimize its efforts to meet the priorities set forth in its Strategic Framework. Such an analysis will add validity to the ARP projects, provide context for future decision-making, and advance APHIS' ability to quantify and predict zoonotic disease dynamics and human risk.

Approach

This literature review was conducted in the PubMed (https://pubmed.ncbi.nlm.nih.gov/) and SCOPUS (https://www.scopus.com/) databases, using the search terms "SARS-CoV-2," OR "SARS-CoV-1", OR "MERS" combined with MeSH terms focusing on the prioritized research categories. Results were limited to studies that were published in English and that addressed research in non-human animal species (initial searches were not limited by date because the relative recency of the viruses created natural date ranges). This search returned a list of 2,883 papers. These papers were manually screened for relevance to SARS-CoV-1, MERS-CoV, or SARS-CoV-2 (e.g., that those viruses were not mere context or analogies). The remaining papers were allocated to the following topic areas and reviewed in detail to develop this report.

Table 1: Literature Search Results Categorization

Research Category	Papers (n)
Epidemiology	469
Molecular Biology and Virology	128
Immunology	136
Pathogenesis	135
Surveillance	29
Diagnostics	168
Vaccines	123
Therapeutics	50
Biosecurity	20
Total	**1,258**

These studies formed the main structure of the report and were supplemented by 140 recently published studies identified as the literature review progressed and through participation in the May 2023 International Conference on Livestock, Companion Animals and Wildlife Coronaviruses. Additional literature searches were performed during writing to provide appropriate citation for all material and, where needed, useful background. Studies were selected for inclusion based on the authors' impressions of their relevance and quality to the goals of controlling emerging zoonotic coronaviruses. More recent studies were given priority within the report. In total, 817 studies are referenced herein.

This review aimed primarily to define trends in the coronavirus literature to inform subsequent identification of knowledge gaps at an APHIS-sponsored workshop taking place in 2023. The literature review thus focuses on presenting both well-established patterns across the literature and novel findings that require further investigation.

Interpretation of literature review results and definition of trends was aided by conversations with coronavirus researchers and experts who were contacted by email and invited to participate in brief interviews about trends in SARS-CoV-1, MERS-CoV, SARS-CoV-2, or pan-coronavirus research. These individuals were selected from participation in the National Institutes of Health Tracking Resistance and Coronavirus Evolution (TRACE) Working Group, through presentation at a related conference, or through being among the list of most prolific authors within the "SARS-CoV-2," or "SARS-CoV-1," or "MERS" database search (defined as

having contributed to at least 3 of the publications selected for inclusion in the review). Information provided by these researchers and experts is incorporated only as corroborated by independent citations within the appropriate report sections.

Epidemiology

Coronavirus Ecology

Reservoirs

Chiropterans as Reservoirs for Related Coronaviruses

The origins of emerging coronaviruses of interest, including SARS-CoV-1, MERS-CoV, and SARS-CoV-2, can be bioinformatically traced back to various species of the order Chiroptera.[5,6] Chiropteran immune systems support long-term, asymptomatic viral infections through two main mechanisms: (1) viral host receptors lacking full compatibility with some viruses and (2) constitutive expression of interferon alpha (IFN-α).[7–11] While constitutive expression of IFN-α would trigger detrimental inflammatory responses in other mammals, Chiropterans appear unaffected; other research groups have hypothesized that this adaptation increases Chiropteran tolerance to increased DNA damage during prolonged flights.[12–16] Chiropterans are often co-infected with multiple viruses, which enables recombination events and the potential creation of novel viruses with broader species tropisms.[17] Researchers have recently recapitulated these characteristics of viral propagation in Chiropteran cell lines.[18]

Certain Chiropteran social behaviors further facilitate the propagation of viral pathogens. Chiropterans reside in close quarters with their colony mates, and breeding and migration can affect their proximity to one another.[19–21] In addition, Chiropteran echolocation is produced from vibrations that may trigger airborne release of viral pathogens.[20,21] The unique combination of high-density social behaviors and immune system characteristics of Chiropterans makes these mammals a large reservoir of coronaviruses, along with other potentially zoonotic pathogens.

SARS-related (SARSr) and MERS-related (MERSr) viral RNAs have been detected in Chiropterans, and some Chiropterans have tested positive for SARSr or MERSr antibodies (Table 2). Most prevalence studies of Chiropterans included in this review assayed for viral RNA, and a minority of studies tested for relevant antibodies. Evidence of SARSr infections in Chiropterans was detected in Australia,[22,23] Bulgaria,[24] Cambodia,[25] China,[5,26–34] Japan,[35] Laos,[36] Nigeria,[37] and Vietnam,[38] while evidence of MERSr was detected in Australia,[23] China,[39–42] Italy,[43] Saudi Arabia,[44] South Africa,[45,46] Switzerland,[47] and Thailand.[48] Other betacoronavirus RNAs were previously detected in Ethiopia,[49] Ghana,[50] Romania,[50] and Ukraine.[50] Notably, the *Rhinolophus* genus frequently tested positive for SARSr infections.[5,25–33,51]

Table 2: Locations of Chiropterans Infected with Emerging Coronaviruses

Virus	Location	Species
SARSr	Australia[22,23]	*Rhinonicteris aurantia*[22]

		Austronomous australis[23**]
		Chalinolobus gouldii[23**]
		Chalinolobus morio[23**]
		Nyctophilus gouldi[23**]
		Nyctophilus major[23**]
		Vespadelus regulus[23**]
	Bulgaria[24]	*Rhinolophus blasii*[24]
		Rhinolophus euryale[24]
		Rhinolophus ferrumequinum[24]
		Rhinolophus mehelyi[24]
	Cambodia[25]	*Rhinolophus shameli*[25]
	China[5,26–34]	*Rhinolophus ferrumequinum*[5,26–28]
		Rhinolophus macrotis[5,27–29*]
		Rhinolophus pearsoni[5*]
		Rhinolophus affinis[26,30]
		Rhinolophus sinicus[26,28,31–33]
		Miniopterus schreibersi[34]
		Aselliscus stoliczkanus[26]
		Rhinolophus marshalli[29]
		Rhinolophus pusillus[29,51*]
		Rhinolophus thomasi[29]
		Chaerephon plicatus[51]
		Rousettus leschenaulti[5**]
	Japan[35]	*Rhinolophus cornutus*[35]
	Laos[36]	*Rhinolophus malayanus*[36]
		Rhinolophus marshalli[36]
		Rhinolophus pusillus[36]
	Nigeria[37]	*Hipposideros commersoni*[37]
	Vietnam[38]	*Rhinolophus acuminatus*[38]
MERSr	Australia[23]	*Chalinolobus gouldii*[23**]
		Chalinolobus morio[23**]
		Vespadelus regulus[23**]
	China[39–42]	*Vespertilio superans*[39–41]
		Ia io[40]
		Pipistrellus abramus[40]
		Pipistrellus pipistrellus[40]
		Hypsugo pulveratus[42]
	Italy[43]	*Eptesicus serotinus*[43]
	Saudi Arabia[44]	*Taphozous perforates*[44]
	South Africa[45,46]	*Neoromicia zuluensis*[45]
		Neromicia capensis[46]
	Switzerland[47]	*Vespertilio murinus*[47]
	Thailand[48]	*Tadarida plicata*[48]
Other Betacoronaviruses	Ethiopia[49]	*Chaerephon pumilus*[49]
		Neoromicia somalica[49]
		Rhinopoma hardwickii[49]
	Ghana[50]	*Nycteris gambiensis*[50]
	Romania[50]	*Pipistrellus nathusii*[50]
		Pipistrellus pipistrellus[50]
		Pipistrellus pygmaeus[50]

	Ukraine[50]	*Pipistrellus nathusii*[50]

* Detected via viral RNA and serology.

** Only detected via serology.

Dromedaries

A large proportion of dromedary camels (*Camelus dromedarius*) in the Middle East and parts of Africa are currently or have been previously infected with MERS-CoV, particularly in larger herds.[52–56] However, *C. dromedarius* MERS-CoV-specific antibodies are short-lived, resulting in potential reinfections;[57] for example, approximately 25% of *C. dromedarius* calves had been reinfected with MERS-CoV.[58] Trade routes and import and export of *C. dromedarius* impact the spread of MERS-CoV in different Middle Eastern and African geographies.[56]

MERS-CoV RNA surveillance studies have identified active infections in *C. dromedarius* in Burkina Faso,[54] Djibouti,[59] Egypt,[53,60] Ethiopia,[54] Kenya,[58,61] Morocco,[54] Oman,[62] Qatar,[63,64] Saudi Arabia,[59,65–68] Somalia,[65] Sudan,[59,65] Tunisia,[69] and United Arab Emirates (Table 3).[70] Notably, a surveillance study in Egypt identified much higher rates of infection in camels located on farms or in slaughterhouses compared to those in live animal markets or raised in free herds.[53]

Table 3: Rates of MERS-CoV Infection in *Camelus dromedarius*

Country	Rate of Infection
Burkina Faso	5%[54]
Djibouti	11%[59]
Egypt	4%[60]
	15%[53]
Ethiopia	10%[54]
Kenya	<1%[61]
	34%[58*]
Morocco	2%[54]
Oman	100%[62**]
Qatar	59%[63]
	21%[64]
Saudi Arabia	15%[65]
	13%[59]
	3%[66]
	45%[67]
	25%[68]
Somalia	7%[65]
Sudan	6%[65]
	14%[59]
Tunisia	80%[69]
United Arab Emirates	5%[70]

*Calves only.

**Camels with respiratory symptoms.

Masked Palm Civets

SARS-CoV-1 has been traced back to spillover from bats to the masked palm civet (*Paguma larvata*).[71,72] *P. larvata* likely experience asymptomatic SARS-CoV-1 infections[73] and are thought to have transmitted SARS-CoV-1 to humans during human–animal contacts during wildlife farming practices and at wet markets.[74–77] Consistent with detection of SARS-CoV-1 RNA and antibodies in *P. larvata*, in vitro experiments have shown that exogenous masked palm civet angiotensin-converting enzyme 2 (ACE2) supports SARS-CoV viral entry.[78] Although SARS-CoV-2 infections have not been detected in *P. larvata*, a variety of in silico predictive studies of infection risk, including modeling of ACE2-receptor binding domain (RBD) binding[79–85] and ACE2 homology,[80,83–98] as well as exogenous in vitro viral entry studies,[91,95–97,99,100] suggest that *P. larvata* may be susceptible to SARS-CoV-2 infection.

Raccoon Dogs

Similar to *P. larvata*, raccoon dogs (*Nyctereutes procyonoides*) at live animal markets tested positive for SARS-CoV-1 infection.[75,101] In addition, in vitro experiments have shown that exogenous *N. procyonoides* ACE2 supports SARS-CoV viral entry.[78]

N. procyonoides may also be susceptible to SARS-CoV-2 infection based on in silico analysis of ACE2 homology[83,86,87,89,91,96] and predictive ACE2-RBD binding.[82,83] In vitro experiments exogenously expressing *N. procyonoides* ACE2 demonstrated that this viral receptor can support both ACE2-RBD binding[91] of SARS-CoV-2 as well as viral entry.[91,96] Althogh *N. procyonoides* can also be experimentally infected with SARS-CoV-2 and transmit the virus to other *N. procyonoides* via direct contact,[102] no evidence of SARS-CoV-2 infection was detected in 11 *N. procyonoides* in Germany.[103] More definitive prevalence studies are needed to determine whether *N. procyonoides* significantly contribute to the spread of SARS-CoV-2.

Candidate Reservoirs for SARS-CoV-2

The animal reservoir for SARS-CoV-2 remains unknown, with evidence suggesting snakes or pangolins as candidate reservoirs. Sequence analysis and relative synonymous codon usage (RSCU) biases suggested snake as a potential SARS-CoV-2 reservoir. RSCU biases for the many-banded krait (*Bungarus multicinctus*) and Chinese cobra (*Naja atra*) were more similar to SARS-CoV-2 than the Chinese rufous horseshoe bat (*Rhinolophus sinicus*), chicken (*Gallus gallus*), Sunda pangolin (*Manis javanica*), and the European hedgehog (*Erinaceus europaeus*).[17] However, multiple research groups strongly disagree that RSCU analysis was sufficient to identify *B. multicinctus* and *N. atra* as potential SARS-CoV-2 reservoirs.[104,105] SARS-CoV-2 has also not been identified in any snake species.

While closely related viruses (e.g., RaTG13) circulate in Chiropterans, these viruses still lack critical features present in SARS-CoV-2, including a furin cleavage site at the S1/S2 junction. This furin cleavage site facilitates conformational changes to promote interaction with ACE2. Researchers have suggested that SARS-CoV-2 arose after recombination events between RaTG13 and another coronavirus containing the furin cleavage site.[106,107] Other coronaviruses

containing sites recognized by furins include MERS-CoV[108,109] and bovine coronavirus (Bov-CoV).[110]

SARS-CoV-2 viruses detected in other species also lack this furin cleavage site. *M. javanica* was previously suspected as a SARS-CoV-2 reservoir, but sequence analysis confirmed the absence of this furin cleavage site, and further evolutionary analysis indicated that RaTG13 from Chiropterans was more closely related to SARS-CoV-2 than pangolin coronaviruses.[107] The origin of this furin cleavage site remains unclear but may provide further insight into potential SARS-CoV-2 reservoirs.

Potential Future Reservoir for SARS-CoV-2: Mustelids

Multiple countries have reported SARS-CoV-2 outbreaks on American mink (*Neogale vison*) farms, including those in Canada,[111] Denmark,[112,113] France,[114] Greece,[111] Italy,[115] Lithuania,[111] Netherlands,[116–118] Poland,[119] Spain,[120] Sweden,[111] and United States.[121–123] These outbreaks likely originated from humans working on these farms,[124] and spillover events likely occurred from farmed *N. vison* to wild *N. vison*[121] as well as cats (*Felis catus*) and possibly dogs (*Canis lupus familiaris*) residing nearby.[125,126] As a result of *N. vison* infections on farms, the Danish government opted to depopulate infected farms, institute mandatory reporting of symptoms, and quarantine and disinfect infected farms.[127] Because of its susceptibility and transmission among populations, *N. vison* may become a SARS-CoV-2 reservoir with global impact, as millions of *N. vison* are farmed across China, Europe, North America, and Russia.[128]

SARS-CoV-2 viral RNA has also been detected in other mustelids, particularly in Spain, including ferrets (*Mustela putorius furo*),[129] and Eurasian river otters (*Lutra lutra*).[130] Previous SARS-CoV-2 infections are likely in mustelids as indicated by antibody detection in European pine martens (*Martes martes*) and European badgers (*Meles meles*) in France[131] and *M. purotrius furo* in Spain.[132] Further research is required to determine whether farmed and wild mustelids have the potential to become SARS-CoV-2 reservoirs.

Possible Modes of Coronavirus Transmission

Airborne Droplets

Coronaviruses are often spread through airborne droplets, and the distance a virus can travel depends on external environmental factors, including air flow, temperature, and humidity.[133–138] Colder temperatures paired with either low or high levels of relative humidity promote overall coronavirus stability.[138,139] This stability of coronaviruses within droplets can therefore dictate maximum transmission distances. Therefore, transmission of coronaviruses among animals under human care and captive wildlife may depend on their housing conditions, including population density.

Animals with the potential to transmit coronaviruses via airborne droplets will likely shed coronavirus oronasally. Detection of viral RNA in oropharyngeal or oronasal swabs is often used

as a readout for viral shedding as well as a common diagnostic tool (see "Diagnostics" for more details).

Fomites

Extensive prior research has demonstrated that MERS-CoV can survive on both plastic and steel surfaces for more than 48 hours, which may play a critical role in transmission, at least among humans.[140–146] SARS-CoV-1 RNA can be detected on hospital surfaces near infected patients, and computational modeling suggests that fomite transmission has contributed to previous outbreaks of SARS-CoV-1 among humans.[147,148] Although fomite transmission of SARS-CoV-2 has been overshadowed by the threat of airborne transmission, replication-competent SARS-CoV-2 is detectable on fomites; however, infectivity of SARS-CoV-2 on fomites is poorly understood.[149] Similar to viruses in droplets, the stability of coronaviruses on fomites can be influenced by environmental conditions.[139] Animals shedding coronaviruses via any route (e.g., oronasal, ocular, fecal) can contaminate nearby fomites, resulting in intraspecies and possibly interspecies transmission.

Feces

Recently, surveillance efforts of SARS-CoV-2 have transitioned from clinical sampling to wastewater sampling. SARS-CoV-2 RNA is readily detectable in wastewater as well as fecal samples isolated from various species, but little is known about whether live, infectious SARS-CoV-1, MERS-CoV, or SARS-CoV-2 can survive digestion. Although previous research has shown that SARS-CoV-1 is inactivated by gastric juices, live coronaviruses have been occasionally isolated from fecal swabs.[150] Researchers have proposed that certain gastrointestinal conditions that reduce gastric pH may enable survival of SARS-CoV-2 in the stomach.[151,152] MERS-CoV can survive and retain infectivity following passage through the human digestive tract.[153] In addition, SARS-CoV-1 has been isolated once from a farmed pig (*Sus scrofa*) fecal sample[154] and twice from fecal/anal swabs from *R. sinicus*.[155,156] Consistent with the potential of fecal shedding of SARS-CoV-1 and SARS-CoV-2, the corresponding viral host receptor, ACE2, is expressed in the gastrointestinal tract of *S. scrofa*,[157,158] *F. catus*,[159] tiger (*Panthera tigris*),[158,159] lion (*Panthera leo*), Eurasian lynx (*Lynx lynx*), common marmoset (*Callithrix jacchus*), gorilla (*Gorilla gorilla*), golden-headed lion tamarin (*Leontopithecus chrysomelas*), horse (*Equus caballus*), and sheep (*Ovis aries*).[158]

If emerging coronaviruses can indeed survive digestion, inadequate wastewater treatment could result in live coronavirus contamination of coastal waters, although coronaviruses may not survive certain aquatic conditions at sufficient concentrations for transmission.[160–164] Some respiratory viruses (e.g., cetacean morbillivirus) can transmit from terrestrial animals to pinnipeds, and from pinnipeds to cetaceans for sustained circulation.[165]

Animal Products

Some animal species that can contract and transmit SARS-CoV-1, MERS-CoV, or SARS-CoV-2 are common livestock species raised for meat and other animal products, such as milk. Although

MERS-CoV RNA is detectable in camel milk, further research has not adequately determined whether milk from an infected camel can transmit MERS-CoV.[166,167] China's Centers for Disease Control reported on tracing efforts that linked a SARS-CoV-2 outbreak to seafood products in 2020.[168] A recent study investigated the stability of SARS-CoV-2 in meat, poultry, and seafood via multiple surrogates: murine hepatitis virus (MHV), transmissible gastroenteritis virus (TGEV), and phi 6 RNA bacteriophage. At least one surrogate was detectable in both refrigerated and frozen meat, poultry, and seafood after 30 days of storage. Post-isolation, these viral particles were likely infectious, because cell culture assays resulted in cytopathic effects.[169] In addition, a recent study demonstrated that SARS-CoV-2 spike (S) protein could bioaccumulate in Pacific oysters; this finding, combined with frequent detection of SARS-CoV-2 RNA in wastewater, indicates that oysters have the potential to transmit SARS-CoV-2.[170] Further research is required to determine whether viral particles detected in animal products retain their infectivity and are present at high enough concentrations to successfully infect a host after consumption.

Arthropods

Although coronavirus-like organisms have been identified in sea bird tick and cat fleas,[171,172] arthropods likely do not biologically or mechanically transmit coronaviruses. Despite expression of SARS-CoV-2 host receptors in ticks (*Ixodes scapularis* and *Ixodes ricinus*) and cat fleas (*Ctenocephalides felis*),[172–176] extensive epidemiological studies of wild-caught arthropods did not detect SARS-CoV-2 in mosquitoes (i.e., *Anopheles* and *Culex* species), as well as arthropods from the following taxonomic families: Asilidae (robber flies), Blattidae (cockroaches), Calliphoridae (blow flies), Ulidiidae (picture-winged flies), Dolichopodidae (long-legged flies), Drosophilidae (pomace flies), Muscidae (houseflies), Phoridae (hump-backed flies), Psychodidae (drain flies), Sarcophagidae (flesh flies, Syrphidae (hoverflies), and Tabanidae (horse flies).[177,178] In addition, no MERS-CoV RNA was detected in *Hyalomma dromedarii* (camel ticks) that previously fed on infected dromedary camels.[179] Researchers in laboratory settings have attempted to infect arthropod species to assess their potential for transmission of SARS-CoV-2; mosquitoes, houseflies (*Musca domestica*) and midges (*Culicoides sonorensis*) cannot mechanically or biologically transmit SARS-CoV-2 in this setting.[180–183] Notably, computation modeling of ectoparasite ACE predicted that body louse, deer tick, and water flea ACE can bind SARS-CoV-2 RBD.[176]

Mechanisms of Coronavirus Evolution

As coronaviruses continue to evolve, mutations may arise that affect their infectivity, transmissibility, severity, and species tropism. Notably, SARS-CoVs have a high level of genetic diversity and rate of recombination, which increases the likelihood of cross-species transmission and expanded species tropism.[184] These intrinsic aspects of SARS-CoVs, paired with the unique Chiropteran immune system (see "Chiropterans" for more details) can facilitate the rapid development of viral threats to other species. For example, researchers suggest that multiple recombination events involving Bat-CoV-RaTG13 resulted in SARS-CoV-2 containing the S1/S2 cleavage site seen in humans.[185]

The mutation rate of RNA viruses Is relatively high compared to other pathogens because of the absence of the corrective function of RNA-dependent RNA polymerase.[74] In addition, because of their larger genomes, coronaviruses are more tolerant of deletion mutations compared to other pathogens.[186] Indeed, the evolutionary rates of SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are similarly high at a magnitude of 10^{-3} mutations per site per year.[187–190] However, these evolutionary rates are not evenly distributed; the SARS-CoV-2 spike gene has a higher evolutionary rate compared to the rest of its genome.[191] Mutation acquisition has expanded SARS-CoV-2's species tropism to include mouse (*Mus musculus*) and *N. vison*.[192]

The presence and activity of certain host innate immune proteins may be impacting the types of mutations seen in SARS-CoV-2. After detecting an overabundance of C-to-U mutations in SARS-CoV-2, researchers proposed a model by which two classes of innate immune proteins—adenosine deaminases acting on RNAs (ADARs) and apolipoprotein B mRNA editing enzyme, catalytic polypeptides (APOBECs)—induce point mutations in foreign genetic material, including SARS-CoV-2 to create a C-to-U mutational bias, impacting the virus's evolution.[193–196]

Evidence Types for Determining Species Susceptibility

This section summarizes the evidence types commonly described in the emerging coronavirus epidemiology literature. The contexts of different evidence types should be considered when making determinations about the vulnerability of a particular species to coronavirus infection; for example, exogenous expression of a viral host factor in vitro may not recapitulate natural infection risk in vivo of a particular species. A combination of evidence types can help identify species at risk for coronavirus infection, which are identified in the "Species Interfaces Relevant to Coronavirus Transmission" section.

Risk of Interspecies Exposure

Various species-intrinsic and species-extrinsic factors place certain species at risk of exposure to emerging coronaviruses.

Species-Intrinsic Factors

Species that prey on various Chiropterans may be at higher risk for contracting coronavirus infections due to direct interspecies contacts, while highly social species and those living in dense groups may also be at high risk for contracting coronavirus infections due to direct intraspecies contacts.[197] Although birds that prey on Chiropterans have tested positive for betacoronaviruses,[1] these have not been identified as closely related to SARS-CoV-1, MERS-CoV, or SARS-CoV-2.[198] In addition, social grooming behaviors in Japanese macaques (*Macaca fuscata yakui*) facilitate transmission of parasitic infections.[199] Mating behaviors and seasonality can also impact direct contact between farmed and wild animals of the same species. For example, wild boar and farmed pig mating interactions may have caused a brucellosis spillover event.[200]

[1] Avian species are mainly hosts for deltacoronaviruses and gammacoronaviruses, not betacoronaviruses.

Species-Extrinsic Factors

Legal and illegal wildlife trade bring various species in direct contact that would not otherwise meet in natural conditions.[201–203] Interspecies contacts have also been disrupted over time due to habitat loss[204] and agricultural practices that increase contact between livestock and wildlife.[205] For example, in Malaysia and Singapore, farmed pigs were sometimes housed near fruit trees that attracted flying foxes (*Pteropus* spp.) that carry Nipah virus. This close contact resulted in transmission of Nipah virus from bats to farmed pigs and then to humans.[206,207] In addition, computational modeling has suggested that transmission of Hendra virus from black flying foxes (*Pteropus alecto*) to *E. caballus* occurs through prolonged exposure to contaminated urine at the base of bat tree roosts.[207,208] In both instances, adjustments to farming practices dramatically reduced interspecies contacts and viral spread.[206–208]

Ongoing climate change can also drastically affect virus transmissibility[139] as well as animal life cycles.[209] Changes in temperature and humidity can impact the transmissibility of coronaviruses via different routes (see "Possible Modes of Coronavirus Transmission"). Climate change greatly impacts seasonality across the globe, which has already been shown to impact interspecies interactions facilitated by factors such as migratory patterns,[210] foraging and predation,[211] and lifecycle synchronicity.[209,212]

Expression of Coronavirus Host Factors

Specific host factors are required for efficient viral entry of different viruses. Although expression of host factors can be used to predict species susceptibility and routes of transmission, expression of canonical viral host factors may not be sufficient to accurately predict host susceptibility.[213] Expression and function of other host factors should be considered, among other evidence types. For further information on the molecular mechanisms of coronavirus host factors, see "Molecular Biology and Virology."

Receptors

ACE2 serves as the canonical viral receptor for SARS-CoV-1 and SARS-CoV-2,[214,215] while dipeptidyl peptidase-4 (DPP4) is the main viral receptor for MERS-CoV.[216] Because these receptors play major roles in facilitating viral entry, many research articles investigated the expression levels and patterns of these proteins as part of assessing potential risk of infection in different species. In addition, some species express soluble extracellular domain isoforms of ACE2 that do not support RBD binding and viral entry.[217,218] A soluble extracellular domain of DPP4 has been extensively studied in the context of metabolic diseases and cancer, but it is unclear whether this form of DPP4 is due to alternative splicing or protein cleavage events.[219] However, a soluble form of DPP4 likely does not support viral entry because of a lack of tethering to cells.

In the absence of canonical viral receptors, coronaviruses may use alternative receptors to infect cells. Notably, species susceptibility research has not thoroughly investigated the expression of these alternative receptors and their relation to susceptibility to coronaviruses. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and

liver/lymph node-specific intracellular adhesion molecules-3-grabbing non-integrin (L-SIGN) (i.e., C-type lectins)[220–223] as well as T-cell immunoglobulin and mucin domain 1 (TIM1)[220,224] and AXL[220,225] can support SARS-CoV-2 viral entry in vitro; however further research has indicated that these proteins cannot support SARS-CoV-2 viral entry in the absence of ACE2.[220,226,227] Therefore, although expression of C-type lectins, TIM1, and AXL are not sufficient to assess whether a species is susceptible to SARS-CoV-1 or SARS-CoV-2 infection, the presence of these factors may still improve the rate of viral entry. Another proposed alternative receptor for SARS-CoVs, cluster of differentiation 147 (CD147), cannot directly bind to the spike (S) protein of SARS-CoV-2, and therefore likely also does not support viral entry in the absence of ACE2.[220,228–230] In addition, neuropilin 1 (NRP1) enhances transmembrane protease, serine 2 (TMPRSS2)-dependent SARS-CoV-2 entry[231,232] and has been proposed to form a complex with ACE2 and SARS-CoV-2.[233] No additional research indicates that NRP1 can facilitate ACE2-dependent viral entry.

A recent genomic receptor screen identified 12 proteins with affinity for SARS-CoV-2. Of these proteins, Kremen1 and ASGR1 supported SARS-CoV-2 viral entry independent from ACE2 in vitro and in mice. Notably, both of these alternative receptors did not support viral entry of SARS-CoV-1 or MERS-CoV in the absence of ACE2. In addition, the presence of Kremen1 or asialoglycoprotein receptor 1 (ASGR1) on certain cell types correlated with cellular susceptibility to SARS-CoV-2 infection.[234] Therefore, further research on the expression of Kremen1 and ASGR1 may provide additional information on the susceptibility of different species to SARS-CoV-2 infection.

Proteases

Prior to viral entry, host cell proteases are required to cleave S protein of SARS-CoV-1 or SARS-CoV-2. Both SARS-CoVs contain two cleavage sites; SARS-CoV-2 contains a furin cleavage site and a site cleaved either by TMPRSS2 or cathepsin L, while SARS-CoV-1 contains two cleavage sites, both of which are cleaved by TMPRSS2 or cathepsin L.[235–237] Other serine proteases help promote infection by respiratory viruses in airway cells, and additional research may identify specific proteases that may indicate increased susceptibility to infection.[220,238]

Computational Modeling of Host Factors

Computational modeling methods have been used extensively to predict species susceptibility to SARS-CoV-2, but not SARS-CoV-1 or MERS-CoV. The main modeling strategies present in the literature are protein sequence alignments, ACE2-RBD docking simulations, and molecular dynamics simulations.

Protein Sequence Alignments

ACE2 protein sequence alignment data available in current literature were generated using versions of Clustal,[83,87,90,92,93,96,98,239–246] Multiple Sequence Comparison by Log- Expectation (MUSCLE),[90,93,247–251] Multiple Alignment using Fast Fourier Transform (MAFFT),[82,84,85,252,253,253,254] BioEdit,[94,255] Molecular Evolutionary Genetics Analysis (MEGA),[250,256–259] Constraint-based Multiple Alignment Tool (COBALT),[93] and Basic Local

Alignment Search Tool (BLAST).[260] Importantly, the types and locations of amino acid substitutions likely have different effects on ACE2-RBD binding, and individual substitutions should ultimately be considered within the context of the full ACE2 protein.

Docking Simulations

ACE2-RBD docking simulation data in the current literature were generated using combinations of High Ambiguity Driven protein-protein DOCKing (HADDOCK),[82,84,255,260,261] AlphaFold,[85] Rosetta,[247,251,253,253] Iterative Threading ASSEmbly Refinement (I-TASSER),[85,87,258,260] Modeller,[81–85,239,243,245,257,261] Proteins, Interfaces, Structures and Assemblies (PISA),[85] SWISS-MODEL,[79,80,86,94,241,242,244,246,256,259,262] Chimera,[89,90,93,241,256,261] Phyre2,[96,98] HDOCK,[81,242,246] Global Range Molecular Matching X (GRAMM-X),[244] PyMOL,[88,90] ClusPro,[259] PRISM,[258] and Visual Molecular Dynamics.[240] Most docking simulations were based on the X-ray crystallography structures of human ACE2-RBD. These data were used to predict the capacity for binding of SARS-CoV-2 RBD by ACE2 from various species.

Molecular Dynamics Simulations

Molecular dynamics simulations have been used to determine the potential stability of ACE2-RBD complexes using GROMACS,[80,81,239,242,255,262] Visual Molecular Dynamics,[241] AMBER,[79,243,257] and Desmond.[241] Researchers have posited that more stable ACE2-RBD interactions are predictive of infection susceptibility.

In Vitro Experimental Infection Capacity

Multiple in vitro assays have been used to assess species susceptibility to coronavirus infection: host receptor-virus binding, viral entry, and viral replication. These assays were performed either in non-permissive cells overexpressing ACE2 (i.e., exogenous) or cell lines derived from the species of interest (i.e., endogenous). Although in vitro assay results do not always recapitulate in vivo risks of infection, these assays can be useful for rapid screening of multiple species.

In Vivo Experimental Infection Capacity

Experimental infections of live animals have been used to identify species capable of sustaining coronavirus infection. Although these infection experiments are performed in live animals, the viral titers and routes of infection may not reflect real-world coronavirus exposures. In addition, experimental infection studies may not be feasible in larger animals. Oral and nasal inoculations more closely mimic respiratory virus exposures than intratracheal and intravenous inoculations.

In Vivo Experimental Transmission Studies

Experimental transmission studies of live animals have been used to determine (1) whether a species sheds sufficient viral loads to infect another, co-housed animal and (2) the level of contact between animals required for viral transmission. For example, SARS-CoV-2 transmitted from separately housed, infected ferrets to naïve ferrets via shared airflow at distances greater than 1 meter, indicating the possibility of airborne transmission among *M. putorius furo*.[263]

Although these transmission studies require a large number of animals and specialized housing, they can closely recapitulate real-world infection and transmission scenarios.

Detection of Natural Infections

Active and past infections can be detected through viral RNA detection methods and serological methods, respectively. Positive coronavirus RNA and antibody assay results from a particular species indicate a risk of infection in other animals of the same species. Detection of viral RNA is indicative of active infection, while detection of specific antibodies suggests previous infection. Specific methods for detecting viral RNA and antibodies are described in detail in "Diagnostics."

Species Interfaces Relevant to Coronavirus Transmission

Interactions with Chiropterans

Because Chiropterans harbor a wide variety of viruses (see "Chiropterans as Reservoirs for Related Coronaviruses"), animals in close contact with them are at risk for zoonotic spillover events. One such contact event is predation of Chiropterans. Although most specialized predators of bats are birds[264] (e.g., bat hawk [*Macheiramphus alcinus*][265–267] and bat falcons [*Falco rufigularis*][268]), opportunistic predators include other raptors[264,269–273] as well as raccoons (*Procyon lotor*),[274,275] *F. catus*,[276] Cebidae monkeys,[277–279] *Cercopithecus* monkeys,[280] otters (*Lutra lutra*),[281] *N. vison*,[282] long-tailed weasels (*Neogale frenata*),[283] and Siberian weasels (*Mustela sibirica*).[284] Another contact event involves exposure of animals to Chiropteran saliva, which is known to spread various Chiropteran viruses such as rabies, lyssavirus, Hendra virus, and Nipah virus. Animals that have contracted viruses originating from Chiropterans include *E. caballus*,[285–287] mules (*Equus mulus*),[288] donkeys (*Equus asinus*),[288] goats (*Capra hircus*),[288] *O. aries*,[286,288] *S. scrofa*,[288,289] *C. lupus familiaris*,[288] poultry,[288] *F. catus*,[286,288,290] *Vulpes vulpes*,[286] skunks (*Mephitis mephitis*),[286,291] cows (*Bos taurus*),[286] and stone martens (*Martes foina*).[292] These spillover events indicate close contacts, and therefore animals that have contracted Chiropteran viruses are at risk for future spillover events of coronaviruses. The susceptibilities of these species (i.e., avian, mustelid, NHP, and farmed ungulate species) to SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are outlined below, and susceptibilities of companion animals are outlined in "Interactions with Humans."

Avian Susceptibility

Avian species that prey on Chiropterans have tested positive for betacoronaviruses, despite normally only contracting gamma- and deltacoronaviruses.[198] However, most computational modeling data predicted low susceptibility of avian species, which is consistent with the inability to infect avians in vivo and in vitro. Computational modeling data of 94 avian species analyzed across 19 computational modeling publications are summarized in Table 4. Very few publications reported any avian species with ACE2-RBD affinity comparable to humans except for Kaushik et al., 2022 and Fischhoff et al., 2021.

Table 4: Summary of Computational Modeling Data on Avian Susceptibility to SARS-CoV-2

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Accipiter nisus	Eurasian sparrowhawk	Low[245]	Low[245]	Not assessed
Amazona collaria	Yellow-billed amazon	Low[245]	Low[245]	Not assessed
Anas platyrhynchos	Mallard	Low[83,84,86,87,93,245,250,257]	Low[82–85,244,245,257]	Not assessed
Anser brachyrhynchus	Pink-footed goose	Low[245]	Low[245]	Not assessed
Anser cygnoides	Swan goose	Low[93,245]	Low,[82] High[245]	Not assessed
Antrostomus carolinensis	Chuck-will's widow	Low[93]	Low,[82] Moderate[85]	Not assessed
Apaloderma vittatum	Bar-tailed trogon	Low[93]	Not assessed	Not assessed
Aptenodytes forsteri	Emperor penguin	Low[83,87,93]	Low[82,83]	Not assessed
Apteryx haastii	Great spotted kiwi	Low[245]	Low[245]	Not assessed
Apteryx owenii	Little spotted kiwi	Low[245]	Low[245]	Not assessed
Apteryx rowi	Okarito kiwi	Low[83,93,245]	Low,[82,245] High[85]	Not assessed
Aquila chrysaetos	Golden eagle	Low[83,93,245]	Low,[82,83,244,245] High[85]	Not assessed
Athene cunicularia	Burrowing owl	Low[83,93,245]	Low[82,83,245]	Not assessed
Aythya fuligula	Tufted duck	Low[83,93,253]	Low[82,83,253]	Low[253]
Buceros rhinoceros	Rhinoceros hornbill	Low[83,93]	Low,[83] High[82]	Not assessed
Calidris pugnax	Ruff	Low[83,93,245]	Low[82,83,245]	Not assessed
Calidris pygmaea	Spoon-billed sandpiper	Low[245]	Low[245]	Not assessed
Calypte anna	Anna's hummingbird	Low[83,93]	Low,[82,83] Moderate[85]	Not assessed
Camarhynchus parvulus	Small tree finch	Low[83,93]	Low,[82,83] High[85]	Not assessed
Cariama cristata	Red-legged seriema	Low[93]	Low[82]	Not assessed
Cathartes aura	Turkey vulture	Low[86]	Not assessed	Not assessed
Catharus ustulatus	Swainson's thrush	Low[83]	Low[82,83]	Not assessed
Centrocercus urophasianus	Greater sage-grouse	Not assessed	Moderate[85]	Not assessed
Chaetura pelagica	Chimney swift	Low[83,93]	Low,[83] High[82]	Not assessed
Charadrius vociferus	Killdeer	Low[83,93]	Low[82,83]	Not assessed
Chiroxiphia lanceolata	Lance-tailed manakin	Low[83]	Low,[83] High[82]	Not assessed
Chlamydotis macqueenii	MacQueen's bustard	Low[83,93]	Low,[83] High[82]	Not assessed
Chloebia gouldiae	Gouldian finch	Low[245]	High[245]	Not assessed
Columba livia	Rock dove	Low[93]	Not assessed	Not assessed
Corapipo altera	White-ruffed manakin	Low[83,93]	Low,[82,83] High[85]	Not assessed

Corvus brachyrhynchos	American crow	Low[93], Moderate[83]	Low[83]	Not assessed
Corvus cornix	Hooded crow	Low[83,93]	Low,[82,83] High[85]	Not assessed
Corvus kubaryi	Mariana crow	Not assessed	High[85]	Not assessed
Corvus moneduloides	New Caledonian crow	Low[83,93]	Low,[82,83] Moderate[85]	Not assessed
Coturnix japonica	Japanese quail	Low[83,93,245]	Low,[82,83,245] Moderate[85]	Not assessed
Cuculus canorus	Common cuckoo	Low[93]	Low[82]	Not assessed
Cyanistes caeruleus	Eurasian blue tit	Low[83,93,245]	Low,[82] Moderate,[83] High[85,245]	Not assessed
Cygnus atratus	Black swan	Low[83]	Low,[83] Moderate[85]	Not assessed
Cygnus olor	Mute swan	Not assessed	Moderate[85]	Not assessed
Dromaius novaehollandiae	Emu	Low[93,245]	Low,[82,245] High[85]	Not assessed
Egretta garzetta	Little egret	Low[93]	Not assessed	Not assessed
Empidonax traillii	Willow flycatcher	Low[83,93]	Low,[82,83] High[85]	Not assessed
Eurypyga Helias	Willow flycatcher	Low[83,93]	Low[82,83]	Not assessed
Falco cherrug	Saker falcon	Low[83,93]	Low,[82,83] Moderate[85]	Not assessed
Falco naumanni	Lesser kestrel	Not assessed	Moderate[85]	Not assessed
Falco peregrinus	Peregrine falcon	Low[93]	Not assessed	Not assessed
Falco rusticolus	Gyrfalcon	Not assessed	Moderate[85]	Not assessed
Ficedula albicollis	Collared flycatcher	Low[83,93,245]	Low[82,83,245]	Not assessed
Fulmarus glacialis	Northern fulmar	Low[83,93]	Low[82,83]	Not assessed
Gallus gallus	Red junglefowl	Low,[83,84,87,90,93,95–97,240,245,253,257] Moderate[86,98,250]	Low,[82–84,244,245,253,257] High[85]	Low[253]
Gavia stellata	Red-throated loon	Low[83,93]	Low[82,83]	Not assessed
Geospiza fortis	Medium ground finch	Low[93]	Low[82]	Not assessed
Haliaeetus albicilla	White-tailed eagle	Low[83,93]	Low,[83] Moderate[244]	Not assessed
Haliaeetus leucocephalus	Bald eagle	Low[83,93]	Low[82,83]	Not assessed
Junco hyemalis	Dark-eyed junco	Low[245]	Low[245]	Not assessed
Lepidothrix coronate	Blue-capped manakin	Low[83,93,245]	Low[82,83,245]	Not assessed
Leptosomus discolor	Cuckoo-roller	Low[93]	High[82]	Not assessed
Lonchura striata	White-rumped munia	Low[83,93,245]	Low,[82,83] Moderate,[85] High[245]	Not assessed
Manacus vitellinus	Golden-collared manakin	Low[83,93,245]	Low,[82,83,245] High[85]	Not assessed
Meleagris gallopavo	Wild turkey	Low,[83,93,245] Moderate[86,250]	Low,[83,245] High[244]	Not assessed
Melopsittacus undulatus	Budgerigar	Low[83,93,245]	Low,[82,83,245] High[85]	Not assessed
Merops nubicus	Northern carmine bee-eater	Low,[93] Moderate[83]	Low,[83] High[82]	Not assessed

Mesitornis unicolor	Brown mesite	Low[83,93]	Low,[83] High[82]	Not assessed
Molothrus ater	Brown-headed cowbird	Not assessed	High[85]	Not assessed
Motacilla alba	White wagtail	Not assessed	Moderate[85]	Not assessed
Neopelma chrysocephalum	Saffron-crested tyrant-manakin	Low[83,93]	Low,[83] High[82,85]	Not assessed
Nipponia nippon	Crested ibis	Low[83,93]	Low,[83] High[82]	Not assessed
Nothoprocta perdicaria	Chilean tinamou	Low,[83,245] Moderate[93]	Low,[82,83] Moderate,[85] High[245]	Not assessed
Numida meleagris	Helmeted guineafowl	Low[83,93,245]	Low,[82,83,245] High[85]	Not assessed
Onychostruthus taczanowskii	White-rumped snowfinch	Not assessed	Moderate[85]	Not assessed
Opisthocomus hoazin	Hoatzin	Low[83,93]	Low,[83] High[82]	Not assessed
Oxyura jamaicensis	Ruddy duck	Low[83]	Low,[83] Moderate[85]	Not assessed
Parus major	Great tit	Low[83,93,245]	Low,[82,83] High[85,245]	Not assessed
Passer montanus	Eurasian tree sparrow	Not assessed	Moderate[85]	Not assessed
Pelecanus crispus	Dalmatian pelican	Low[93]	Low[82]	Not assessed
Phaethon lepturus	White-tailed tropicbird	Low[83,93]	Low[82,83]	Not assessed
Phalacrocorax carbo	Great cormorant	Low[93]	Low[82]	Not assessed
Phasianus colchicus	Ring-necked pheasant	Low[83,93,245]	Low,[82,83,245] High[85]	Not assessed
Pipra filicauda	Wire-tailed manakin	Low[83,93]	Low,[82,83] High[85]	Not assessed
Pseudopodoces humilis	Ground tit	Low[83,93]	Low[82,83]	Not assessed
Pterocles gutturalis	Yellow-throated sandgrouse	Low[93]	Low[82]	Not assessed
Pyrgilauda ruficollis	Rufous-necked snowfinch	Not assessed	High[85]	Not assessed
Pygoscelis adeliae	Adélie penguin	Low[83,93]	Low[82,83]	Not assessed
Serinus canaria	Atlantic canary	Low,[93,245] moderate[83,84]	Low,[82–84,245] High[85]	Not assessed
Strigops habroptila	Kākāpō	Low[83,93,245]	Low,[82,83,245] High[85]	Not assessed
Struthio camelus	Common ostrich	Low[83,93,245]	Low[82,83,245]	Not assessed
Sturnus vulgaris	Common starling	Low[83,93]	Low,[83] High[82]	Not assessed
Taeniopygia guttata	Australian zebra finch	Low[83,93,245]	Low,[83,245] High[82,85]	Not assessed
Tauraco erythrolophus	Red-crested turaco	Low[93]	High[82]	Not assessed
Tinamus guttatus	White-throated tinamou	Low[93]	Low[82]	Not assessed
Tyto alba	Barn owl	Low[83,93]	Low,[82,83] High[85]	Not assessed

Zonotrichia albicollis	White-throated sparrow	Low[83,93,245]	Low,[82,83,245] Moderate[85]	Not assessed

Previous experiments determined that all avian species tested (i.e., mallard [*Anas platyrhynchos*],[293,294] swan goose [*Anser cygnoides*],[293] Japanese quail [*Coturnix japonica*],[293] *G. gallus*,[293–296] and wild turkey [*Meleagris gallopavo*][293]) could not be successfully infected with MERS-CoV or SARS-CoV-2. Exogenous expression of ACE2 from *G. gallus*[91,95,97,100] and *M. gallopavo*[100] in vitro also failed to facilitate ACE2-SARS-CoV RBD binding and viral entry. However, one in vitro study demonstrated that *G. gallus* ACE2-expressing cells did manage to support some SARS-CoV-2 replication but at a much lower rate than highly susceptible species.[100] In addition seroprevlance studies of more than 400 pintails (*Anas acuta*), Eurasian wigeon (*Anas penelope*), and *G. gallus* did not detect any MERS-CoV antibodies.[297,298] Serology and viral RNA testing also failed to detect evidence of SARS-CoV-1 infections in *A. platyrhynchos* and *G. gallus.* Together, computational modeling, experimental infection, and surveillance studies indicate that most avian species are at minimal risk for SARS-CoV-1, MERS-CoV, and SARS-CoV-2 infections. However, because of some species' close contact events with Chiropterans, researchers should consider adding screening for these coronaviruses to existing avian surveillance studies.

Mustelid Susceptibility

SARS-CoV-2 infections on fur farms of *N. vison* have been widely reported during the pandemic, and other research evidence, including computational modeling data, suggests that other mustelids may be susceptible to coronavirus infections. Computational modeling data of 14 mustelid species analyzed across 30 publications are summarized in Table 5.

Table 5: Summary of Computational Modeling Data on Mustelid Susceptibility to SARS-CoV-2

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity Evidence**	**ACE2-RBD Molecular Dynamics**
Arctonyx collaris	Greater hog badger	Moderate[96]	Not assessed	Not assessed
Lontra canadensis	North American river otter	Moderate,[83] High[259]	Low,[259] High[83,85,244]	Not assessed
Lutra lutra	Eurasian otter	Moderate[90]	Not assessed	Not assessed
Mellivora capensis	Honey badger	Moderate[93]	Not assessed	Not assessed
Melogale moschata	Chinese ferret-badger	Moderate[96]	Not assessed	Not assessed
Mustela erminea	Stoat	Moderate,[83,86,93] High[88,89,248,259]	Low,[259] High[82,83,85]	Not assessed
Mustela lutreola	European mink	Moderate,[93,247] High[248]	Moderate[247]	Not assessed
Mustela nigripes	Black-footed ferret	Moderate[93]	Not assessed	Not assessed

Mustela putorius furo	Ferret	Low,[240] Moderate,[83–85,90,92,98,245,250–253,261] High[86,88,259]	Low,[259] Moderate,[81] High[82–85,90,244,245,253,260,261]	Moderate,[255] High[253]
Neogale vison	American mink	Moderate,[85,245] High[88]	Low,[79] Moderate,[85] High[82,245]	Not assessed
Pteronura brasiliensis	Giant otter	Moderate[93]	Not assessed	Not assessed
Taxidea taxus	American badger	Moderate[93]	Not assessed	Not assessed

While *M. putorius furo* DPP4-expressing cells did not support MERS-CoV viral entry,[299] expression of ACE2 did support moderate SARS-CoV-1 binding, entry, and replication.[99,100,300] Intratracheal inoculation of *M. putorius furo* was also sufficient for symptomatic SARS-CoV-1 infection, and direct contact was sufficient to transmit SARS-CoV-1.[301,302] For SARS-CoV-2, similar in vitro overexpression experiments showed that *M. putorius furo* ACE2 supported minimal to some viral binding,[99,240,248] entry,[99,100,248] and replication.[303] Endogenous *M. putorius furo* cells supported some SARS-CoV-2 replication[304] but resulted in no cellular lysis.[304] Similarly, *N. vison* ACE2-expressing cells did not support SARS-CoV-2 binding and supported some viral entry.[248] Additional overexpression in vitro studies demonstrated moderate SARS-CoV-2 binding and viral entry in greater hog badger (*Arctonyx collaris*) and Chinese ferret-badger (*Melogale moschata*) ACE2-expressing cells.[96]

Strong in vivo evidence indicates that *M. putorius furo* are susceptible to SARS-CoV-2 infection. In vivo intranasal inoculation resulted in minimally symptomatic SARS-CoV-2 infections in *M. putorius furo* with nasal shedding.[263,294,295,305–312] In addition, these animals transmitted the infection to others via direct contact[295,309,311,312] as well as airborne respiratory droplets.[263,309,310,312] Consistent with these results, *M. putorius furo* respiratory tract tissue samples expressed both ACE2[158,303,313] and TMPRSS2.[303] Based on computational data, *M. putorius furo* only expresses full-length ACE2 and not truncated forms possibly capable of binding SARS-CoV-2 without facilitating viral infection (see “Receptors” for more detail on ACE2 isoforms).[256] Additional experimental infection and transmission experiments are needed for other mustelids to more accurately assess whether their susceptibility to these coronaviruses is similar to *M. putorius furo*.

Some mustelids have been tested for both active and previous infections of SARS-CoV-1 and SARS-CoV-2. *A. collaris* and *M. moschata* found at live markets in China tested negative for SARS-CoV-1 infection, but surveillance for SARS-CoV-1 in animals has only been performed at small scales. Seropositivity and viral RNA studies have identified active or previous SARS-CoV-2 infections in Asian small-clawed otter (*Aonyx cinereus*), *L. lutra*, *M. martes*, *M. putorius furo*, and *N. vison*, although at relatively low rates. Consistent with detection in *N. vison*, these animals also express ACE2 in the respiratory tract.[158]

A viral RNA and seropositivity study of 87 and 12 *M. meles*, respectively, found no evidence of SARS-CoV-2 infection. However, ACE2 was detected in respiratory tracts of *M. meles*, indicating risk of SARS-CoV-2 infection.[158] In addition, 57 *M. mephitis* samples, 59 European mink (*Mustela lutreola*) samples, and 2 least weasel (*Mustela nivalis*) samples all tested negative for SARS-CoV-

2 RNA. No SARS-CoV-2 antibodies were detected in 101 *M. lutreola*, 3 *M. nivalis*, and 3 *M. putorius*[2] samples. Overall, because of the susceptibility of mustelids to SARS-CoV-1 and SARS-CoV-2 as determined by computational modeling, in vitro and in vivo infection studies, and surveillance data, mustelids with habitats near known Chiropteran roosts are at risk for contracting emerging coronaviruses.

Non-Human Primate Susceptibility

Because of close evolutionary ties, non-human primate (NHP) susceptibilities to SARS-CoV-1, MERS-CoV, and SARS-CoV-2 likely reflect human susceptibilities. Computational modeling presented across 31 publications for 89 species predicted high susceptibility for most NHPs, and these data are summarized in Table 6. Great apes, lesser apes, Old World monkeys, and New World monkeys were mostly predicted as highly susceptible for SARS-CoV-2; notably, lemurs and lorises were mostly predicted as moderately susceptible.

Table 6: Summary of Computational Modeling Data on Non-Human Primate Susceptibility to SARS-CoV-2

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Great Apes				
Gorilla gorilla	Western gorilla	High[83,85,86,88,93,94,241,245,248,252,258,259]	Low,[259] High[82,83,85,239,245,260]	High[239]
Pan paniscus	Bonobo	High[83,86,88,93,241,245,248,252,259]	Low,[259,260] High[82,83,85,245]	Not assessed
Pan troglodytes	Chimpanzee	Low,[257] Moderate,[85] High[83,84,86,88,89,93,243,245,248,252,259]	Low,[259] High[82–85,243–245,257,260]	Not assessed
Pongo abelii	Sumatran orangutan	High[83–86,88,89,93,241,245,248,252,259]	Low,[259] High[82–85,245,260]	Not assessed
Lesser Apes				
Hylobates moloch	Silvery gibbon	High[83,85,88,241,252,259]	Low,[259] Moderate,[85] High[82,83]	Not assessed
Nomascus leucogenys	Northern white-cheeked gibbon	High[83,86,88,93,241,245,248,252,259]	Low,[259] High[82,83,85,245]	Not assessed
Old World				
Cercocebus atys	Sooty mangabey	High[83,86,88,93,245,248,252,259]	Low,[259] High[82,83,245]	Not assessed
Chlorocebus aethiops	Grivet	High[86,88]	High[82,85]	Not assessed
Chlorocebus sabaeus	Green monkey	High[83,86,88,93,245,248,252,259]	Low,[259] High[82,83,85,245,260]	Not assessed
Colobus angolensis	Angola colobus	High[93,245]	High[245]	Not assessed
Erythrocebus patas	Common patas monkey	High[93]	Not assessed	Not assessed
Macaca fascicularis	Crab-eating macaque	High[83,86–88,93,96,240,245,248,252,259]	Moderate,[259] High[81–83,244,245]	Not assessed

[2] European polecat, the likely ancestor of now domesticated *M. putorius furo*

Macaca mulatta	Indochinese rhesus macaque	Low,[257] Moderate,[85] High[83,84,86–90,93,94,96,98,240,243,245,248,251–253,259]	Low,[259] Moderate,[85,243] High[82–84,239,244,245,253,257,260]	High[239,253]
Macaca nemestrina	Southern pig-tailed macaque	High[83,86,88,93,245,248,252,258,259]	Low,[259] Moderate,[85] High[82,83,244,245]	Not assessed
Mandrillus leucophaeus	Drill	High[83,86,93,245,248,252,259]	Low,[259] High[82,83,245]	Not assessed
Mandrillus sphinx	Mandrill	High[88]	Not assessed	Not assessed
Nasalis larvatus	Proboscis monkey	High[93]	Not assessed	Not assessed
Papio anubis	Olive baboon	High[83,85,86,88,89,93,245,248,252,259]	Low,[259] High[82,83,85,244,245]	Not assessed
Piliocolobus tephrosceles	Ugandan red colobus	Moderate[85], High[83,88,93,245,248,252,259]	Low,[259] High[82,83,85,245]	Not assessed
Pygathrix nemaeus	Red-shanked douc	High[93]	Not assessed	Not assessed
Rhinopithecus bieti	Black-and-white snub-nosed monkey	Low,[248] High[88,259]	High[259]	Not assessed
Rhinopithecus roxellana	Golden snub-nosed monkey	High[83,85,88,89,93,245,248,252,259]	Low,[259] High[83,85,245]	Not assessed
Theropithecus gelada	Gelada	High[83,85,88,93,245,248,252,259]	Low,[259] High[82,83,85,245]	Not assessed
Trachypithecus francoisi	Francois' leaf monkey	High[259]	Low[259]	Not assessed
New World				
Alouatta palliata	Mantled howler monkey	High[93,252]	Not assessed	Not assessed
Aotus nancymaae	Nancy Ma's night monkey	High[83,86,88,93,245,248,252,259]	Low,[259] Moderate,[83,85] High[82,245]	Not assessed
Ateles geoffroyi	Black-handed spider monkey	High[93]	Not assessed	Not assessed
Callicebus donacophilus	White-eared titi monkey	High[93]	Not assessed	Not assessed
Callithrix jacchus	Common marmoset	Moderate,[85] High[83,86,88,89,93,245,248,251,252,259]	Low,[259] Moderate,[83,85] High[82,245]	Not assessed
Cebus capucinus	Columbian white-faced capuchin	High[86,88,93,245,248,252]	High[82,245]	Not assessed
Cebus imitator	Panamanian white-faced capuchin	High[259]	Low,[259] Moderate[85]	Not assessed
Pithecia pithecia	White-faced saki	High[93]	Not assessed	Not assessed
Saguinus imperator	Emperor tamarin	High[93]	Not assessed	Not assessed
Saimiri boliviensis	Black-capped squirrel monkey	High[83,86,88,93,245,248,252,259]	Low,[259] Moderate,[83,85] High[82,245]	Not assessed
Sapajus apella	Tufted capuchin	High[83,88,93,248,252,259]	Low,[259] Moderate,[83,85] High[82]	Not assessed
Lemurs and Lorises				

Arctocebus calabarensis	Calabar angwantibo	Moderate[254]	Moderate[254]	Not assessed
Avahi laniger	Woolly indri	High[254]	High[254]	Not assessed
Avahi peyrierasi	Peyrieras's woolly lemur	High[254]	High[254]	Not assessed
Cheirogaleus major	Greater dwarf lemur	Moderate[254]	Low[254]	Not assessed
Cheirogaleus medius	Fat-tailed dwarf lemur	Moderate,[254] High[93]	Low[254]	Not assessed
Daubentonia madagascariensis	Aye-aye	High[93,252,254]	High[254]	Not assessed
Eulemur albifrons	White-headed lemur	High[254]	Moderate[254]	Not assessed
Eulemur collaris	Collared brown lemur	High[254]	Moderate[254]	Not assessed
Eulemur coronatus	Crowned lemur	High[254]	Moderate[254]	Not assessed
Eulemur flavifrons	Blue-eyed black lemur	High[93,252,254]	Moderate[254]	Not assessed
Eulemur fulvus	Common brown lemur	High[254]	Moderate[254]	Not assessed
Eulemur macaco	Black lemur	High[254]	Moderate[254]	Not assessed
Eulemur mongoz	Mongoose lemur	High[254]	Moderate[254]	Not assessed
Eulemur rubriventer	Red-bellied lemur	High[254]	Moderate[254]	Not assessed
Eulemur rufus	Red lemur	High[254]	Moderate[254]	Not assessed
Eulemur sanfordi	Sanford's brown lemur	High[254]	Moderate[254]	Not assessed
Hapalemur alaotrensis	Lac Alaotra bamboo lemur	High[254]	Moderate[254]	Not assessed
Hapalemur gilberti	Bamboo lemur	High[254]	Moderate[254]	Not assessed
Hapalemur griseus	Eastern lesser bamboo lemur	High[254]	Moderate[254]	Not assessed
Hapalemur meridionalis	Southern lesser bamboo lemur	High[254]	Moderate[254]	Not assessed
Hapalemur occidentalis	Western lesser bamboo lemur	High[254]	Moderate[254]	Not assessed
Indri indri	Indri	High[93]	Not assessed	Not assessed
Lemur catta	Ring-tailed lemur	High[254]	Moderate[254]	Not assessed
Lepilemur ankaranensis	Ankarana sportive lemur	Moderate[254]	Moderate[254]	Not assessed
Lepilemur dorsalis	Gray-backed sportive lemur	Moderate[254]	Moderate[254]	Not assessed
Lepilemur ruficaudatus	Red-tailed sportive lemur	Moderate[254]	Moderate[254]	Not assessed

Lepilemur septentrionalis	Northern sportive lemur	Moderate[254]	Moderate[254]	Not assessed
Loris lydekkerianus	Gray slender loris	Moderate[254]	Moderate[254]	Not assessed
Loris tardigradus	Red slender loris	Moderate[254]	Moderate[254]	Not assessed
Microcebus murinus	Gray mouse lemur	Low,[248] Moderate,[83,93,245,254] High[252,259]	Low,[259] Moderate,[83,254] High[245]	Not assessed
Mirza coquereli	Coquerel's giant mouse lemur	High[93]	Not assessed	Not assessed
Mirza zaza	Northern giant mouse lemur	Moderate[254]	Moderate[254]	Not assessed
Nycticebus bengalensis	Bengal slow loris	Moderate[254]	Moderate[254]	Not assessed
Nycticebus pygmaeus	Pygmy slow loris	Moderate[254]	Moderate[254]	Not assessed
Otolemur crassicaudatus	Brown greater galago	Moderate[254]	Moderate[254]	Not assessed
Otolemur garnetti	Northern greater galago	Low,[248] Moderate,[83,86,93,245,252,254] High[259]	Low,[259] Moderate,[83,254] High[82,85,245]	Not assessed
Otolemur Monteiro	Silvery greater galago	Moderate[254]	Moderate[254]	Not assessed
Perodicticus ibeanus	East African potto	Moderate[254]	Moderate[254]	Not assessed
Perodicticus potto	Potto	Moderate[254]	Moderate[254]	Not assessed
Protolemur simus	Greater bamboo lemur	High[245,254]	Moderate,[254] High[245]	Not assessed
Propithecus coquereli	Coquerel's sifaka	High[83,86,88,93,245,248,252,254,259]	Low,[259] High[82,83,245,254]	Not assessed
Propithecus coronatus	Crowned sifaka	High[254]	High[254]	Not assessed
Propithecus diadema	Diademed sifaka	High[254]	High[254]	Not assessed
Propithecus edwardsi	Milne-Edwards's sifaka	High[254]	High[254]	Not assessed
Propithecus perrieri	Perrier's sifaka	High[254]	High[254]	Not assessed
Propithecus tattersalli	Golden-crowned sifaka	High[254]	High[254]	Not assessed
Propithecus verreauxi	Verreaux's sifaka	High[254]	High[254]	Not assessed
Varecia rubra	Red ruffed lemur	High[254]	Moderate[254]	Not assessed
Varecia variegata	Black-and-white ruffed lemur	High[254]	Moderate[254]	Not assessed
Other				

Carlito syrichta	Philippine tarsier	Low,[248] Moderate,[245] High[83,88,89,252,259]	Low,[259] Moderate,[83] High[82,85,245]	Not assessed
Galago moholi	Mohol bushbaby	Moderate[254]	Moderate[254]	Not assessed
Galago senegalensis	Senegal bushbaby	Moderate[254]	Moderate[254]	Not assessed
Galagoides demidovii	Prince Demidoff's bushbaby	Moderate[254]	Moderate[254]	Not assessed

Numerous NHP species have been tested in vitro for viral susceptibility. Grivet (*Chlorocebus aethiops*)-derived cells supported lytic viral replication of SARS-CoV-1,[155,156,314] MERS-CoV entry and replication,[108,315] and SARS-CoV-2 replication,[100] indicating susceptibility to all three viruses. Rhesus macaque (*Macaca mulatta*)-derived cells supported viral entry of SARS-CoV-1[156] as well as lytic cell replication of SARS-CoV-2.[304] Exogenous expression of *M. mulatta* ACE2 also supported binding, entry, and replication of SARS-CoV-2.[96,304] The ACE2 of another closely related species, crab-eating macaque (*Macaca fascicularis*), also supported binding and entry of SARS-CoV-1 and SARS-CoV-2.[91,248] Additional screening of NHP ACE2 proteins demonstrated that the following species supported SARS-CoV-2 binding and entry: *G. gorilla*, northern white-cheeked gibbon (*Nomascus leucogenys*), chimpanzee (*Pan troglodytes*), olive baboon (*Papio anubis*), Ugandan red colobus (*Piliocolobus tephrosceles*), Sumatran orangutan (*Pongo abelii*), golden snub-nosed monkey (*Rhinopithecus roxellana*), and gelada (*Theropithecus gelada*). Additional studies are required to further explore the susceptibility of great apes to SARS-CoV-2; preliminary studies identified TMPRSS2 and ACE2 expression in *P. abelii* respiratory tract,[303] but not in the respiratory tract of *G. gorilla*.[158] Notably, based on sequence data, *P. abelii*—and other NHPs (i.e., *M. fascicularis, M. mulatta,* drill [*Mandrillus leucophaeus*], bonobo [*Pan paniscus*], chimpanzee [*Pan troglodytes*], and *R. roxellana*)—ACE2 isoforms all support SARS-CoV-2 infection.[256] Conversely, ACE2 from *C. jacchus*, black-capped squirrel monkey (*Saimiri boliviensis*), and tufted capuchin (*Sapajus apella*) did not support SARS-CoV-2 binding or entry.[248] Notably, all NHP species that supported SARS-CoV-2 binding and entry are New World primates and great apes, while the species that did not support binding and entry are Old World primates.

In vivo experimental infection experiments largely agree with in vitro infection results. The following species experienced symptomatic SARS-CoV-2 infections after combination inoculation (e.g., intratracheal plus intranasal, intratracheal plus intranasal plus conjunctival, etc.): *C. aethiops*,[316–318] *M. fascicularis*,[319,320] *M. mulatta*,[316,318,319,321] and southern pig-tailed macaque (*Macaca nemestrina*).[318] Although computational modeling predicted *Callithrix* and *Saimiri* species as highly susceptible, both *C. jacchus*[319] and *S. sciureus*[318] were mostly asymptomatic after experimental infection with minimal viral shedding. Consistent with these lack of symptoms, *C. jacchus* ACE2 expression was absent from the respiratory tract but detectable in the small intestine.[158] Both *C. aethiops* and *M. mulatta* are also susceptible to aerosol-based SARS-CoV-2.[316] Notably, the degree of viral shedding does not always correlate with severity of symptoms. *M. fascicularis* experienced asymptomatic infections after combination inoculation with SARS-CoV-1 or MERS-CoV. These monkeys seroconverted for both

viruses, but these antibodies were not always protective against reinfection with SARS-CoV-1.[322,323 324] *M. mulatta* was susceptible to MERS-CoV combination inoculation but experienced no symptoms following combination inoculation with SARS-CoV-1. Monkeys infected with MERS-CoV or SARS-CoV-1 seroconverted, but this was not protective against SARS-CoV-1 reinfection.[322–324] *C. aethiops* also remained asymptomatic after infection with SARS-CoV-1 but did seroconvert.[324]

Some NHPs have been tested for active and previous SARS-CoV-2 coronaviruses. No evidence of natural active or previous infections of SARS-CoV-2 was detected in *Alouatta palliata*,[325] *C. jacchus*,[325] collared mangabey (*Cercocebus torquatus*),[326] *M. mulatta,*[327] *P. troglodytes*,[326] and pied tamarin (*Saguinus bicolor*).[325] However, some captive NHPs have been naturally infected with SARS-CoV-2, including sun-tailed monkey (*Allochrocebus solatus*),[326] *G. gorilla*, mandrill (*Mandrillus sphinx*), and Guianan squirrel monkey (*Saimiri sciureus*).[328]

Farmed Ungulate Susceptibility

Depending on farm location, farmed ungulates can be at risk for spillovers from Chiropterans, other wild animals, and even farmers. The computational modeling data for 13 farmed ungulates are summarized in Table 7.

Table 7: Summary of Computational Modeling Data for Farmed Ungulates

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Bos grunniens	Domestic yak	Moderate[245]	High[245]	Not assessed
Bos indicus	Zebu	High[83,87,93,248,259]	High[82,83,244,259]	Not assessed
Bos taurus	Domestic cattle	Low,[240,257] Moderate,[85,245,246] High[80,83,84,86,87,89,90,93,95–97,242,243,248,250,253,259]	Low,[81,84] Moderate,[85,239,253] High[80,82,83,85,90,243–245,257,259,260]	Moderate[239,253]
Bubalus bubalis	Domestic water buffalo	Moderate,[246] High[83,93,248,259]	Low,[82] High[83,244,259,260]	Not assessed
Camelus bactrianus	Bactrian camel	Low,[248] High[83,93,95–97,253,259]	Low,[82,253,259] Moderate,[83] High[244]	High[253]
Camelus dromedarius	Dromedary camel	Low,[89,248] Moderate,[241,245,246] High[83,84,86,93,253,259]	Low,[82,84,253,259] Moderate,[83] High[85,244,245]	Moderate[253]
Capra hircus	Goat	Low,[240] Moderate,[245,246] High[83,84,86,87,90,93,95–97,248,253,259]	Low,[84,259] High[82,83,90,244,245,253,260]	High[253]
Equus asinus	Donkey	Low,[240] Moderate,[83,93,95,97,245,246] High[84,248,259]	Low,[82,259] High[83,84,244,245]	Not assessed
Equus caballus	Horse	Low,[240] Moderate,[83,85,86,90,95,97,241,243,245,246] High[80,84,87–89,93,96,248,253,259]	Low,[80,82,243,259] Moderate,[81,85,253] High[83,84,90,244,245]	High[253]
Odocoileus virginianus	White-tailed deer	High[93,250,259]	Low,[259] High[82,83,85,257]	Not assessed
Ovis aries	Sheep	Low,[240,257] Moderate,[85,245,246] High[80,83,84,86,87,89,90,93,95–97,241,243,248,253,259]	Low,[243,244,259] Moderate,[80,85,239] High[82–84,90,245,253,257,260]	Moderate,[239] High[253]

Sus scrofa	Pig	Low,[240,246,257] Moderate,[85,90,98,241,245] High[80,83,84,86,87,89,93,96,243,248,250–253,259]	Low,[80,84,243,244,259] Moderate,[85,239] High[82,83,90,245,253,257,260]	Moderate[239,253]
Vicugna pacos	Alpaca	Low,[248] Moderate,[90,241] High[83,88,93,96,250,259]	Low,[82,259] Moderate,[83] High[85]	Not assessed

Overexpression of ACE2 proteins from different farmed ungulates identified that following species as potentially susceptible to SARS-CoV-1 based on ACE2 binding and entry: *B. taurus*,[97] Bactrian camel (*Camelus bactrianus*),[91,95] *C. hircus*,[91,95,97,100] *E. caballus*,[91,95,97,100,329] *O. aries*,[91,95,97,100] *S. scrofa*,[91,99,100,314] and alpaca (*Vicugna pacos*).[91] Notably, *E. asinus* ACE2 did not support SARS-CoV-1 binding or entry,[95,97] while *S. scrofa*-derived cells with endogenous ACE2 supported viral replication.[155,156] In addition, *C. dromedarius*-derived[315] and *E. caballus*-derived cells[330] both supported MERS-CoV infection, while *O. aries*-derived cells did not.[156,315]

Exogenous and endogenous in vitro results were inconsistent for some farmed ungulate species (i.e., *B. taurus*, *C. hircus*, *E. caballus*, *O. aries*, *S. scrofa*, *V. pacos*). Overexpression of species-specific ACE2 mostly indicated susceptibility, while species-derived cell culture models mostly showed minimal susceptibility. Importantly, results for white-tailed deer (*Odocoileus virginianus*) were consistent across both experimental contexts. These differing results are summarized in Table 8. Because in vivo experimental infection studies of *B. taurus*[331] and *S. scrofa*[294,295,311,332,333] indicated low susceptibility as well, using species-derived cell culture models to assess susceptibility may provide results more relevant to in vivo contexts.

Table 8: Summary of In Vitro Data for Farmed Ungulates

	Exogenous Results			**Endogenous Results**		
Species	**ACE2 Binding**	**Viral Entry**	**Viral Replication**	**ACE2 Binding**	**Viral Entry**	**Viral Replication**
Bos taurus	High[91,97,240,248]	High[91,95,97,100,248]	Moderate[303]	Not assessed	Not assessed	None,[100] Moderate[304]
Camelus bactrianus	Moderate[91,97]	Moderate[91,95,97]	Low[303]	Not assessed	Not assessed	Low[304]
Capra hircus	High[91,97,248]	High[91,95,97,100,248,334,335]	Not assessed	Not assessed	Not assessed	None[304]
Equus asinus	None[95,97]	None[95,97]	Not assessed	Not assessed	Not assessed	Not assessed
Equus caballus	High[91,97,248,329]	Moderate[91,95,97,100,248,334]	Not assessed	Not assessed	Not assessed	None[100]
Odocoileus virginianus	Not assessed	High[336]	Not assessed	Not assessed	Not assessed	High[337]
Ovis aries	High[91,97,248]	High[91,95,97,100,248]	Not assessed	Not assessed	Not assessed	None[100]
Sus scrofa	High[91,99,248]	High[91,99,100,248,335]	High[100]	Not assessed	Not assessed	None,[303] Moderate[100,304]
Vicugna pacos	Moderate[91]	Moderate[91]	Not assessed	Not assessed	Not assessed	Low[303]

In vivo expression studies identified the following farmed ungulates as having ACE2 and TMPRSS2 expression in respiratory tracts: *B. taurus*,[158,303,313] *C. bactrianus*,[303] *C. hircus*,[303,338] and *O. aries*.[158,303] Studies of ACE2 expression in *E. caballus*[158,303] and *V. pacos*[158,303] obtained mixed results of ACE2 in the respiratory tract, and *S. scrofa* contains low ACE2 expression in respiratory organs. Notably, *E. caballus* and *S. scrofa*[158] also express ACE2 in the small intestine, suggestive of potential gastrointestinal symptoms and fecal shedding.

Most experimental infection studies in farmed ungulates focused on MERS-CoV. As expected, *C. dromedarius* can be infected with MERS-CoV intranasal inoculation,[339] which correlates well with high expression of the viral receptor DPP4 in the upper respiratory tract.[340] Although no studies have identified natural MERS-CoV infections in *C. bactrianus*, these camels can also be intranasally infected.[341] After intranasal inoculation, *E. caballus* and *O. aries* experience minimal symptoms and viral shedding that do not result in seroconversion.[342,343] *C. hircus*,[342] *S. scrofa*,[343,344] and *V. pacos*[339,345,346] also experience minimal symptoms from intranasal inoculation, but these animals develop MERS-CoV antibodies. One experimental infection study of farmed ungulates for SARS-CoV-1 demonstrated that only combination inoculation including the intravenous route resulted in seroconversion without symptoms.[296]

Recent surveillance studies have detected evidence of SARS-CoV-2 in *O. virginianus.* Experimental infection studies have demonstrated that *O. virginianus* can be infected intranasally, resulting in subclinical infection and seroconversion.[336,337,347] In addition, these animals can transmit SARS-CoV-2 via close contact[347] and can transmit the virus to fetuses.[337] In agreement with these experimental studies, surveillance studies have identified evidence of active and past SARS-CoV-2 infections in *O. virginianus* in Illinois,[348] Iowa,[349] Michigan,[348] New York,[348,350] Ohio,[351,352] Pennsylvania,[348,353] South Carolina,[354] and Texas.[355–357] Both free-roaming and captive *O. virginianus* tested positive for current or previous SARS-CoV-2 infections,[349,355] and further study of viral sequences isolated from Ohio identified multiple likely human-to-deer spillover events as well as deer-to-deer transmission.[351,352,358] In addition, further sequence analysis demonstrated the SARS-CoV-2 evolutionary rate in *O. virginianus* is three times faster than the rate in humans with different mutational biases and selection pressures, suggesting that *O. virginianus* may become a significant SARS-CoV-2 reservoir. Although no phenotypic changes were observed for SARS-CoV-2 in *O. virginianus*, the faster evolutionary rate may result in phenotypic changes with significant impacts on virulence and species tropism.[352] Additional surveillance studies have identified evidence of SARS-CoV-2 in *C. hircus* and *O. aries* at very low rates in Italy[359] as well as seropositivity in *S. scrofa* in one animal in Gabon and six animals in Croatia.[326,360] Consistent with these low infection rates, *C. hircus* ACE2 has two isoforms, only one of which can support SARS-CoV-2 infection. Similarly, *S. scrofa* ACE2 has three isoforms, but only one can fully bind and facilitate SARS-CoV-2 infection.[90,256]

While only one study identified SARS-CoV-1 antibodies in *S. scrofa* but not *B. taurus* in China,[154] numerous MERS-CoV surveillance studies have been conducted in farmed ungulates in mostly Middle Eastern regions. For a summary of MERS-CoV surveillance studies in *C. dromedarius*, see "Dromedaries." Notably, some farmed ungulates positive for MERS-CoV RNA or antibodies were housed in close proximity with *C. dromedarius*, suggesting transmission from infected *C.*

dromedarius. Neutralizing antibodies for MERS-CoV in *B. taurus* were identified in Egypt,[361] but not in Jordan,[362] Netherlands,[363] Saudi Arabia,[298] Tunisia,[361] and United Arab Emirates (UAE).[364] and In *C. bactrianus*, evidence of MERS-CoV neutralizing antibodies was only found in China,[365] but not Japan,[366] Kazakhstan,[367] and Mongolia.[368] Neutralizing antibodies in *C. hircus* were detected in Egypt and Tunisia, and one active infection was found in Senegal;[361] however, no evidence of current or previous MERS-CoV infections in *C. hircus* was found in Jordan,[362] Netherlands,[363] Saudi Arabia,[68,298] Spain,[363] and UAE.[364] Only one active infection in *E. asinus* was detected in Egypt,[361] but no evidence of infection was found in Spain[330] and Tunisia.[361] One *E. caballus* animal had neutralizing antibodies in Tunisia,[361] but no other evidence of infection was found in Egypt,[60,361] Spain,[330] and UAE.[330] *Ovis aries* animals in Egypt,[60,361] Jordan,[362] Senegal,[361] Tunisia,[361] and UAE[364] had very low rates of current or past MERS-CoV infections, while *O. aries* in Netherlands[363] and Saudi Arabia[68,298] were negative for MERS-CoV infections and antibodies. In addition, *V. pacos* in Israel[369] and Qatar[370] had MERS-CoV neutralizing antibodies, while *S. scrofa* in China showed no evidence of current or past MERS-CoV infections.[297]

Interactions with Humans

Humans that contract zoonotic viruses can transmit them to other animals as well. Multiple reports have shown that Chiropterans have transmitted to humans Henipavirus in Cameroon,[371] Melaka and Pulau viruses in Malaysia,[372] and Filovirus in India.[373] Human–Chiropteran interactions facilitated these zoonoses, and emerging coronaviruses could also transmit from Chiropteran to human. In addition, avian species have transmitted multiple avian influenza subtype A viruses to humans,[374] and *C. dromedarius* have transmitted MERS-CoV to humans.[375] Reverse zoonoses can also occur when infected humans are in close contact with naïve animals. Examples of reverse zoonoses of respiratory viruses include H1N1 transmission to farmed *S. scrofa*,[376–378] companion *M. putorius furo*,[379] and farmed *M. gallopavo*;[380] metapneumovirus to wild *P. troglodytes*;[381] and adenoviruses to captive *M. fascicularis*, *M. mulatta,* and mantled guereza (*Colobus guereza*).[382] The susceptibilities of animals with close contacts to humans (i.e., captive animals, companion animals, and other wildlife) to SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are outlined below, and susceptibilities of farmed animals are summarized in "Interactions with Chiropterans."

Companion Animal Susceptibility

Companion animals that spend time outdoors are at risk for close Chiropteran contacts, especially if left unattended, and they are also at risk because of close contact with humans. Common companion animal species include *C. lupus familiaris*, *F. catus*, birds, and small mammals (e.g., rodents, rabbits [*Oryctolagus cuniculus*], *M. putorius furo*). Susceptibilities of *C. lupus familiaris*, *F. catus*, and *O. cuniculus* are described in this section, while birds (see "Avian Susceptibility"), rodents (see "Rodent Susceptibility"), and *M. putorius furo* (see "Mustelid Susceptibility") are described elsewhere. Computational modeling data for *C. lupus familiaris*, *F. catus*, and *O. cuniculus* are summarized in Table 9.

Table 9: Summary of Computational Modeling Data for Select Companion Animals

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Canis lupus familiaris	Dog	Low,[240,249,257] Moderate,[85,94,241,246,252,258,261] High[80,83,84,86–90,93,96,243,245,248,250,253,259]	Low,[243,249,253,259–261] Moderate,[80,239] High[81–85,89,90,244,245,257]	Low,[249,253] Moderate,[255] High[239]
Felis catus	Cat	Low,[240,257] Moderate,[241,246,249] High[80,83–98,243,245,248,250,252,253,258,259,261]	Low,[257,259] Moderate,[81,239,249,253] High[80,82–85,89,90,243–245,260–262]	Low,[249,253] Moderate,[239,255] High[262]
Oryctolagus cuniculus	Rabbit	Low,[240,250,257] Moderate,[85,241,246] High[80,83,84,86–90,93–97,243,245,248,251,259]	Low,[82,243,259] Moderate,[80,85] High[83,84,244,245,257,260]	Not assessed

In vitro experimental infection data indicate potential companion animal susceptibilities to some emerging coronaviruses. Exogenous expression of *C. lupus familiaris* ACE2 supported moderate to high viral binding and high viral entry of both SARS-CoV-1[91,100] and SARS-CoV-2.[91,96,100,248,335] In vitro studies of *C. lupus familiaris*-derived cells resulted in mixed results of either no SARS-CoV-2 replication[100] or some replication.[303,304] In addition, overexpression of *F. catus* ACE2 resulted in moderate to high viral binding and entry but no viral replication of both SARS-CoV-1[91,95,97,100,314,383] and SARS-CoV-2.[91,95–97,100,240,248,334,335,384] However, *F. catus*-derived cells did support SARS-CoV-2 replication that resulted in cell lysis.[304] For *O. cuniculus*, overexpression of ACE2 supports high viral binding and entry for SARS-CoV-1[91,95,97,100,314] and SARS-CoV-2, but replication only occurred for SARS-CoV-2.[91,95–97,100,248] *O. cuniculus*-derived cells supported MERS-CoV[385] infection as well as some SARS-CoV-2 replication.[304]

Some viral receptor and protease expression studies detected ACE2 and TMPRSS2 in the respiratory tracts of *C. lupus familiaris*,[303,313,338,386] *F. catus*,[158,313,338] and *O. cuniculus*.[303] However, other studies failed to detect expression of these genes in *C. lupus familiaris*[158] and *O. cuniculus*[313,338] respiratory tracts. *C. lupus familiaris* ACE2 isoforms include one truncated isoform that may actually block ACE2-SARS-CoV-2 binding, impeding infection, while all isoforms predicted for *F. catus* and *O. cuniculus* ACE2 can facilitate SARS-CoV-2 infection.[90,256] In addition, ACE2 was detected in *F. catus* digestive tracts, suggesting potential gastrointestinal symptoms and fecal shedding.[159] Consistent with these expression data, experimental infection with SARS-CoV-2 resulted in seroconversion in *C. lupus familiaris*,[294,387] *F. catus*,[294,387–391] and *O. cuniculus*.[392] In addition, *O. cuniculus* can be infected with MERS-CoV via combination inoculation, resulting in viral shedding and seroconversion.[385] *F. catus* can also be infected with SARS-CoV-1 via intratracheal inoculation,[301,302] and similar to SARS-CoV-2,[387,390,391] *F. catus* can transmit SARS-CoV-1 via direct contact.[302]

Consistent with experimental infection results, surveillance studies have found evidence of natural SARS-CoV-2 infections in *C. lupus familiaris*, *F. catus*, and *O. cuniculus*. Evidence of active or previous SARS-CoV-2 infections in *C. lupus familiaris* were reported in Croatia,[393,394]

Ecuador,[395] Egypt,[396] Poland,[397] France,[398] Gabon,[326] Italy,[399–401] Spain,[402–404] Thailand,[405] and United States,[406–408] but not in France,[409] Indonesia,[410] Mexico,[411] or Turkey.[412] For *F. catus*, SARS-CoV-2 infections were detected in Colombia,[413] China,[414,415] Croatia,[393] Ecuador,[395] Egypt,[396] France,[398,409,416] Germany,[417,418] Iran,[419] Israel,[420] Italy,[399,401,421] Mexico,[411] Peru,[422] Poland,[397] Spain,[404,423,424] Switzerland,[425] United States,[406–408] Thailand,[405] and Turkey,[426] but not Gabon.[326]. Multiple case study reports detailed evidence of human-to-animal transmission events for both *C. lupus familiaris*[396,407,427–433] and *F. catus*,[395,407,416,425,428,431,434–442] and one study provided evidence of potential transmission from *M. putorius furo*.[126] Fewer surveillance studies were performed for *O. cuniculus*, but one such study identified a very low rate of seropositivity in France.

Rodent Susceptibility

A diverse set of rodents were analyzed for susceptibility to SARS-CoV-1, MERS-CoV, and SARS-CoV-2. Importantly, different rodent species interact with humans in multiple ways as companion animals, research subjects, and wildlife encounters. Computational modeling data for 59 rodent species from 41 articles are summarized in Table 10.

Table 10: Summary of Computational Modeling Data for Rodent Species

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Acomys cahirinus	Cairo spiny mouse	Moderate[93]	Not assessed	Not assessed
Allactaga bullata	Gobi jerboa	High[93]	Not assessed	Not assessed
Arvicanthis niloticus	African grass rat	Moderate,[83] High[259]	Low,[83,259] High[85]	Not assessed
Arvicola amphibius	European water vole	High[259]	Low,[259] Moderate[85]	Not assessed
Cavia porcellus	Guinea pig	Low,[89,248] Moderate,[93,95–97,245,259] High[84,86,87]	Low,[82] High[84,245,259]	Not assessed
Cavia tschudii	Montane guinea pig	Moderate[93]	Not assessed	Not assessed
Chinchilla lanigera	Long-tailed chinchilla	Low,[248,257] Moderate,[245] High[83,86,88,93,259]	Low,[257,259,260] Moderate,[83] High[82,245]	Not assessed
Cricetomys gambianus	Gambian pouched rat	High[93]	Not assessed	Not assessed
Cricetulus griseus	Chinese hamster	Low,[240] High[83,85,86,88,89,92,93,245,248,258,259,261]	Low,[259,261] High[82,83,85,89,244,245]	Not assessed
Ctenodactylus gundi	Common gundi	High[93]	Not assessed	Not assessed
Ctenomys sociabilis	Social tuco-tuco	Moderate[93]	Not assessed	Not assessed
Cynomys marmota	Prairie dog	Low[257]	High[257]	Not assessed
Dasyprocta punctata	Central American agouti	Moderate[93]	Not assessed	Not assessed
Dipodomys ordii	Ord's kangaroo rat	Low,[89,248] High[83,86,88,93,245,259]	Low,[82,259] Moderate,[83] High[245]	Not assessed
Dipodomys spectabilis	Banner-tailed kangaroo rat	Not assessed	High[85]	Not assessed

Dipodomys stephensi	Stephens's kangaroo rat	High[93]	Not assessed	Not assessed
Dolichotis patagonum	Patagonian mara	Moderate[93]	Not assessed	Not assessed
Ellobius lutescens	Transcaucasian mole vole	High[93]	Not assessed	Not assessed
Fukomys damarensis	Damara mole-rat	Low,[248] Moderate,[245] High[83,88,93,259]	Low,[259] High[79,82,83,85,245]	Not assessed
Grammomys surdaster	African woodland thicket rat	Low,[248] Moderate,[83,93] High[259]	Low,[83,259] High[82,85]	Not assessed
Graphiurus murinus	Woodland dormouse	High[93]	Not assessed	Not assessed
Heterocephalus glaber	Naked mole-rat	Low,[248] Moderate,[245] High[83,86,88,89,93,259]	Low,[259] High[82,83,85,245,260]	Not assessed
Hydrochoerus hydrochaeris	Capybara	High[93]	Not assessed	Not assessed
Hystrix cristata	Crested porcupine	High[93]	Not assessed	Not assessed
Ictidomys tridecemlineatus	Thirteen-lined ground squirrel	Low,[248] Moderate,[245] High[83,86,88,89,93,259]	Low,[82,259] High[83,85,245]	Not assessed
Jaculus jaculus	Lesser Egyptian jerboa	Moderate,[245] High[83,88,93,248,259]	Low,[259] High[82,83,245]	Not assessed
Marmota flaviventris	Yellow-bellied marmot	Low,[248] High[83,88,93,259]	Low,[82,259] High[83,85]	Not assessed
Marmota marmota	Alpine marmot	Low,[248] Moderate,[245] High[83,88,93,259]	Low,[82,259] High[83,245]	Not assessed
Mastomys coucha	Southern multimammate mouse	Low,[248] Moderate,[83,93] High[259]	Low,[82,83,259] Moderate[85]	Not assessed
Meriones unguiculatus	Mongolian gerbil	Moderate[93]	Not assessed	Not assessed
Mesocricetus auratus	Golden hamster	Low,[240] Moderate,[85] High[83,84,86–89,92,93,241,245,247,248,251,253,259]	Low,[259] High[81–85,245,247,253,260]	Moderate[253]
Microtus ochrogaster	Prairie vole	Low,[248] Moderate,[92,245] High[83,88,93,259]	Moderate,[79] High[82,83,85,245,259]	Not assessed
Microtus oregoni	Creeping vole	High[259]	Moderate,[85] High[259]	Not assessed
Mus caroli	Ryukyu mouse	Low,[245,248] Moderate,[83,93] High[259]	Low,[79,82,83,245] High[85,259]	Not assessed
Mus musculus	House mouse	Low,[89,240,245,246,248,257] Moderate,[80,83,84,86,87,90–98,243,250,251,253,258] High[259]	Low,[79–84,239,243–245,257,262] Moderate,[253] High[85,90,259,260]	Low,[239,262] Moderate[253]
Mus pahari	Gairdner's shrewmouse	Low,[245,248] Moderate,[83,93] High[259]	Low,[83,245,259] Moderate,[79] High[82,85]	Not assessed
Mus spicilegus	Steppe mouse	Low[245]	Low[245]	Not assessed
Mus spretus	Algerian mouse	Low,[245] Moderate[93]	High[245]	Not assessed
Myocastor coypus	Nutria	Moderate[93]	Not assessed	Not assessed
Nannospalax ehrenbergi	Middle East blind mole-rat	High[245]	High[245]	Not assessed
Nannospalax galili	Upper Galilee mountains blind mole rat	High[259]	Low[259]	Not assessed
Octodon degus	Common degu	Low,[248] Moderate,[93,245] High[83,88,259]	Low,[259,260] Moderate,[83] High[82,245]	Not assessed
Ondatra zibethicus	Muskrat	High[93]	Not assessed	Not assessed

Onychomys torridus	Southern grasshopper mouse	High[259]	Low,[259] High[85]	Not assessed
Perognathus longimembris	Little pocket mouse	High[93]	Not assessed	Not assessed
Peromyscus leucopus	White-footed mouse	High[83,85,92,93,248,259]	Moderate,[85] High[82,83,259]	Not assessed
Peromyscus maniculatus	Deer mouse	Moderate,[245] High[83,85,88,92,93,248,259]	Low,[259] High[82,83,85,245]	Not assessed
Petromus typicus	Dassie rat	Moderate[93]	Not assessed	Not assessed
Phodopus campbelli	Campbell's dwarf hamster	High[86,89,241]	High[82]	Not assessed
Phodopus roborovskii	Roborovski dwarf hamster	High[247]	High[247]	Not assessed
Psammomys obesus	Fat sand rat	Moderate[93]	Not assessed	Not assessed
Rattus norvegicus	Brown rat	Low,[89,245,248] Moderate,[80,83,86,90,91,93–97,250,251] High[84,259]	Low,[79,80,82–84,239,244,245,259] High[85]	Low[239]
Rattus rattus	Black rat	Low,[246] Moderate,[83,98] High[259]	Low[83,259]	Not assessed
Rhizomys pruinosus	Hoary bamboo rat	High[79]	Not assessed	Not assessed
Spermophilus dauricus	Daurian ground squirrel	Moderate,[245] High[93]	High[245]	Not assessed
Thryonomys swinderianus	Greater cane rat	Moderate[93]	Not assessed	Not assessed
Urocitellus parryii	Arctic ground squirrel	Low,[248] Moderate,[85,245] High[83,88,93,259]	Low,[82,259] Moderate,[85] High[83,245]	Not assessed
Zapus hudsonius	Meadow jumping mouse	Moderate[93]	Not assessed	Not assessed

In vitro experimental data using rodent ACE2 and rodent-derived cell culture models indicate a diverse set of susceptibilities across species to SARS-CoV-1, MERS-CoV, and SARS-CoV-2. For guinea pigs (*Cavia porcellus*), overexpression of DPP4 supported only minimal MERS-CoV entry,[299] and overexpression of ACE2 resulted in minimal to no viral binding and moderate to no viral entry for both SARS-CoV-1 and SARS-CoV-2,[91,95,97,99] indicating a general lack of susceptibility of this species to these coronaviruses. A similar lack of susceptibility was concluded for brown rats (*Rattus norvegicus*) for SARS-CoV-1 and SARS-CoV-2 as well.[96,97,99,100] In vitro results for *M. musculus* viral receptor overexpression studies provided varied results. Although DPP4 overexpression resulted in minimal viral entry,[299] ACE2 overexpression supported varied levels of SARS-CoV-1[91,95,97,99] and SARS-CoV-2[91,95–97,99,248,335,384] binding and viral entry. Ultimately, however, *M. musculus*-derived cells did not support viral replication of SARS-CoV-1 or SARS-CoV-2,[100,314] indicating overall low susceptibility. Similarly, overexpression of Chinese hamster (*Cricetulus griseus*) and Syrian hamster (*Mesocricetus auratus*) ACE2s supported high viral entry of both SARS viruses,[100,248,334] but infection of corresponding species-derived cells did not yield any viral replication.[100,155,314]

Other rodent species ACE2s supported binding and viral entry for either SARS-CoV-1 or SARS-CoV-2. Long-tailed chinchilla (*Chinchilla lanigera*)[100] and Arctic ground squirrel (*Urocitellus parryii*)[99] ACE2s supported high viral entry for both SARS-CoV-1 and SARS-CoV-2. Lesser jerboa (*Jaculus jaculus*) and white-footed mouse (*Peromyscus leucopus*) ACE2s were only tested for

SARS-CoV-2, and both supported high viral entry.[248] Notably, no species-derived cell lines for these four species (i.e., *C. lanigera*, *U. parryii*, *J. jaculus*, and *P. leucopus*) have been used to confirm these susceptibility findings. However, in vivo infection studies of two related species—Wyoming ground squirrel (*Urocitellus elegans*) and eastern deer mouse (*Peromyscus maniculatus*)—determined that *P. maniculatus* was susceptible to intranasal infection of SARS-CoV-2 resulting in seroconversion and the ability to transmit to other animals via direct contact;[92,443,444] *Urocitellus elegans* could not be intranasally infected with SARS-CoV-2.[444] In addition to *P. maniculatus*, bank vole (*Myodes glareolus*),[445] black-tailed prairie dog (*Cynomys ludovicianus*),[446] and fox squirrel (*Sciurus niger*)[447] also live in close proximity to *O. virginianus* and other likely susceptible ungulates (see "Farmed Ungulate Susceptibility"), but only *M. glareolus* could be intranasally infected with SARS-CoV-2.[444,448]

While no in vivo SARS-CoV-2 infection studies of *R. norvegicus* have been published, low expression of ACE2 and TMPRSS2[449] further confirm low susceptibility findings from in vitro infection studies. In vivo infection studies of *M. musculus* demonstrated an inability to infect intranasally with SARS-CoV-2,[444] which confirmed the lack of susceptibility findings from in vitro experiments. Minimal expression of ACE2 and TMPRSS2 in mice likely contributes to this lack of susceptibility.[449] *M. musculus* cannot be intranasally infected with MERS-CoV, likely due to low DPP4 lung expression.[450]

Four different hamster species (i.e., *M. auratus*,[251,451–453] Campbell's dwarf hamster [*Phodopus campbelli*], Roborovski hamster [*Phodopus roborovskii*], and winter white dwarf hamster [*Phodopus sungorus*][454]) can all be intranasally infected with SARS-CoV-2. All four species experience symptoms, although *P. roborovskii* reportedly had more severe symptoms than the other two *Phodopus* species.[454] Transmission studies using *M. auratus* further demonstrated SARS-CoV-2 transmission to other animals via direct contact, aerosol, and fomites.[251,452] Consistent with *M. auratus* in vivo susceptibility, ACE2 and TMPRSS2 were coexpressed in the same lung cells as demonstrated by single cell RNA-sequencing,[338] and all predicted *M. auratus* ACE2 isoforms can facilitate SARS-CoV-2 infection.[256] Notably, the susceptibility of *M. auratus* in vivo was not recapitulated in *M. auratus*-derived cell lines, suggesting that all in vitro infection studies for SARS-CoV-2 susceptibility should be confirmed with in vivo testing when possible.

Researchers in some countries have performed sporadic surveillance studies for SARS-CoV-1 and SARS-CoV-2 in rodent species. For SARS-CoV-1, Eurasian beavers (*Castor fiber*) in a live market as well as chestnut white-bellied rats (*Niviventer fulvescens*), black rats (*Rattus rattus*), and Sikkim rats (*Rattus sikkimensis*) in China tested negative.[33 75] Also in China, SARS-CoV-2 remains at a low rate of positivity in hamsters, although individual species were not specified.[247,455] In addition, a study of *R. norvegicus* in Belgium found no evidence of SARS-CoV-2 infection,[456] while a case study of one *C. porcellus* found no evidence of SARS-CoV-2 transmission from its positive owner.[434] Of rodent species tested near a mink farm containing SARS-CoV-2-positive *N. vison*, only one *M. musculus* tested positive for SARS-CoV-2, and both rock squirrel (*Otospermophilus variegatus*) and *P. maniculatus* animals tested negative.[121]

Non-Farmed Ungulate and Ursid Susceptibility

Large mammals can encounter humans in farm and captive contexts as well as wildlife encounters. For a summary of susceptibilities of farmed large mammals, see "Farmed Ungulate Susceptibility." Susceptibilities of other large mammals are summarized within this section, including computational modeling data for 31 species summarized in Table 11.

Table 11: Summary of Computational Modeling Data for Large Mammals, Excluding Farmed Ungulates

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Ailuropoda melanoleuca	Giant panda	Moderate,[90,245,246] High[80,83,86–88,93,248,259]	Low,[259] Moderate,[80] High[83,245]	Not assessed
Ammotragus lervia	Barbary sheep	High[93]	Not assessed	Not assessed
Antilocapra americana	Pronghorn	High[93]	Not assessed	Not assessed
Beatragus hunter	Hirola	High[93]	Not assessed	Not assessed
Bison bison	American bison	High[83,90,93,248,259]	Low,[244] High[83,259]	Not assessed
Bos mutus	Wild yak	Moderate,[245] High[83,86,87,93,248,259]	Low,[259] High[82,83,245,260]	Not assessed
Camelus ferus	Wild Bactrian camel	Low,[248] Moderate,[85] High[83,87,88,93,259]	Low,[82,259] Moderate,[83] High[85]	Not assessed
Capra aegagrus	Wild goat	High[93]	Not assessed	Not assessed
Catagonus wagneri	Chacoan peccary	Moderate,[245] High[93]	High[245]	Not assessed
Ceratotherium simum	White rhinoceros	High[83,88,93,93,248,259]	Low,[259] High[82,83]	Not assessed
Dicerorhinus sumatrensis	Sumatran rhinoceros	High[93]	Not assessed	Not assessed
Diceros bicornis	Black rhinoceros	High[93]	Not assessed	Not assessed
Elaphurus davidianus	Père David's deer	High[93]	Not assessed	Not assessed
Equus przewalskii	Przewalski's horse	Moderate,[83,241] High[88,93,248,259]	Low,[82,259] High[83]	Not assessed
Giraffa camelopardalis	Masai giraffe	High[93]	Not assessed	Not assessed
Hemitragus hylocrius	Nilgiri tahr	High[93]	Not assessed	Not assessed
Hippopotamus amphibius	Hippopotamus	High[93]	Not assessed	Not assessed
Loxodonta africana	African bush elephant	Low,[89,246,248] Moderate,[87,93,245] High[83,86,259]	Low,[82,244,259] Moderate,[83] High[245]	Not assessed
Moschus moschiferus	Siberian musk deer	Moderate,[245] High[93]	High[245]	Not assessed
Muntiacus muntjak	Southern red muntjac	High[87]	High[82]	Not assessed
Nanger dama	Dama gazelle	High[93]	Not assessed	Not assessed
Okapia johnstoni	Okapi	High[93]	Not assessed	Not assessed
Oryx dammah	Scimitar oryx	High[93,259]	Low,[259] High[85]	Not assessed
Pantholops hodgsonii	Tibetan antelope	High[93]	Not assessed	Not assessed
Rangifer tarandus	Reindeer	High[93]	Not assessed	Not assessed
Tapirus indicus	Malayan tapir	High[93]	Not assessed	Not assessed

Tapirus terrestris	South American tapir	High[93]	Not assessed	Not assessed
Tragulus javanicus	Java mouse-deer	High[93]	Not assessed	Not assessed
Ursus americanus	American black bear	Moderate,[245] High[86]	High[245]	Not assessed
Ursus arctos	Brown bear	High[83,86–88,93,248,259]	Low,[259] High[82,83,85,260]	Not assessed
Ursus maritimus	Polar bear	Moderate,[85,245] High[83,86–88,93,162,248,259]	Low,[162,259] High[82,83,85,245]	Not assessed

The only experimental infection studies in large mammals have been performed in vitro. Similar to *O. virginianus,* mule deer (*Odocoileus hemionus*)-derived cells supported replication of SARS-CoV-2.[337] However, cells from another cervid, elk (*Cervus canadensis*), cannot support SARS-CoV-2 replication.[337] ACE2 overexpression from the moose (*Alces alces*) cervid also cannot support SARS-CoV-2 replication.[303] Respiratory tissues from *A. alces*[303] and sika deer (*Cervus nippon*)[338] expressed ACE2 and TMPRSS2, suggesting that both species may still be susceptible to SARS-CoV-2 infections despite in vitro results.[303] Overexpression of two bovid ACE2s—from wild yak (*Bos mutus*)[248] and nyala (*Tragelaphus angasii*)[303]—support high viral binding of SARS-CoV-2 and minimal viral replication, respectively; *T. angasii* lung tissue also expressed ACE2 and TMPRSS2.[303] In addition, overexpression of ACE2s from giant panda (*Ailuropoda melanoleuca*), brown bear (*Ursus arctos*), and white rhinoceros (*Ceratotherium simum*) supported SARS-CoV-2 binding and viral entry,[248] while overexpression of northern giraffe (*Giraffa camelopardalis*) ACE2 overexpression only supported minimal SARS-CoV-2 replication.[303] However, because of ACE2 and TMPRSS2 positivity in *G. camelopardalis* respiratory tracts, these animals may still be susceptible to SARS-CoV-2 infections.[303] Although no ACE2 or TMPRSS2 expression data have been published for ursids, polar bear (*Ursus maritimus*) ACE2 consists of nine isoforms, and multiple truncated isoforms cannot fully interact with SARS-CoV-2 RBD, which may reduce susceptibility.[256] Similar to its domesticated counterpart, wild camel (*Camelus ferus*) ACE2 overexpression supported SARS-CoV-2 as well as SARS-CoV-1 binding and viral entry.[99]

Surveillance studies of large mammals for SARS-CoV-2 have been conducted for chital (*Axis axis*) in a captive U.S. facility;[355] roe deer (*Capreolus capreolus*) in Austria,[457] Germany,[458] and United Kingdom;[459] *C. canadensis* in the United States;[354] red deer (*Cervus elaphus*) in Austria,[457] Germany,[458] Poland,[460] and United Kingdom;[459] *C. nippon* in the United Kingdom;[459] European fallow deer (*Dama dama*) in the United Kingdom;[459] water deer (*Hydropotes inermis*) in the United Kingdom;[459] Reeve's muntjac (*Muntiacus reevesi*) in the United Kingdom;[459] and mouflon (*Ovis gmelina*) in Germany.[458] A low rate of seropositivity in *C. capreolus* and *C. elaphus* was detected in Germany, although these data are likely due to cross-reactivity with other coronavirus antibodies;[458] a low rate of seropositivity in these animals was also reported for the United Kingdom.[459] Both *D. dama* and *M. reevesi* animals in the United Kingdom also were seropositive for SARS-CoV-2.[459]

Small and Medium-Sized Carnivore and Omnivore Susceptibility

As small carnivores and omnivores, *P. larvata, N. procyonoides*, mustelids, *F.* catus, and *C. lupus familiaris* are highly susceptible to emerging coronaviruses (see "Reservoirs," "Mustelid Susceptibility," and "Companion Animal Susceptibility" for more details); other small and medium-sized carnivores and omnivores could be at similar risk levels for contracting viruses through contact with infected prey. Susceptibility data from other small carnivores and omnivores are summarized in this section, including computational modeling data for 26 species in Table 12.

Table 12: Summary of Computational Modeling Data for Small and Medium-Sized Carnivores and Omnivores

		Evidence Types		
Species	**Common Name**	**ACE2 Homology**	**ACE2-RBD Affinity**	**ACE2-RBD Molecular Dynamics**
Acinonyx jubatus	Cheetah	High[83,88,93,248,259]	Low,[82,259] High[83,85]	Not assessed
Canis lupus dingo	Dingo	Moderate,[241,245] High[83,87,93,248,259]	Low,[259] High[82,83,85,245]	Not assessed
Chrysocyon brachyurus	Maned wolf	High[93]	Not assessed	Not assessed
Crocuta Crocuta	Spotted hyena	Moderate,[87] High[88]	High[82]	Not assessed
Cryptoprocta ferox	Fossa	Moderate[93]	Not assessed	Not assessed
Helogale parvula	Common dwarf mongoose	Moderate[93]	Not assessed	Not assessed
Hyaena hyaena	Striped hyena	Moderate,[93] High[259]	Low,[259] High[85]	Not assessed
Lynx canadensis	Canada lynx	Moderate,[85] High[83,88,93,245,248,259]	Low,[82,259] High[83,85,245]	Not assessed
Lynx pardinus	Iberian lynx	High[86,88]	High[82]	Not assessed
Mungos mungo	Banded mongoose	Moderate[93]	Not assessed	Not assessed
Neofelis diardi	Sunda clouded leopard	High[93]	Not assessed	Not assessed
Neofelis nebulosa	Clouded leopard	High[93]	Not assessed	Not assessed
Nyctereutes procyonoides	Common raccoon dog	Low,[89] Moderate[83,86,87,96]	High[82,83]	Not assessed
Paguma larvata	Masked palm civet	Moderate,[80,83–87,90–98] High[88,89]	Low,[79–81] Moderate,[83] High[82,84,85]	Not assessed
Panthera leo	Lion	High[88,253]	Moderate[253]	Moderate[253]
Panthera onca	Jaguar	High[93]	Not assessed	Not assessed
Panthera pardus	Leopard	Moderate,[85] High[83,88,93,245,248,259]	Low,[259] Moderate,[85] High[82,83,245]	Not assessed
Panthera tigris	Tiger	Low,[257] Moderate,[241,246] High[80,83,84,87,88,90,93,248,253,259,261]	Low,[81,259] Moderate,[80,253] High[82–84,244,257,260,261]	Low[253]
Paradoxurus hermaphroditus	Asian palm civet	Moderate[93]	Not assessed	Not assessed
Procyon lotor	Raccoon	Low,[89] Moderate[86,87]	Low[82]	Not assessed
Puma concolor	Cougar	Low,[257] Moderate,[85] High[83,87,88,93,248,259]	Low,[259] High[82,83,85,257]	Not assessed

Puma yagouaroundi	Jaguarundi	High[259]	Low,[259] High[85]	Not assessed
Speothos venaticus	Bush dog	High[93]	Not assessed	Not assessed
Suricata suricatta	Meerkat	Low,[89,245,248] Moderate,[83,86,87,93] High[259]	Low,[82,259] Moderate,[83] High[85,245]	Not assessed
Vulpes lagopus	Arctic fox	High[93,259]	Low,[259] High[85]	Not assessed
Vulpes vulpes	Red fox	Moderate,[85,87,245] High[83,86,89,93,96,248,259]	Low,[259,260] Moderate,[85] High[82,83,244,245]	Not assessed

Most in vitro studies of carnivores and omnivores utilized overexpression of species-specific ACE2s and not species-derived cell lines. Similar to *F. catus*, ACE2s from four other feline species—Canada lynx (*Lynx canadensis*), leopard (*Panthera pardus*), cheetah (*Acinonyx jubatus*), and cougar (*Puma concolor*)—also supported high SARS-CoV-2 binding and viral entry.[248] ACE2 and TMPRSS2 expression in respiratory tracts and small intestines of *P. leo* and *P. tigris* further suggest feline susceptibility to SARS-CoV-2 and potential fecal shedding.[158,159,303,313,338] *P. larvata* ACE2 overexpression supported SARS-CoV-1 binding and entry,[91,95,97,99,100] but multiple studies for this species reported varied results for SARS-CoV-2 ranging from no viral binding and entry to moderate binding and entry;[91,95–97,99,100] *P. larvata* ACE2 overexpression also supported SARS-CoV-1 replication.[155,156] Exogenous *V. vulpes* and *N. procyonoides* ACE2s also supported viral binding and entry of both SARS-CoV-1 and SARS-CoV-2.[78,91,99,248]

In vivo infection studies of carnivores and omnivores have only utilized *N. procyonoides* and *P. lotor*. In agreement with in vitro data, *N. procyonoides* was susceptible to intranasal infection with SARS-CoV-2 and could transmit the virus via direct contact with other naïve animals.[102] Despite low ACE2 expression in the respiratory tract,[303] *P. lotor* was susceptible to asymptomatic, intranasal SARS-CoV-2 infection resulting in seroconversion.[461] However, *P. lotor* could not transmit SARS-CoV-2 to other animals via direct contact,[461] and a second study determined that this species could not be infected with SARS-CoV-2 via the intranasal route.[444]

Globally, sporadic reports of natural infections in captive carnivores and omnivores indicated anecdotal SARS-CoV-2 infections in binturong (*Arctictis binturong*),[462] spotted hyena (*Crocuta crocuta*), *L. canadensis*, white-nosed coati (*Nasua narica*),[462] *P. leo*,[462–468] *P. tigris*,[462–464,469–472] snow leopard (*Panthera uncia*),[462,473] *P. concolor*,[468] and fishing cat (*Prionailurus viverrinus*).[462] In addition, India reported one SARS-CoV-2 infection in a wild *P. pardus*.[474] Low seropositivity rates for wild golden jackel (*Canis aureus*) and *V. vulpes* were reported in Croatia, although *Canis aureus* antibodies failed to neutralize SARS-CoV-2.[360] Screening of Asian palm civet (*Paradoxurus hermaphroditus*) in Cambodia[25] and Vietnam,[475] *P. lotor* in the United States[121] and Canada,[476] large-spotted civet (*Viverra megaspila*) in Cambodia,[25] *V. vulpes* in Netherlands,[118] and *P. larvata* and *N. procyonoides* in Cambodia[25] did not detect any evidence of SARS-CoV-2 infections. However, past surveillance studies for SARS-CoV-1 detected high rates of active infections in *N. procyonoides* and *P. larvata* in live animal markets in China.[71,73,75,77]

Molecular Biology and Virology

SARS-CoV-1 and SARS-CoV-2 enter cells via S protein (i.e., a class I viral fusion protein) interaction with the host ACE2 receptor found on pulmonary and extra-pulmonary cell types.[477] MERS-CoV gains access to host cells by engaging the S protein with transmembrane dipeptidylpeptidase (DPP4), also referred to as CD26.[478] MERS-CoV-1, SARS-CoV-1, and SARS-CoV-2 fuse at the plasma membrane and depend on cell surface proteases—such as endosomal cathespins, cell surface transmembrane proteases/serine proteases (TMPRSS), furin, and trypsin—to activate viral fusion proteins and prime the S protein at the S1 and S2 interface.[220,479,480] Interestingly, the cleavage efficiency of the S protein in the S1 and S2 subunits modulates the SARS-CoV-2 infection.[481,482] Only SARS-CoV-2 utilizes the furin protease, as this virus has a polybasic furin cleavage motif, unlike MERS-CoV and SARS-CoV-1.[250] The RBD position also differs between SARS-CoV-1 and SARS-CoV-2. The position of the RBD in SARS-CoV-2 is frequently angled at a position that favors evasion of the host immune response, while the RBD in SAR-CoV-1 is frequently in the "up" conformation and demonstrates a lower binding affinity to ACE2 compared to SARS-CoV-2.[220,483,484] Notably, the Omicron SARS-CoV-2 variant enters host cells via the endocytic route, has reduced TMPRSS usage, and enhanced usage of membrane-type matrix metalloproteinase, leading to weaker cell-cell fusion activity.[220,485]

Molecular Biology Implications for Intermediate Hosts

SARS-CoV-1, MERS-CoV, and SARS-CoV-2 genomes share similarity, with SARS-CoV-2 sharing 79% genome sequence identity with SARS-CoV and 50% with MERS-CoV.[486] All three viruses have high sequence homologies to Chiropteran coronaviruses, and therefore likely originated from these animals. SARS-CoV-2 shares more than 90% and 93.3% sequence identity with the Chiropteran coronaviruses RaTG13 and RmYN02, respectively.[486] For SARS-CoV-1 and MERS-CoV, the over 99% genome sequence identity to coronaviruses isolated from palm civets and camels, respectively, indicates that these animals were the intermediate hosts. Pangolin-CoV is linked to SARS-CoV-2 with 90.7-92.6% overall sequence identity and one amino acid variation from SARS-CoV-2 in the RBD.[487] However, pangolins are not considered an intermediate host for SARS-CoV-2, in part because SARSr-CoV sequences isolated from pangolins lack the polybasic furin cleavage motif.[107]

The S protein of SARS-CoV-2 is distinct from MERS-CoV and SARS-CoV-1 and known bat SARSr-CoVs. It shares 76.7-77% amino acid sequence with SARS-CoVs from civets and humans, 75-97.7% with bat coronaviruses, and 90.7-92.6% with pangolin coronaviruses.[483] Additionally, SARS-CoV-2 spike contains a distinct four amino acid insertion between the S1 and S2 domains at the priming loop,, which is seen in other SARSr-CoVs of the betacoronavirus lineage.[484] Molecular surveillance, molecular dynamic simulations, and comparative in silico analyses for genetic diversity, particularly at the S protein level, provide clues to the origin, early evolution, intermediate hosts, and host adaptation and predictive range of SARS-CoV-1, MERS-CoV, and SARS-Cov-2 (See "Computational Modeling of Host Factors").[241,250,488]

Mutations that Affect Host Range

The S protein is the key to SARS-CoV-2 receptor binding and cell membrane fusion, making it also a molecular determinant for host tropism and viral transmission.[484] Mutations in the

receptor binding motif of the S protein promote adaptive diversity and increase the host range of SARS-CoV-2; this motif is the structural determinant that engages with the ACE2 host receptor. S protein mutations can also enhance binding to the host ACE2 receptor. More than 3,561 viral S protein mutations have been identified. However, mutations that enhance infectivity, replication, transmissibility, and resistance to neutralization are found within five variants, known as alpha (B.1.1.7), beta (B.1.351; mutations include L18F, D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G, A701V), gamma (P.1; mutations include L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F), omicron (B.1.529; mutations include D614G, E484A, N501Y, Q493K, K417N, S477N, Y505H G496S) and delta (B.1.617.2; mutations include T19R, G142D, Δ156–157, R158G, L452R, T478K, D614G, P681R, D950N).[489–491]

The original strain of SARS-CoV-2 was unable to employ murine and non-mammalian ACE2 receptors for viral entry.[100,492] However, SARS-CoV-2 spike mutations can expand the host range of infectivity, thereby creating new viral reservoirs. Thakur et al. (2022) assessed whether the S protein of four different variants (alpha, beta, gamma, and delta) could broaden the host range to *M. musculus*, *R. norvegicus*, and *P. larvata* ACE2.[489] The alpha, beta, gamma, and delta strains demonstrated increased usage of *M. musculus* ACE2 compared to the wildtype Wuhan strain (D614).[489] The N501Y-containing variants (alpha, beta, and gamma) permitted binding to the rat ACE2 receptor, and the beta variant of concern conferred a small increase in binding to the civet ACE2.[489] Other studies observed similar results with strains containing the N501Y mutation (present in all omicron sublineages) in the RBD.

Mutant strains with either a single N501Y mutation or combination of N501Y, K417N, and K417N in the RBD also acquired the ability to interact with the *M. musculus* and *N. vison* ACE2 receptors in vitro.[192] Additionally, laboratory studies generated mouse adapted SARS-CoV-2 strains through serial infections, and these strains contained K417N and/or N501Y mutations (See "SARS-CoV-1, SARS-CoV-2, and MERS-CoV Immune Responses in Select Susceptible Species", "Mouse").[493] The N501Y mutation combined with the T4781 mutation in a pseudovirus enables utilization of *G. gallus* ACE2. The delta variant, which lacks the N501Y mutation, was unable to utilize *G. Gallus* ACE2, but the N501Y-containing omicron variant was able to employ *M. gallopavo* and *G. gallus* ACE2 receptors. The T4781 mutation combined with the A262A mutation increased utilization of the ACE2 receptors in *S. scrofa*, *B. taurus*, *O. cuniculus*, *F. catus*, *C. lupus familiaris*, *C. hircus*, and *Equus caballus*; however, fish and reptilian ACE2 receptors remained incompatible with these mutations.[492] The D614G mutation is present in all SARS-CoV-2 variants and when combined with either A262S or T4781 mutations, enhanced utilization for human and NHP ACE2 has been observed.[492]

The Y453F and N501T SARS-CoV-2 mutations increased *M. putorius furo* ACE2 usage. The experiments that yielded this result also identified a new genetic variant, Y453F, with five amino acid changes in the S protein. Notably, a reverse zoonotic event has already occurred from humans to minks, and subsequently the mink-associated coronavirus 2 (miSARS-CoV-2) was transmitted back to humans containing a Y453F spike mutation in the RBD, which enhanced cell entry and ACE2 binding in minks, other mustelid species, and humans.[494,495] (This

transmission is the source of one of 76 SARS-CoV-2 variants found in humans.[496]) Prolonged interspecies contacts may result in acquisition of new mutations that further increase host species tropism.

Recombination Events

Recombination events of betacoronaviruses in wild and domestic animals are the evolutionary driving force in the emergence of SARS-CoV-1, MERS-CoV, and SARS-CoV-2, and provide an opportunity for zoonosis, reverse zoonosis, and creation of novel lineages. Frequent recombination events can take place during coinfections, which commonly occur in Chiropterans (see "Chiroptorans"). Both SARS-CoV-1 and SARS-CoV-2 arose after recombination events among Chiropteran coronaviruses. In contrast to SARS-CoV-1 and SARS-CoV-2, MERS-CoV arose from recombination events in both *C. dromedarius* and Chiropterans.[497]

So et al. (2019) analyzed an example of recombination events occurring between coronaviruses in dromedary camels. They found evidence of recombination of DcCov-HKU23 dromedary isolates with viruses from rodents, *O. cuniculus*, and *B. taurus* in Nigeria, Morocco, and Ethiopia,[498] and identified several genomic positions indicative of cross-species virus active recombination events among betacoronaviruses. Recombination signals were observed with: (1) BcoV-DB2 at the NS2a gene, (2) rabbit coronavirus (RbCoV-HKU14) at the hemagglutinin esterase gene, and (3) rodentCoV-IM2014 at ORFa, ORFb, and NS5a genes.

To date, there is no evidence that MERS-CoV-2 and SARS-CoV-2 have recombined.[499] However, future recombination events are likely to occur because: (1) both viruses are co-circulating in the same region, (2) both viruses can infect type II alveolar cells, (3) SARS-CoV-2 has a high recombination rate, and (4) SARS-CoV-2 RBD is compatible with ACE2 receptors of diverse species. Recombination events between MERS-CoV-2 and SARS-CoV-2 are also possible due to their identical transcription regulatory sequences and clusters of high sequence homology at ORF1a and ORF1b.

Molecular Determinants of SARS-CoV-1 and SARS-CoV-2 Host Ranges

ACE2 receptor recognition is a necessary component of viral entry for SARS-CoV-1 and SARS-CoV-2, and this receptor is conserved across a variety of species with high similarities identified at major binding sites.[252] The ACE2 receptor has a signal sequence at the N terminus, a transmembrane sequence at the C terminus, and an extracellular region that contains a zinc metallopeptidase domain.[252] The polymorphism of ACE2 receptors contributes to differences in SARS-CoV-2 susceptibility across various species. However, high compatibility between the SARS-CoV-2 S protein and various host ACE2 receptors highlights adaptive diversity and leads to reverse zoonosis as observed between minks and humans. Six amino residues within the receptor-binding domain of the S protein are important for species tropism as well as progression.[253]

Host susceptibility is impacted by the presence or absence of amino acids that are key for ACE2-RBD binding. Analysis of amino acid differences in the RBM can help researchers identify host ranges (see "Computational Modeling of Host Factors"). For example, the host ranges of SARS-

CoV-1 and SARS-CoV-2 are dissimilar due to distinct ACE2-interacting residues within the RBD and differences in host proteases required for activation and virus uptake.[500] Palm civets are thought to be an unlikely intermediate host due to the absence of amino acids needed to interact with ACE2.[86]However, substitution of the two amino acid residues of SARSr-CoV isolated from palm civets made it capable of infecting human ACE2-expressing cells.[501]

Yet ACE2 RBD homology is not *sufficient* to determine host range. For example, there are 18 interacting amino acids between human ACE2 and RBD of SARS-CoV-2; although nine of these sites differ in the ferret ACE2, the latter still supports SARS-CoV-2 infection.[502] A comparative analysis revealed the differing key amino acids between RBD of SARS-CoV-1 and SARS-CoV-2 for interaction with the ACE2 receptor: the amino acids needed for SARS-CoV-1-ACE2 binding are Y442, L472, N479, D480, T487, and Y491, whereas the key amino acids needed for SARS-CoV-2 are L455, F486, Q493, S494, N501, and Y505. Alexander et al. identified ACE2 residues in SARS-CoV-2 that distinguish susceptible from non-susceptible species (Leu79, HIS34, Tyr83, Gln24, Lys31, Asp30, and Glu329).[253] In silico analysis also suggests that species with K31, Y41, N90, and K353 are likely to be susceptible to SARS-CoV-2 infection.[98] The authors examined amino acid substitutions in 14 species of mammals, birds, reptiles, and amphibians that were proposed to be intermediate hosts for SARS-CoV-2, and discovered that N90 is possibly a critical position in ACE2 for SARS-CoV-2 binding, because the substitution of N90T destroys a major N-glycosylation site.[98] There is a high correlation between in silico analysis of ACE2 binding prediction and in vivo SARS-CoV-2 infection in human, NHPs, *F. catus*, *M. putorius furo*, and *S. scrofa* (although SARS-CoV-2 replicates poorly in *S. scrofa*). Researchers have determined that ACE2 genetic diversity is broader among bat species compared to humans and other mammals susceptible to SARS-CoV related viruses, which aligns with Chiropterans serving as coronavirus reservoirs.

Different animal species express various ACE2 isoforms, some of which do not support SARS-CoV-2-ACE2 binding and impact overall infection susceptibility.[256] Researchers have demonstrated that the cytoplasmic tail of ACE2 is not critical for SARS-CoV-2 infection. There are five canine isoforms, including one that lacks a transmembrane domain and a soluble canine ACE2; soluble ACE2 proteins impede the interaction between full-length ACE2 and SARS-CoV-2. The ACE2 isoform found in *S. scrofa* lacks the first 122 residues in the N terminus, which is crucial for binding. In contrast, *M. mulatta*, *M. fascicularis*, and *M. putorius furo* have one ACE2 isoform that retains the critical amino acids for interaction with the SARS-CoV-2 S protein. Notably, the only ACE2 isoform found in *O. aries*, *R. roxellana*, *M. leucophaeus*, *Physeter macrocephalus*, and *Delphinapterus leucas* contains the key residues in human ACE2 needed for binding to SARS-CoV-2.[256]

Host range is also determined by host proteins that are required for infection. Poston et al. demonstrated that a vacuolar protein sorting gene, VPS20, is required for infectivity for human and animal CoVs.[503] Shang et al. (2020) demonstrated that the host furin protease increases the types of cells, such as lung epithelial and lung fibroblast cells, that SARS-CoV-2 can infect, because the virus becomes less dependent on target cells to highly express other host proteases such as TMPRSS2 and/or lysosomal cathepsins that are used for viral entry.[504]

Codon usage bias, or preferential selection for a codon in highly expressed genes, changes based on the host environment of a virus.[505] Structural proteins of SARS-CoV-2 showed codon usage patterns similar to coronaviruses that infect horseshoe bats.[107] Interestingly, the codon usage pattern of the S protein in SARS-CoV-2 is closely related to the SARSr-CoV-BtKY72 found in Kenya.[506] This finding correlates with affinity binding and molecular dynamic simulation data. A group led by Yuzhou Gong reported that snakes were suspected to be a likely source of coronaviruses. However, codon usage bias has not been shown to be useful for successful viral infection; in fact, several studies have disproved this utility by demonstrating that the similarity of RSCU between virus and host is not sufficient to identify host species.[105,192]

Immunology

Natural immunity to SARS-CoV-1, MERS-CoV, and SARS-CoV-2 induced after viral infection involves both the innate and adaptive immune systems. The robustness of these immune responses varies greatly based on both the viral strain and the host species.[294,507–509] The generation of protective immunity (i.e., immunological memory) against subsequent reinfections with the same or related strain consists of both antigen-specific memory B and T lymphocytes as well as the production of neutralizing antibodies. In addition, T cells contribute to protection against reinfection, and tissue resident memory T cells play an important role in the immune response to both SARS-CoV-1 and SARS-CoV-2.

Many detailed studies have characterized *human* immune responses to SARS-CoV-2, in particular. In the early phases of the SARS-CoV-2 pandemic, therapeutic strategies and vaccine formulations were inadequate, so animal models susceptible to SARS-CoV-2 were studied to further characterize immune responses and symptoms; current research studies focus largely on how viral infections and vaccination provide protection against subsequent reinfections with newly mutated variants of SARS-CoV-2.[510] Although such studies are typically not designed to address coronavirus in animals *per se*, they have generated data relevant to understanding coronavirus in animals. While studies of less commonly used animal models have helped evaluate vaccine and therapeutic efficacy, most immunological studies have used rodents and NHP animal models. In addition, in the past 3 years, new SARS-CoV-2 variant lineages have emerged including alpha, beta, gamma, delta, and most recently, omicron—each of which has different pathogenic characteristics in different species, which must be considered when assessing animal susceptibilities. The immunological response of several species to infection with SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are summarized below.

Innate Immune Response

Upon viral infection, the host's innate immune system recognizes the pathogenic single-stranded RNA via the toll-like receptor-7 (TLR-7) and toll-like receptor-8 (TLR-8). These receptors activate downstream signaling cascades to activate IFN regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain enhancer (NFκB) of activated cells, which regulate both type I and type III IFN responses. Activation of these IFN responses results in upregulation of IFN stimulated genes (ISGs) and the production of various cytokines and chemokines to eliminate the viral infection. Earlier studies of SARS-CoV-1, MERS-CoV, and other coronaviruses demonstrated that betacoronaviruses have the capacity to evade the innate immune system by

inhibiting NFκB signaling.[511,512] Because SARS-CoV-1 and SARS-CoV-2 proteins are homologous, ongoing analyses are focused on the roles of different SARS-CoV-2 proteins to determine whether they modulate the IFN response similar to SARS-CoV-1. Type I IFNs (IFN-α and interleukin 28 receptor alpha/beta [IL28RA/IL10Rβ]) lead to the formation of both STAT1-STAT2 heterodimers and STAT1 homodimers, which induce transcription of ISGs. Type III IFN (i.e., IFN-γ) signaling results in the formation of additional STAT1 homodimers. ORF-10 of SARS-CoV-2 has been found to suppress the type I IFN expression and downstream ISGs through a mitochondrial antiviral signaling (MAVS) protein.[513] Ongoing research aims to elucidate the roles of these overlapping IFN signaling cascades and their roles in the immune response to SARS-CoV-2 infection.[514]

SARS-CoV-2 viral infection of lung cells results in the recruitment of both macrophages and monocytes to the alveolar tissue, resulting in the production of various cytokines that prime the adaptive immune response. Most individuals infected with SARS-CoV-2 eventually clear the viral infection; their immune response dampens and resolves, allowing recovery. However, some individuals develop more severe infections with more exaggerated innate immune responses than individuals who more rapidly resolve SARS-CoV-2 infections. This heightened response involves increased levels of inflammatory monocyte-derived macrophages ($CD14^+$ $CD16^+$ double positive). These macrophages are recruited to the lung tissue where they secrete various inflammatory cytokines including IP-10, macrophage inflammatory protein 1 α (MIP-1α), and monocyte chemoattractant protein 1 (MCP-1).[515,516] Inflammatory cell infiltration by macrophages, neutrophils, and activated T cells is associated with lung tissue damage. This infiltration as well as enhanced release of cytokines often results in acute respiratory distress syndrome (ARDS) in many cases.[517,518] Individuals with severe COVID-19 disease often have either autoimmune diseases that elevate levels of autoantibodies to IFN-1 or mutations that disrupt IFN responses to infection, which highlights the importance of IFN responses for timely clearance of SARS-COV-2.[519,520]

Numerous studies have shown that early IFN responses are critical in the innate immune response and are important in the early stages of disease.[512] Dysregulation of IFN response timing may lead to more severe disease due to higher plasma levels of pro-inflammatory cytokines including interleukin (IL)-2, IL-7, IL-10, granulocyte colony stimulating factor (G-CSF), IFN gamma-induced protein 10 (IP-10), MCP-1, MIP1α, and tumor necrosis factor (TNF).[521,522] In addition, elevated systemic levels of the pro-inflammatory cytokine IL-6 correlate with severe COVID-19 disease. Additional studies have suggested that inflammasome activation can elevate levels of IL-1 and IL-18, which can also promote inflammation.[523] These pro-inflammatory responses contribute to the cytokine storm that is observed in severe COVID-19 disease in some individuals, and targeted immunosuppressive treatments can help modulate production of these inflammatory cytokines.[517] Studies in animal models are ongoing to better understand the role of the IFN response in SARS-CoV-2 replication and lung pathology.

Similar to SARS-CoV-1 and -2, infection with MERS-CoV affects the respiratory tract. MERS-CoV infection in camels typically causes mild symptoms including nasal and ocular discharge. Analysis of sera from camels revealed the presence of MERS-CoV neutralizing antibodies that

did not cross-react with the SARS-CoV-1 antigen.[362,363] One hundred immune-response genes were analyzed in 121 camels that had been infected with the MERS-CoV-1 virus. Transcriptional profiling of 121 MERS-CoV-infected camels identified several genes with adaptive immune system functions (major histocompatibility complex class I and II) and innate immune functions. The results suggested that MERS-CoV infection involves multiple host factor pathways that are seen in other coronavirus infections in other host species.[524] In addition, type I IFNs have been identified as critical to the innate immune response to MERS-CoV in camels.[525,526]

Adaptive Immune Response

The generation of immunological memory against subsequent SARS-CoV-1, MERS-CoV, and SARS-CoV-2 reinfections involves the adaptive immune response, which consists of virus specific memory B and T lymphocytes functions. B cells generate the humoral response by producing neutralizing antibodies that can prevent reinfection with the same or related strains of the virus.[527] In addition, both $CD4^+$T cells and $CD8^+$T cells contribute to viral clearance. $CD8^+$ cytotoxic T cells kill infected cells, while $CD4^+$ helper T cells prime the B cell response as well as produce cytokines for immune cell recruitment. Immunological memory involves several immune cell types including memory B cells, plasma B cells, and tissue resident memory T cells that allow for a rapid immune response and accelerated clearance of infection when the host encounters the virus in a subsequent infection. SARS-CoV-2 elicits a robust B cell response, and most individuals seroconvert and produce antibodies 7 to 10 days post infection (dpi). Antibodies include virus-specific immunoglobulin (Ig)M, IgG, and IgA that recognize the external S protein or internal N protein. Neutralizing antibodies against the RBD can prevent viral entry into cells and subsequent infection.[528,529] Neutralizing antibodies that bind RBDs of SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are distinct for each virus. However, there is some degree of cross-reactivity between antibodies to the S and N proteins of the coronaviruses. These cross-reactive antibodies target the stem helix portion of the spike S2 fusion subunit, which is present in the prefusion conformation in different viruses.[530,531]

The longevity of protection afforded post-infection for SARS-CoV-1, MERS-CoV, and SARS-CoV-2 in animals is poorly understood. However, studies of human patients who have recovered from SARS-CoV-2 infection determined that while specific plasma and memory B cells are present, they begin to decline approximately 3 months post-infection. In addition, SARS-CoV-1 neutralizing antibodies decrease significantly within a few years of infection to nearly undetectable levels in some individuals.[532] Studies conducted on camel serum have shown the presence of antibodies highly specific for the MERS-CoV S protein.[363] The durability of this antibody protection is poorly understood; however, a study of calves identified reinfections with a median time between infections of only 59 days.[58]

SARS-CoV-1, MERS-CoV, and SARS-CoV-2 Immune Responses in Select Susceptible Species

Because NHP immune systems closely recapitulate human innate immune responses to pathogens, most immunological studies are performed in these animal models rather than rodent models. As a result, information about immune responses to SARS-CoV-1, MERS-CoV,

and SARS-CoV-2 in animals is limited primarily to NHPs. However, additional studies of SARS-CoV-2 have been performed in cats, hamsters, ferrets, and transgenic mice expression human angiotensin I converting enzyme 2 (hACE2).[507,508,533,534] The immune response elicited by infection with SARS-CoV-1 and SARS-CoV-2 will be summarized for several host species below.

Non-Human Primates

Immunology research on SARS-CoV-1, MERS-CoV, and SARS-CoV-2 has been performed in multiple different NHPs (e.g., *M. mulatta, M. fascicularis*, *P. anubis, C. sabaeus*). *M. mulatta* are susceptible to SARS-CoV-1 infection[535] as well as MERS-CoV infection.[323,536,537] *M. mulatta* have also been used in numerous studies of SARS-CoV-2 and its emerging variants.[316,320,538,539] In response to SARS-CoV-2 infection, *M. mulatta* develop clinical symptoms including systemic inflammation and elevation of cytokines accompanied by interstitial and alveolar pneumonitis within the first week of infection. Analysis of BAL samples collected from infected *M. mulatta* indicates that they mount an early antiviral response including an inflammatory phenotype with the presence of both innate and adaptive immune cells, myeloid cells, and a type I IFN response. Cytokines including IFN-α, IFN-γ, IL-6, IL-8, perforin, IP-10, MIP1-α, and MIP1-β were elevated in these BAL samples as well. Both type I IFNs and IL-6 are signatures of a cytokine storm that contributes to the development of ARDS (see "Innate Immune Response"). In addition, IFN-α was elevated, which resulted in downstream expression of the type I ISG IP-10 (i.e., CXCL-10); increased IP-10 can facilitate recruitment of T regulatory $CXCR3^+$ Th1 T cells, which are found at sites of inflammation. Further analysis of lung tissue revealed extensive infiltration of interstitial lymphocytes, macrophages, plasma cells, and eosinophils, which led to an expansion of the alveolar space. Both $CD4^+$T cells and $CD8^+$T cells exhibited both proliferative and memory cell markers that increased after infection with the virus. By contrast, markers of naive T cells and effector T cells were reduced, indicating that an induction of robust CD4+ and CD8+ T cell responses had taken place after infection and was maintained up to 9 dpi.[540] Viral antigens were detected in alveolar epithelial cells and alveolar macrophages, suggesting engulfment of infected cells. Following infection with SARS-CoV-2, $CD4^+$T cells isolated from BAL samples expressed high levels of PD-1 and LAG-3, indicating a high level of T cell exhaustion. Additionally, $CD8^+$T cells in BAL fluid had increased PD-1 and LAG-3 expression, which was correlated with the BAL viral titer load. It appears that rapid recruitment of myeloid cells expressing Type I IFNs aids in controlling the viral replication of SARS-CoV-2, but the remaining viral antigens promote the recruitment of effector T lymphocytes. *M. mulatta* also developed T cell memory for SARS-CoV-2.[540]

In SARS-CoV-2 challenge experiments using previously infected *M. mulatta*, the animals had a marked reduction in median viral loads compared to the primary infection with a concomitant increase in B and T cell responses. This protective adaptive immune response involving both B and T cells was elicited by 21 days after infection and afforded a full protection against reinfection,[539] indicating that SARS-CoV-2 infection induces protective immunity against re-exposure in *M. mulatta.* In addition, the antibodies exhibit a range of effector functions, including antibody-dependent complement deposition, antibody-dependent cellular phagocytosis, and antibody-dependent natural killer (NK) cell degranulation (NK CD107a).[539] However, many questions remain regarding the durability of protection, and studies are

ongoing to determine the level of protection after a several month period. Other studies indicate that this protective response is $CD8^+$ T cell-dependent.[539]

M. fascicularis infected with SARS-CoV-2 exhibit mild clinical symptoms, including mild fever and weight loss, nasal discharge, and high levels of viral RNA present in the respiratory tract. Viral loads were lower than those observed in the *M. mulatta*, and viral clearance occurs more quickly in this species.[319,320] Infected animals also developed diffuse alveolar damage.[541]

Additional studies found that *P. anubis* are generally more susceptible to SARS-CoV-2 infection than *M. mulatta* and display more extensive lung infection (see "Non-Human Primates"). Histopathological analysis of BAL samples revealed the presence of plasma cells, interstitial lymphocytes, macrophages, the alveolar space. There is also evidence of alveolar wall thickening and collagen deposition. *P. anubis* also exhibit more prolonged viral shedding with higher levels of virus being present when compared to rhesus macaques.

Studies conducted on *C. jacchus* indicated less severe pathology and limited viral shedding after SARS-CoV-2 infection when compared to rhesus macaques or baboons.[540,542] Histopathologic analysis of BAL samples indicated a lower number of interstitial lymphocytes and macrophages in the alveolar space, indicating less recruitment to lung tissues (see "Non-Human Primates"). SARS-CoV-1-infected marmosets were found to develop interstitial pneumonia and may also develop additional pathologies involving liver and renal organs and gastrointestinal involvement.[543]

In SARS-CoV-2 infection studies, *C. sabaeus* developed moderate viral titers as evidenced by the presence of viral RNA and infectious virus in nasal, BAL, oral, and rectal swabs by day 2 post-infection. In addition, the animals developed more severe respiratory disease and exhibited interstitial pneumonia with diffuse alveolar damage, hyaline membranes, and multinucleate epithelial cells by day 5 post-infection.[317] All animals in the study seroconverted at day 5 post-infection and were protected in challenge experiments conducted 2 months after the primary infection. Transcriptome analysis of BAL samples demonstrated stimulation of pro-inflammatory IFN and IL-6 pathways similar to other NHPs. In addition, these animals had increased serum concentrations of interleukins as well as other pro-inflammatory cytokines and chemokines.[317]

The immune responses in aged NHPs have been studied in some NHPs. While both young and aged *M. mulatta* fully recovered by 2 weeks post infection, aged *M. mulatta* had lower SARS-CoV-2 IgG titers compared to their young counterparts. For *P. anubis,* SARS-CoV-2 infections in aged animals yielded higher SARS-CoV-2 viral titers and more severe pathology compared to young animals. Although *C. jacchus* exhibit mild disease when infected with SARS-CoV-2, aged *C. jacchus* exhibited some degree of pulmonary inflammation although they still recover quickly.[540]

Results on these NHPs indicate that different species mount differing immune responses to SARS-CoV-2, with the *P. anubis* exhibiting more severe disease than both *M. mulatta* and *C.*

jacchus. Notably, *M. mulatta* and *P. anubis* share multiple SARS-CoV-2 immune response features.

Syrian Hamster

Hamsters are naturally susceptible to infection with SARS-CoV-2, and *M. auratus* has been used extensively as an animal model to study the immune response to SARS-CoV-2 because infection with the virus mimics many characteristics of human COVID-19 disease. *M. auratus* develop moderate disease following intranasal infection with low doses of the virus but recover within 2 weeks.[544] SARS-CoV-2 RNA was detected at high levels in nasal turbinates, trachea, and lungs with lower levels measured in other organs including intestines, heart, liver, spleen, and kidney. Additionally, SARS-CoV-2 was detected in brain tissue of hamsters and acute inflammation of the olfactory epithelium is seen with infection of mature and immature olfactory neurons. This may relate to the olfactory function impairment seen with COVID-19 in patients.[545]

Severity of pulmonary disease correlates with the infectious dose of virus, and the most severe pathology is observed at day 5 post-infection, followed by clearance of viral antigen and a reduction in inflammation by 2 weeks post infection.[251] In addition, infected hamsters display both enteric necrosis as well as cardiac myofiber degeneration not observed in other rodent species. Neutralizing antibodies were detectable at approximately 1 week post-infection. Viral replication in hamsters could be suppressed by administration of early convalescent serum collected from previously infected hamsters. However, convalescent serum did not reduce the lung pathology observed.[546]

In additional experiments, previously infected Syrian hamsters were protected against subsequent reinfection of SARS-CoV-2, as evidenced by absence of viral replication and lung pathology.[547,548] Additional reinfection experiments with newly emerging Alpha and Beta variants showed that previously infected Syrian hamsters were protected against viral replication in the lower respiratory tract and severe lung pathology. However, viral replication was still observed in the upper respiratory tract, suggesting that these animals could still transmit SARS-CoV-2.[549]

The role of type I and type III IFNs in controlling SARS-CoV-2 replication was investigated in STAT2 knockout hamster lines that lack this key type I and type III IFN downstream signaling protein. Viral titers in blood were higher in STAT2-deficient hamsters compared to wildtype controls, although the severe lung pathology was not observed in these animals. These results suggest that STAT2-dependent IFN responses play a key role in limiting viral dissemination yet contribute to the development of lung pathology observed upon infection.[550] Additional studies have demonstrated the role of the adaptive immune response using RAG2 hamster knockout strains[551] and IL-2 R deficient hamsters.[544] Studies of both genetic backgrounds showed that functional B and/or T cells are required for clearance of the virus. Prolonged viral presence of up to 24 days was evident in the IL-2 R knockout hamster strain, which lacked T cells, NK cells, and mature B cells, suggesting that the innate immune system plays a role in decreasing viral replication, and that the adaptive immune system is needed to completely clear the viral infection. Comparison of *M. auratus* model to other rodent models indicates that the SARS-

CoV-2 virus replicates to higher levels and for a longer period of time in the respiratory tracts of hamsters. In addition, hamsters also display more significant lung pathology with spread of virus to other organs and tissues.[552,553]

Ferret

M. putorius furo are highly susceptible to SARS-CoV-1 and SARS-CoV-2 (see "Mustelid Susceptibility").[294,309] Upon infection with SARS-CoV-2, ferrets display elevated body temperatures and shed the virus through nasal secretions. SARS-CoV-2 is detectable in saliva, urine, and fecal specimens. Viral antigens were also detected in nasal turbinate, lung tissues, and intestine until 8 days after infection. Infected ferrets could also transmit SARS-CoV-2 to naive ferrets via direct contact.[309] Analysis of age-related disease severity in ferrets demonstrated that aged ferrets older than age 3 years exhibited higher viral loads and longer periods of viral shedding accompanied by a more prominent lung inflammatory cell infiltration when compared to young animals.[312] Lung tissues from SARS-CoV-2-infected, aged ferrets showed enhanced type I IFN activity as well as an increase in activated T cells and macrophage responses when compared to young animals.[309] Together, these data indicate that age is a critical factor in SARS-CoV-2 severity in *M. putorius furo*. Infected ferrets develop antibodies against SARS-CoV-2,[294,309] and challenge of recovered ferrets with SARS-CoV-2 was accompanied by a reduction in viral shedding from the upper respiratory tract compared to the prior infection, indicating protection from reinfection.[307]

Mink

Previous research has indicated that *N. vison* can be infected by both SARS-CoV-1[554] and SARS-CoV-2.[116,555,556] SARS-CoV-2 causes severe respiratory disease in *N. vison* with high mortality rates. Virus can be detected in nasal, oral and rectal swabs, and SARS-CoV-2 transmission between *N. vison* and humans has occurred extensively.[557,558] Infected minks often exhibit labored breathing, with interstitial pneumonia and extensive neutrophil, macrophage and lymphocyte infiltration in lung tissue with resulting alveolar damage.

Mouse

Wildtype mouse susceptibility differs for SARS-CoV-1 and SARS-CoV-2.[559] SARS-CoV-1 can infect several laboratory strains of mice including BALB/c and CC57BL/6 mice, and viral replication can be detected in the respiratory tract of mice. The mice are asymptomatic and do not display severe pathology of lung tissue with infection and exhibit only mild infiltration of inflammatory cells. Virus is cleared within 1 week, and neutralizing antibodies can be detected. However, these laboratory strains of mice are not readily infected with older strains of SARS-CoV-2. Recent reports have investigated newly emerging variants of SARS-CoV-2 and found that the B.1.351 variant containing the N501Y mutation in the S protein has allowed recognition of the mouse ACE2 receptor, allowing entry of the virus and subsequent infection of wild type mice. In addition, the presence of serum SARS-CoV-2 specific neutralizing antibodies was detected at 14 dpi in mice that had been infected with the variant strain but not in mice infected with wildtype SARS-CoV-2. Levels of neutralizing antibody increased after rechallenge with the variant species of the virus.[560]

Numerous mouse models susceptible to SARS-CoV-1 and SARS-CoV-2 infections have been generated through various means: (1) adaptation of viruses through serial infections in mice,[561] (2) transgenesis of mice to insert human ACE2,[562–565] and (3) transduction of mice with human ACE2 via adenovirus 5.[566,567] Transgenesis has also been used to generate human DPP4 expressing mice that are susceptible to MERS-CoV infection.[568] Several mouse-adapted strains of both the SARS-CoV-1[569] and SARS-CoV-2 virus have been generated in the past few years that would allow infection and efficient replication of the virus in both the upper and lower respiratory tracts in laboratory strains of mice.[570–573] The SARS-CoV-2 WuHan-Hu-1 strain of virus was used to intranasally inoculate BALB/c mice, and the virus was passaged for 11 times, generating a viral strain that was infective in the mice and caused interstitial pneumonia. Sequence analysis of the virus revealed mutations in the RBD of the S protein, which resulted in increased binding affinity for the mouse ACE2 receptor.[574] Similar methods have been used to generate several different mouse-adapted strains of the virus that accumulate multiple mutations at different sites in the RBD with repeated passaging. The more virulent strains of the virus result in acute lung injury with significant lung inflammation accompanied by infiltrating immune cells.[570–573] Ongoing research of SARS-CoV-2 focuses on host immunology and its ability to prevent viral infection. However, because these studies have mainly used transgenic, humanized mouse models, there is a paucity of immunological studies conducted in wildtype rodent species that may actually be susceptible to SARS-CoV-2.

Pathogenesis

Hamster

Golden Syrian

SARS-CoV-2

Studies containing pathogenesis data on coronaviruses in hamsters primarily focus on *M. auratus* as a model for human disease. Aside from humans and *M. mulatta*, *M. auratus* ACE2 exhibited the highest binding affinity to the S protein of SARS-CoV-2 from in silico modeling (see "Rodent Susceptibility" for more details).[251] *M. auratus* inoculated with SARS-CoV-2 primarily lost weight or had reduced weight gain, as well as lethargy, ruffled fur, a hunched posture, and dyspnea.[251,452,575–577] However, Yuan et al. (2021) found that many female *M. auratus* showed no significant weight loss, while male *M. auratus* showed up to 9.6 percent mean weight loss through 7 dpi.[578] In addition, *M. auratus* inoculated with the Delta variant (B.1.617.3) showed the least weight gain compared to those inoculated with the B.1 D614G variant, while *M. auratus* inoculated with the Omicron variant (B.1.1.529) experienced no weight loss.[579,580] These data suggest that *M. auratus* susceptibility to infection of SARS-CoV-2 is dependent on both *M. auratus* sex and SARS-CoV-2 variant.

Viral RNA was detected primarily in the nasal turbinates and lungs of SARS-CoV-2 inoculated *M. auratus*, with males showing the most sensitivity to infection and viral replication compared to females.[251,452,578] From immunohistochemistry (IHC) analysis of tissue samples, viral antigen was detected in bronchial epithelial cells and pneumocytes, nasal epithelial cells, olfactory sensory

neurons, and duodenum cells.[452] In Delta-inoculated *M. auratus*, viral RNA was detected in nasal turbinates, trachea, and lungs up to 14 dpi, while Omicron-inoculated *M. auratus* showed significantly less viral burden in lung tissue compared to Delta-inoculated *M. auratus*.[579,580]

Blood hematology of SARS-CoV-2-inoculated *M. auratus* showed significant increases in neutrophils, red blood cells, and hemoglobin, as well as intracardiac platelet and fibrin aggregates at 8 dpi, which is potentially indicative of hypercoagulation. Blood chemistry showed elevated markers of renal disease and blood lipids, such as uric acid, triglyceride, and low-density lipoprotein. Furthermore, metabolic markers, such as total protein and albumin, significantly decreased. Together, these blood chemistry changes indicate a potential dysregulation of extrapulmonary organs during acute infection.[575]

Histopathological analysis of lung tissues from SARS-CoV-2-inoculated *M. auratus* showed broncho-interstitial pneumonia (correlated with continued weight loss), diffuse alveolar damage, protein-rich fluid exudate, hyaline membrane formation, cellular debris in bronchiolar lumen, alveolar collapse with hemorrhage, and damage to pulmonary vasculature.[251,575,576] Nasal turbinates showed inflammatory cell infiltration and blood vessel congestion, though the epithelium was generally intact.[251,452] The bronchial and mesenteric lymph nodes show subcapsular and medullary lymphatic sinus ectasia, and the trachea shows epithelial cell swelling, focal cilia loss, and mononuclear cell infiltration.[251] In lung tissue from Omicron-inoculated *M. auratus*, congestion and hemorrhages were absent because of less efficient viral replication compared to Delta-inoculated *M. auratus*, which showed severe pathological changes.[579,580]

One study evaluated *M. auratus* that lacked the *STAT2* gene (*STAT2*$^{-/-}$ *M. auratus*) to determine the role of *STAT2* signaling in SARS-COV-2 pathogenesis.[550] After SARS-CoV-2 inoculation, *STAT2*$^{-/-}$ *M. auratus* showed higher viral titers and detectable viral RNA in the blood, spleen, and liver not typically found in SARS-CoV-2-inoculated wildtype *M. auratus*. In contrast, the lung pathology in *STAT2*$^{-/-}$ *M. auratus* was significantly attenuated compared to wildtype *M. auratus*. Furthermore, pulmonary consolidations were not present in *STAT2*$^{-/-}$ *M. auratus*. These findings indicate that *STAT2* potentially not only restricts the systemic spread of SARS-CoV-2 infection, but also drives severe lung injury.[550]

SARS-CoV-1

M. auratus inoculated with SARS-CoV-1 (Urbani strain) did not show clinical symptoms; however, high viral titers were detected in the upper and lower respiratory tracts for up to 5 dpi, while lower levels of viral titers were detected in the liver and spleen. At 1 to 2 dpi, viral titers could be transiently detected in blood and plasma but are no longer detected by 3 dpi. From IHC analysis, viral antigens were found in endothelial cells of the nasal turbinates and mucosal glands of the trachea. From histopathological analysis, epithelial cells of the nasal turbinates, trachea, and bronchi showed swelling with mononuclear inflammatory cell infiltrates in the submucosa of bronchioles, which is indicative of pneumonitis. In addition, the nasal passages contained mild ulcers and the trachea had a focal loss of cilia. However, the lungs of SARS-CoV-1-inoculated *M. auratus* recovered without detectable viral antigen by 14

dpi. Upon rechallenge of SARS-CoV-1 inoculation, *M. auratus* showed no clinical symptoms, a lack of detectable viral antigen in all tissues, and a lack of pneumonitis.[581]

Although SARS-CoV-1-inoculated *M. auratus* developed only mild infections, *M. auratus* immunosuppressed with cyclophosphamide treatment experienced increased weight loss and mortality. High viral titers were detected at 2 dpi and were eventually detectable in the lungs, liver, spleen, and kidneys by 19 dpi. From histopathological analysis, lungs showed moderate bronchointerstitial pneumonia with multifocal infiltrations of macrophages, neutrophils, lymphocytes, and plasma cells. In extrapulmonary tissues samples, multifocal myocardial inflammation was present, as well as dilation of renal cortical tubules, tubular degeneration, and renal necrosis. Together, these findings suggest that immunosuppressed *M. auratus* are susceptible to broader pathogenesis of SARS-CoV-2 infection compared to healthy *M. auratus*.[582]

MERS-CoV

M. auratus inoculated with MERS-CoV lacked indications of clinical symptoms, viral replication, histopathological lesions in the lungs, cytokine upregulation, or seroconversion of antibodies, indicating that MERS-CoV cannot effectively replicate in *M. auratus*.[323]

Roborovski

SARS-CoV-2

P. roborovskii inoculated with SARS-CoV-2 show severe clinical symptoms, including snuffling, dyspnea, cough, sneeze, ruffled fur, reduced activity, hunched posture, and weight loss.[454,583] Interestingly, although Zhai et al. (2021) did not find any change in body temperature, Trimpert et al. (2020) found that body temperature significantly *decreased*. In addition, multiple *P. roborovskii* were deemed terminally ill and were euthanized at 3 dpi.[454] Viral RNA and titers were primarily detected in the homogenates of the lung and trachea, with lower levels detected in the brain, stomach, intestine, liver, heart, kidney, spleen, and blood. From IHC analysis, viral antigen was detected in alveolar epithelial cells, bronchial epithelial cells, macrophages infiltrating the bronchi, liver cells, subarachnoid cells of the brain, and blood leukocytes.[454,583] Blood analysis also revealed elevated levels of fibrin degradation product and D-dimer in plasma, suggesting thrombosis and fibrinolysis.[583] These viral RNA, IHC, and blood analysis data suggest a severe systemic infection in SARS-CoV-2-inoculated *P. roborovskii*.

From histopathological analysis, lung tissues showed severe inflammatory lesions, multifocal interstitial pneumonia with thickened alveolar septa and infiltration of fibrin and mononuclear cells, diffuse alveolar damage, hyaline membrane formation, edema, and cellular debris within alveoli.[454,583] In extrapulmonary organs, liver tissues showed multifocal fatty changes and portal lymphocytic infiltration, and brain tissues showed focal infiltration of lymphocyte and subarachnoid hemorrhage. However, despite high levels of viral RNA detected in the trachea, tissue damage was not detected. In addition, no obvious histopathological changes were observed in the stomach, intestine, heart, kidney, and spleen.[583]

Together, these data suggest that *P. roborovskii* develop severe or fatal disease from SARS-CoV-2 inoculation and are substantially more susceptible to infection than *M. auratus*. From genome sequencing analysis, the ACE-2 receptors of *P. roborovskii* and *M. auratus* show minor differences. However, these differences do not reside in any amino acids associated with SARS-CoV-2 binding, and thus cannot account for the viral susceptibility differences between *P. roborovskii* and *M. auratus*.[454]

Mouse

SARS-CoV-2

M. musculus inoculated with SARS-CoV-2 (WA1/2020) do not show significant signs of infection characterized by clinical symptoms or pathological analysis because of insufficient binding affinity between the SARS-CoV-2 S protein and murine ACE2 receptors.[500] However, multiple variants, including Beta (B.1.351) and Alpha (B.1.1.7), showed infectious potential in mice. Both variants contain an N501Y substitution in the S protein, which may increase the binding affinity of the S protein to murine ACE2 receptors.[560,584,585]

Both BALB/c and C57BL6/J *M. musculus* inoculated with the Beta variant lost weight in a dose-dependent manner, while older BALB/c *M. musculus* showed ruffled fur, hunched postures, and mortality.[584] BALB/c *M. musculus* also contained higher viral RNA and titer levels in the lungs compared to C57BL6/J *M. musculus*, with IHC analysis showing infection of bronchial epithelial cells, macrophages, and stroma cells in BALB/c *M. musculus*. From histopathology analysis, the lungs of BALB/c *M. musculus* had lesions in both lobes, perivascular and interstitial edema, and hemorrhages, as well as alveolar wall thickening due to macrophage infiltration.[584,585] Similarly, C57BL6/J *M. musculus* inoculated with the Alpha variant showed viral RNA in the nasal turbinates, lungs, spleen, colon tissues, and brain, with alveolar wall congestion, inflammatory infiltration, and hemorrhages in the lungs.[560]

However, Currey et al. (2022) reported conflicting findings when inoculating C57BL6/J *M. musculus* with the Beta variant; C57BL6/J *M. musculus* did not show clinical symptoms or pathological changes in the lungs.[586] In addition, BALB/c *M. musculus* inoculated with the Omicron (B.1.1.529) variant (which also contains an N501Y substitution) did not show clinical symptoms or changes in pulmonary function on whole-body plethysmography. Omicron-inoculated *M. musculus* also showed significantly lower viral titers in the lungs and nasal turbinates compared to Beta variant-inoculated *M. musculus*.[580] Although mouse-adapted SARS-CoV-2 strains similarly contained the N501Y substitution and caused increased virulence in *M. musculus*,[587] these conflicting findings make it unclear whether *M. musculus* are susceptible to infection from N501Y variants.

SARS-CoV-1

BALB/c *M. musculus* showed age-dependent signs of infection when inoculated with SARS-CoV-1.[588,589] Young BALB/c *M. musculus* experienced mild clinical symptoms and were often asymptomatic,[590] while older BALB/c *M. musculus* had clinical symptoms of weight loss,

hunched posture, ruffled fur, and mild dehydration.[588] In older BALB/c *M. musculus*, viral titers were detected in the lungs, nasal turbinates, and liver, with IHC detecting viral antigen in epithelial cells of nasal turbinates and bronchioles and alveolar pneumocytes, but not in whole blood or spleen. From histopathological analysis, the lungs had pneumonitis, alveolar damage, hyaline membrane formation, intra-alveolar edema, and fibrotic foci.[588]

Older BALB/c *M. musculus* inoculated with SARS-CoV-1 also showed a higher number of differentially regulated host cellular genes than younger *M. musculus*. Furthermore, the number of differentially expressed genes in lung tissues of older SARS-CoV-1-inoculated *M. musculus* were significantly greater compared to younger *M. musculus*. A set of genes associated with cell cycle (e.g., DNA repair, cell development, and cell death) were downregulated in younger *M. musculus* but upregulated in older *M. musculus*. The upregulation of these cell cycle genes, along with continuous upregulation of genes associated with immune response through 7 dpi, may contribute to immunopathology and delays in viral clearance in older SARS-CoV-1-inoculated *M. musculus*.[589]

Non-Human Primates

Rhesus Macaque

SARS-CoV-2

Studies on *M. mulatta* inoculated with SARS-CoV-2 report conflicting findings. Some studies found that SARS-CoV-2-infected *M. mulatta* showed no clinical symptoms, including no increases in body temperature or decreases in weight,[576,591] while others found changes in respiratory pattern, increased body temperature, reduced appetite, hunched posture, pale appearance, and dehydration.[592,593] Choudhary et al. (2022) and Munster et al. (2020) both inoculated *M. mulatta* with WA1/2020 SARS-CoV-2 at similar doses via intratracheal and intranasal routes; however, ocular inoculation alone did not affect weight or body temperature.[321] When inoculated with the Beta (B.1.351) variant, *M. mulatta* experienced weight loss and increased body temperature, as well as hematological changes indicative of acute viral infection.[594]

Studies confer viral shedding is detected from swabs of the nose, oropharyngeal, rectum, and bronchoalveolar lavage (BAL), with no viral shedding detected in urine.[576,592,593] Viral RNA was detected transiently in blood, as well as in the gastrointestinal tract and lymphoid tissues.[592,593] From IHC analysis, viral antigen was found in type I and II pneumocytes and alveolar macrophages in lung tissue, as well as lymphocytes and macrophages in intestinal tract tissue.[593] Beta variant-inoculated *M. mulatta* supported more efficient viral replication in the lower respiratory tract and lung tissue compared to those inoculated with prototype SARS-CoV-2 strain GD108.[594] After ocular inoculation of *M. mulatta* with SARS-CoV-2, viral RNA was detected in the conjunctiva, lacrimal gland, nasal cavity, and throat, all of which form a bridge between ocular and respiratory tissue.[321]

From histopathology analysis, lung tissues contained multifocal lesions, interstitial pneumonia, diffuse alveolar damage, thickening of alveolar septa, alveolar oedema, fibrin with formation of hyaline membranes, and type II pneumocyte hyperplasia. In addition, some endothelial cells of blood vessels were necrotic with edematous vessel walls containing cellular debris, which is indicative of both vasculitis and endotheliitis.[576,592,593] In extrapulmonary tissues, one *M. mulatta* showed evidence of encephalitis, with brain tissue histopathology revealing multifocal inflammatory cell infiltrates and blood vessel cuffing. However, no other SARS-CoV-2-related histopathological changes were reported in other organ tissues.[576]

SARS-CoV-1

M. mulatta inoculated with SARS-CoV-1 showed no clinical symptoms of illness.[324,595] Viral titers were detected from nose and throat swabs and tracheal lavage samples, but not in plasma, urine, or fecal samples.[324] In addition, viral RNA is detected in tissues from lymph nodes, trachea, and lungs. Blood hematology and chemistry of infected *M. mulatta* often revealed low platelet counts, but no other remarkable changes. From histopathological analysis, lung tissues had lesions, focal consolidation, mild interstitial edema, alveolar inflammation, but no diffuse alveolar damage.[595]

MERS-CoV

M. mulatta inoculated with MERS-CoV displayed clinical symptoms of transient increased body temperature and decreased water intake. Viral RNA was detected in tissue homogenates of the lungs, but not in nasal turbinate, oropharyngeal, and rectal swabs. From IHC analysis, viral antigen was found extensively across type I and II pneumocytes, alveolar macrophages, eosinophils, and bronchial epithelial cells. From histopathological analysis, lung tissues had focal interstitial pneumonia, focal degeneration and necrosis of pneumocytes and bronchial epithelial cells, focal pulmonary oedema, and mild hemorrhage.[322,596] Extrapulmonary tissues did not exhibit any histopathological changes.[322]

Cynomolgus Macaque

SARS-CoV-2

Similar to *M. mulatta*, studies of *M. fascicularis* inoculated with SARS-CoV-2 report a range of clinical symptoms, with some reporting no changes in weight, body temperature, or adverse clinical signs,[500,591] and others reporting increased body temperature and decreased appetite.[597,598] In comparison to *M. mulatta*, infected *M. fascicularis* had similar viral RNA levels from nasal washes, throat swabs, and bronchiolar lavage, peaking at 3 dpi. Histopathological changes in the lungs of *M. fascicularis* were also comparable to *M. mulatta*, showing multifocal areas of pneumonitis with alveolar necrosis, alveolar wall thickening, alveolar oedema, and inflammatory infiltration. However, Bixler et al. (2022) found that lung tissues from *M. fascicularis* showed more severe pulmonary lesions compared to *M. mulatta*. Furthermore, *M. fascicularis* still contained viral antigen in lung tissues upon IHC analysis at 9 dpi, while most *M.*

mulatta no longer had viral antigen.[591] These findings suggest that *M. fascicularis* are potentially susceptible to more severe SARS-CoV-2 infection than *M. mulatta*.

SARS-CoV-1

M. fascicularis inoculated with SARS-CoV-1 show similar clinical symptoms to SARS-CoV-2 infection, as well as decreased activity, decreased appetite, and dyspnea;[595,599,600] however, one study reported that *M. fascicularis* were asymptomatic.[324] Viral RNA and titers were detected from nasal, throat, and rectal swabs in higher levels than those from *M. mulatta*, as well as urine (unlike SARS-CoV-2) and occasionally blood samples.[324,599] Hematology revealed minimal change to blood cell counts, and blood chemistry indicated elevated alkaline phosphatase, which may or may not necessarily reflect hepatic injury.[599] In addition, viral RNA was detected in tissues from lymph nodes, trachea, and lungs.[595] From histopathological analysis, lung tissues showed interstitial pneumonia similar to SARS-CoV-2 infection, with diffuse alveolar damage, alveolar edema, and necrosis of alveolar and bronchiolar epithelium.[600]

Pigtail Macaque

SARS-CoV-2

M. nemestrina inoculated with SARS-CoV-2 experienced mild clinical symptoms of decreased appetite, soft stool, and mild cough and dyspnea, but no change in weight, body temperature, or blood oxygen saturation levels. Viral titers in *M. nemestrina* were found in nasal, pharyngeal, and rectal swabs, with *M. nemestrina* showing higher titers from nasal swabs and lower titers from pharyngeal swabs compared to *M. mulatta*. From histopathological analysis, lung tissues had interstitial pneumonia with expanded alveolar septa lined by type II pneumocytes and occasional alveolar fibrin rafts, similar to mild histopathological findings in *M. mulatta*. However, mild residual interstitial pneumonia was observed in lung tissue of *M. nemestrina* even after viral antigen is no longer detected at 21 dpi, suggesting longer-term respiratory complications. In addition, D-dimer levels were elevated in blood samples from *M. nemestrina* within the first week of infection, indicating potential coagulopathy. These findings potentially suggest a more robust response from SARS-CoV-2 infection in *M. nemestrina* than *M. mulatta*.[601]

African Green

SARS-CoV-2

C. aethiops inoculated with SARS-CoV-2 exhibited clinical symptoms of decreased appetite and activity, increased body temperature, and mild dyspnea.[602,603] In both *C. aethiops* and *M. mulatta*, aerosol inoculation resulted in less severe and delayed onset of symptoms compared to multi-route inoculation via intranasal and intratracheal delivery.[603] Viral RNA and titers were detected from nasal swabs, pharyngeal swabs, rectal swabs, and bronchiolar lavage in comparable levels to *M. mulatta*, but were not detected in whole blood.[602,603] Similar to *M. nemestrina*, *C. aethiops* showed evidence of transient coagulopathy from increases in partial

thromboplastin time and circulating levels of fibrinogen. From histopathological analysis, lung tissues had mild multifocal pneumonia, characterized by inflammation of terminal bronchioles, alveolar edema, alveolar hemorrhage, and alveolar spaces lined with neutrophils, macrophages, and fibrin. The trachea also contained ulcerated with multifocal epithelial erosion and associated hemorrhage and fibrin. By 34 dpi, lung tissue damage had progressed, showing moderate multifocal chronic pneumonia, despite a lack of viral antigen upon IHC analysis.[602] These findings suggest that *C. aethiops* are susceptible to the most severe underlying pathology of SARS-CoV-2 infection compared to *M. mulatta*, *M. fascicularis,* and *M. nemestrina*.

SARS-CoV-1

C. aethiops inoculated with SARS-CoV-1 displayed no clinical symptoms, except for one reported case of transiently increased body temperature. Viral titers were detected in the throat, nose, and trachea in *C. aethiops* at higher levels than either *M. mulatta* or *M. fascicularis*. However, unlike *M. fascicularis*, viral RNA was not detected in plasma or urine from *C. aethiops*. Upon rechallenge of SARS-CoV-1 inoculation, viral replication was restricted to the upper respiratory tract. From IHC analysis, viral antigen was detected in type I pneumocytes and macrophages in lung tissues, but not in any extrapulmonary tissues. From histopathological analysis, lung tissues showed focal interstitial infiltrates indicative of pneumonia and edema.[324]

Marmoset

MERS-CoV

C. jacchus inoculated with MERS-CoV exhibited more severe clinical symptoms than *M. mulatta*, including weight loss, severe respiratory symptoms, and decreased water intake. From IHC analysis, lung tissues showed moderate levels of antigen in pneumocytes, with increased levels of antigen in alveolar macrophages. Compared to *M. mulatta*, histopathological analysis of *C. jacchus* lung tissues had more widespread pulmonary oedema and diffuse alveolar hemorrhage. Despite viral antigen levels being slightly lower in lung tissues and comparable infiltration of inflammatory cells,[596] these findings suggest that *C. jacchus* are susceptible to more severe MERS-CoV infection than *M. mulatta*.

Ferret

SARS-CoV-2

M. putorius furo showed age-dependent pathogenic characteristics from SARS-CoV-2 infection. *M. putorius furo* aged 1-2 years that were inoculated with SARS-CoV-2 exhibited more severe clinical symptoms than those aged 6 months, including elevated body temperature and greater weight loss, lethargy, and respiratory symptoms.[604] Other studies showed that younger *M. putorius furo* developed mild clinical symptoms, such as stagnated weight gain and ruffled fur (an indication of lethargy), but did not experience elevated body temperatures.[307] Variation in clinical symptoms between studies may also be affected by inoculation dose and route, with higher doses and intranasal inoculation resulting in more severe symptoms.[307,605] Interestingly,

M. putorius furo that were rechallenged with SARS-CoV-2 inoculation showed weight loss, lethargy, and ruffled fur that were not observed during the initial challenge.[307]

Older *M. putorius furo* also contained the highest viral titers from nasal turbinates compared to younger *M. putorius furo*, as well as higher viral RNA levels from fecal samples, indicating higher viral loads within the gastrointestinal tract. Younger *M. putorius furo* also had reduced viral RNA levels in the lower respiratory tract compared to older *M. putorius furo*, which researchers concluded was not associated with reduction in ACE2 receptor expression.[604] In addition, viral RNA has been detected in the BAL, tonsils, trachea, lung, and olfactory bulb, as well as cerebrum and cerebellum from intranasal inoculation. Despite showing increased clinical symptoms, viral shedding and lung pathology were significantly reduced in *M. putorius furo* rechallenged with SARS-CoV-2 inoculation.[307,605]

From histopathology of lung tissue, older *M. putorius furo* contained increased inflammatory cell infiltration and widened, edematous, and congested alveolar septa compared to younger *M. putorius furo*.[604] Lung tissue from both older and younger *M. putorius furo* showed mild peribronchitis with infiltrating cells in the sub-mucosa of bronchi.[605] Epithelial lining of nasal cavities were damaged, characterized by hypertrophy, hyperplasia, and squamous metaplasia, especially in *M. putorius furo* inoculated with higher doses of SARS-CoV-2.[307,605] In addition, the liver showed multifocal inflammatory cell infiltration, consisting of macrophages, lymphocytes, and plasma cells.[307]

SARS-CoV-1

M. putorius furo inoculated with SARS-CoV-1 displayed clinical symptoms of lethargy, with multiple studies having at least one *M. putorius furo* die before 4 dpi.[301,302] Viral RNA was detectable from pharyngeal swabs but not from either nasal or rectal swabs.[302] From immunofluorescence analysis, SARS-CoV-1 antigen was detected in the alveolus, in which antigen appeared in type II pneumocytes and alveolar macrophages. IHC analysis revealed that ACE2 receptors were additionally expressed in bronchiole, bronchus, trachea, and pulmonary blood vessel tissues, where SARS-CoV-1 antigen was not observed. Lastly, histopathology revealed lesions in tissues of the lung, liver, spleen, and bronchial lymph nodes. In lung tissue, histopathology revealed multifocal, mild-to-severe diffuse alveolar damage with macrophages and neutrophils present, as well as proteinaceous exudate in alveolar and bronchiolar lumina.[301] Considering the consistency of clinical and pathological features, SARS-CoV-1 pathogenesis is potentially more severe than SARS-CoV-2 pathogenesis in *M. putorius furo*.

Civet

SARS-CoV-1

P. larvata inoculated with SARS-CoV-1 have shown clinical symptoms of lethargy, reduced aggression, and elevated body temperatures starting at 3 dpi and remaining until 7 dpi. Viral RNA was detected in the lungs, liver, kidney, spleen, heart, and cerebrum and remained detectable in the lymph nodes and spleen up to 35 dpi. In addition, virus was detected in low

levels from blood samples, in which leucopenia was also observed. From histopathology analysis of lung tissue at 3 dpi, researchers found interstitial inflammatory infiltrates and congestion of the alveolar septa. Furthermore, the lumina of alveoli and bronchioles were filled with oedema fluid, erythrocytes, cellular debris, and lymphocytes. Findings from other tissues include spleens with extensive necrosis and atrophy of white pulp lymphoid aggregates; livers with diffuse congestion and renal cortices; and small intestines with focal hemorrhages. In addition, tissues from the cerebrum showed evidence of neuronal degeneration and mild neuronophagia, in which glial cell apoptosis occured.[606,607] Together, these findings suggest that *P. larvata* are highly susceptible to SARS-CoV-1 infection and show substantial disease pathogenesis compared to other species.

Companion Animals

Cat

SARS-CoV-2

F. catus inoculated with SARS-CoV-2 are typically asymptomatic;[387,388] however, in one case study, a 7-month-old *F. catus* presented with symptoms of dullness, lethargy, elevated temperature, and respiratory signs of coughing, wheezing, and dyspnea. Although no SARS-CoV-2 RNA was detected on nasal, oropharyngeal, and rectal swabs, and viral antigen was not detected on post-mortem IHC, SARS-CoV-2 antibodies were detected upon serum analysis. The *F. catus* was also positive for feline parvovirus, which typically causes panleukopenia, suggesting that a combination of SARS-CoV-2 and parvovirus infection may have contributed to death.[608] In another case study, an F*. catus* presented with respiratory and gastrointestinal symptoms and tested positive for SARS-CoV-2 infection. The *F. catus* showed a mild increase in red blood cells and reticulocytes and a mild decrease in platelets. After receiving amoxicillin and prednisone for 10 days, the cat no longer presented with any clinical symptoms.[609]

When infected with SARS-CoV-2, *F. catus* shed virus orally, nasally, and rectally. However, when rechallenged with SARS-CoV-2, *F. catus* did not shed virus. Upon histology analysis, the nasal turbinates showed ulcerative, lymphoplasmacytic and neutrophilic rhinitis, and lymphoplasmacytic tracheitis. The lungs also had mild histological changes, including interstitial pneumonia with peribronchiolar and perivascular lymphocytic cuffing and alveolar histiocytosis.[387,390] In the case of the 7-month-old symptomatic cat, computed tomography (CT) images of the lungs revealed two heterogenous lesions and bilateral ground-glass opacities. In addition, post-mortem histopathology revealed interstitial pneumonia and type II pneumocyte hyperplasia, consistent with prior studies of SARS-CoV-2 infection in *F. catus*.[608]

Although *F. catus* typically do not show clinical symptoms when inoculated with wildtype SARS-CoV-2 variants, other variants of SARS-CoV-2 have caused symptoms.[610,611] Cats inoculated with the Delta (B.1.617.2) variant showed significant clinical symptoms compared to prior studies of *F. catus* inoculated with wildtype SARS-CoV-2, while *F. catus* inoculated with Omicron (BA.1.1) also remained asymptomatic. Similar to wildtype SARS-CoV-2 infection, *F. catus* infected with Delta and Omicron variants shed virus from nasal, oropharyngeal, and rectal swab, but virus

was detected at higher levels in *F. catus* infected with the Delta variant from samples of nasal turbinate, tonsil, retropharyngeal lymph node, trachea, lung, mediastinal lymph node, heart, liver, spleen, kidney, small intestine, and mesenteric lymph node. Gross post-mortem and histopathology findings were also more severe in *F. catus* inoculated with Delta in the lungs. Delta-inoculated *F. catus* showed dark red pulmonary consolidation, hemorrhage, and pulmonary edema, as well as diffuse alveolar damage and disruption of vascular architecture by infiltrating neutrophils and lymphocytes.[387,388,610]

From RNASeq analysis of lung samples from Delta-inoculated *F. catus*, many genes associated with activation of innate immunity and SARS-CoV-2 disease severity were upregulated. In addition, analysis of differentially expressed genes identified several clusters of dysregulated genes associated with clinical symptoms and lung pathology during the acute phase of infection. For example, differentially expressed gene analysis identified the gene encoding aurora kinase B (AURKB), which is associated with cell cycle progression and chromosome segregation and may contribute to SARS-CoV-2 N-protein mutation. Several genes associated with neurodegenerative diseases were also upregulated during the recovery phase of infection in Delta-inoculated *F. catus*.[387,388,610]

SARS-CoV-1

Similar to SARS-CoV-2 infection, *F. catus* inoculated with SARS-CoV-1 do not show any clinical symptoms. Although viral RNA was not detectable from nasal or rectal swabs, SARS-CoV-1-inoculated *F. catus* shed virus from the pharynx. In addition, low levels of SARS-CoV-1 antigen were detected in the respiratory tract, including titers in lung homogenates, and the intestine. In the respiratory tract, SARS-CoV-1 antigen and ACE2 receptor expression were primarily detected in type I and II pneumocytes and serous cells of tracheo-bronchial submucosal glands.[301,302]

From post-mortem histology analysis, lesions were observed in tracheo-bronchial submucosal glands, spleen, mesenteric lymph nodes, and Peyer's patches. Similar to SARS-CoV-2 histology, SARS-CoV-1-inoculated *F. catus* had multifocal diffuse alveolar damage in the lungs, characterized by cellular debris in the alveolar lumen and epithelial cells with karyorrhexis, karyopyknosis, multifocal necrosis, sparse type II pneumocyte hyperplasia, and infiltration with few neutrophils in the alveolar septa.[301,302]

Dog

SARS-CoV-2

C. lupus familiaris inoculated with or exposed to SARS-CoV-2 have shown clinical presentations ranging from asymptomatic to increases in body temperature, decreases in weight, and respiratory symptoms. Although Bosco-Lauth et al. (2020) did not detect any viral shedding by plaque assay at any time point post infection, Lyoo et al. (2023) detected viral RNA from nasal, rectal, and urethral swabs and demonstrated the ability to cultivate SARS-CoV-2 from these samples.[387,396,612] IHC of the lung tissue also showed SARS-CoV-2 antigen in alveolar

macrophages and neutrophils.[612] However, these studies provide evidence that *C. lupus familiaris* are less susceptible to SARS-CoV-2 infection than cats.[387,396,612]

Although Bosco-Lauth et al. (2020) reported not observing any gross lesions in SARS-CoV-inoculated *C. lupus familiaris*, post-mortem histopathology analyses from other studies have revealed mild interstitial pneumonia and perivascular infiltration of lymphocytes, macrophages, and neutrophils.[387,396,612] In addition, blood samples revealed decreases in platelet counts and increases in inflammatory factors, fibrinolysis, and clotting factors. These parameters are indicative of thrombocytopenia and lymphocytopenia, which may occur because of to pulmonary embolism, systemic thrombosis, and/or lymphocyte apoptosis.[396]

In a case study of a West Highland Terrier that presented with hemorrhagic diarrhea, the *C. lupus familiaris* tested positive for SARS-CoV-2 infection (B.1.177) upon next-generation sequencing of a fecal sample. The sequencing of the fecal sample also revealed an I402V substitution in the S protein of the virus, which the authors speculate may have affected the gastrointestinal tract.[402]

MERS-CoV

Similar to SARS-CoV-2, *C. lupus familiaris* inoculated with MERS-CoV showed increased body temperature and weight loss. MERS-CoV was detected from nasal, rectal, and urethral swabs, but not to the level of SARS-CoV-2 detection, indicating that *C. lupus familiaris* may not shed infectious MERS-CoV. Blood samples from MERS-CoV-inoculated *C. lupus familiaris* showed decreases in platelets and increases in lactate dehydrogenase levels, suggesting potential tissue damage. Similar to SARS-CoV-2-inoculated *C. lupus familiaris*, histopathology analysis of lung samples from MERS-CoV-inoculated *C. lupus familiaris* revealed mild interstitial pneumonia and focal bronchiolitis with perivascular infiltration of lymphocytes, macrophages, and neutrophils. However, MERS-CoV antigen was not detected in lung tissue from IHC analysis. Together, these data indicate that *C. lupus familiaris* are potentially less susceptible to MERS-CoV infection compared to SARS-CoV-2.[612]

Mink

SARS-CoV-2

Multiple studies of SARS-CoV-2 in mink populations have reported mixed findings of infection signals. A study of both wild and captive-bred minks (*N. vison* and *M. lutreola*) from Northern Spain found no evidence of systemic symptoms from general physical examinations, with no detection of SARS-CoV-2 RNA from swab samples nor SARS-CoV-2 antibodies from serum samples.[613] However, studies of farmed *N. vison* across Europe and North American have reported findings from SARS-CoV-2 positive cases.[614,615] Viral RNA has been detected from throat and rectal swab samples, as well as lung, conchae, liver, and intestine tissue samples. Clinical symptoms from SARS-CoV-2-positive *N. vison* include reduced food intake, nasal discharge, sneezing, and coughing, as well as respiratory distress.[615] *N. vison* that were

experimentally inoculated with SARS-CoV-2 also showed weight loss; however, viral RNA was not detected from concha or rectal swabs.[310]

Post-mortem histopathological findings from *N. vison* that died on farms revealed acute diffuse interstitial pneumonia with hyaline membrane formation and focal micro-hemorrhages in the alveolar septa, as well as fibrin thrombi formation in the lungs and other tissues.[614,615] Histopathological findings from tissue samples of experimentally inoculated minks showed similar results, as well as interstitial inflammatory infiltrates and intra-alveolar edema.[310]

SARS-CoV-2 genome sequencing from mink farms in Denmark, the Netherlands, and Greece all independently revealed a Y453F mutation of the S protein, suggesting that this variant is mink-specific and may have a higher binding affinity to *N. vison* ACE2 receptors.[614,615]

Sheep and Swine

Neither *O. aries* nor *S. scrofa* are susceptible to infection from SARS-CoV-2.[616,617] SARS-CoV-2 antibodies were not detected in *O. aries* that were in close contact with a veterinary student community from June 2020 to March 2021. However, direct detection of the virus using RT-PCR was not performed.[616] SARS-CoV-2 can infect porcine kidney and testicle cells, but viral RNA has not been detected from any swab or blood samples from inoculated *S. scrofa*. Gross and histopathological analyses of *S. scrofa* lung tissue did not identify any pathological lesions, SARS-CoV-2 antigen, or SARS-CoV-2 RNA. SAR-CoV-2 also failed to replicate in the respiratory and digestive tract of inoculated *S. scrofa*. More refined predictive analysis of the binding potential of SARS-CoV-2 with ACE2 receptors of *O. aries* and *S. scrofa* could provide insight into the lack of infection susceptibility, as well as additional studies on experimental infection.[616,617]

Camelid

MERS-CoV

C. dromedarius are commonly known as a primary reservoir for the MERS-CoV virus and have been studied because of the risk of zoonotic transmission of the virus to humans. MERS-CoV infection in camelids is characterized by minor clinical symptoms comprised of mild to moderate nasal discharge. MERS-CoV is primarily shed in these nasal secretions, but has not been detected in urine, whole blood, or serum. Viral RNA has been detected in feces, but the low level of detection indicates that feces are not likely a contributing factor in transmission.[618]

Viral antigen is primarily detected in the upper respiratory tract, with few reports of viral antigen present in the lower respiratory tree. Primary histopathological lesions are limited to the upper respiratory tract in MERS-CoV-infected camelids, resulting in epithelial cell necrosis, mucosal ulcerations, increased mucous production, and neutrophil accumulation in the nasal mucosa. During the acute phase of infection, inflammatory processes in the lower respiratory tract are limited to epithelial necrosis, lymphocytic infiltration, and squamous metaplasia.[618] One study noted that MERS-CoV infection in both New (i.e., *L. glama* and *V. pacos*) and Old World camelids (i.e., *C. dromedarius* and *C. bactrianus*) was associated with ciliocytophthoria

(i.e., ciliary loss) and depletion of DPP4.[619] Identifying mechanisms in which cilia presence and function are lost may be a key focus for future investigations in upper respiratory infections.[618]

Surveillance

Future surveillance of various animal species can be used to detect novel coronaviruses as well as outbreaks of specific coronaviruses. Optimal surveillance systems should enable early detection of coronaviruses while minimizing costs for sample collection and analysis. Different components of viral surveillance systems, their advantages and disadvantages, and their relevance to different surveillance goals are outlined below.

Geographies

Regions at high risk for zoonotic coronavirus spillover events should be actively surveilled for coronaviruses. Various environmental factors can impact risks for spillover events. Zoonotic spillovers to humans are associated with changes in land use that place humans in closer proximity to infected animals.[620] Therefore, areas of risk for interspecies transmission are those where one infected species, particularly a Chiropteran species, comes into contact with another species. The geographical distributions of different species are impacted by wildlife trade and live markets, climate change, and domestic species introductions (e.g., establishment of farms near wildlife populations).[621] Species interfaces critical for coronavirus transmission are outlined in detail in "Species Interfaces Relevant to Coronavirus Transmission."

Species

Species at risk for coronavirus infection should be prioritized for regular surveillance activities. Risk can be determined based on various criteria, including those outlined in "Evidence Types for Determining Species Susceptibility." Sentinel surveillance strategies can also be employed. For example, farm workers can serve as sentinels for cattle coronavirus infection; zookeepers as sentinels for captive animals; and veterinarians and veterinary technicians as sentinels for companion animals.

Clinical Evaluations and Monitoring

In animals with symptomatic infections, surveillance systems can use symptom data to guide additional testing (see "Assays") or as an early warning system of viral outbreaks. For example, biosensors on farmed pigs can detect temperature changes in real time associated with porcine reproductive and respiratory syndrome virus[622] as well as African swine fever.[623] Observations of clinical signs in farmed, captive, and companion animals can be reported to a central entity as part of a larger surveillance system

Assays

Different detection assays can be used to meet certain surveillance goals. Although sequencing methods may require higher monetary investment than reverse transcription-polymerase chain reaction (RT-PCR) methods, they do provide the most flexibility for detection of multiple pathogens. Metagenomic sequencing paired with strong bioinformatic analysis pipelines can detect various novel viruses within even a single sample. Targeted sequencing using conserved primers detects multiple viruses within specific viral groups. For example, targeted sequencing

of RNA-dependent RNA polymerase (RdRp) is used to detect novel coronaviruses in Chiropterans (see "Diagnostics" and "Viral RNA Detection"). When used as part of a surveillance system, these sequencing techniques can be used to monitor coronavirus evolution and detect mutations that may impact infectivity, transmissibility, and host species tropism.

RT-PCR methods are used alone to screen for the presence of specific coronavirus RNA. RT-PCR alone cannot provide detailed information about acquired viral mutations, but it can serve as a scalable, cost-effective method for screening large numbers of samples (see "RT-PCR" in the "Diagnostics" Section). Compared to costs between $7.20 to $43.30 per sample and duration of 20 hours for targeted sequencing of RdRp, RT-PCR costs approximately $12 per sample and takes only 4 hours to complete.[624] Primers used to detect a specific coronavirus should not cross-react with RNA from other coronaviruses or host genetic material to ensure specific and exclusive detection. When used as part of a surveillance system, large-scale RT-PCR methods can detect local outbreaks of specific coronaviruses in specific species of interest.

RT-PCR and sequencing methods are typically used to detect active infections, while serology methods detect past infections. Antibody detection methods are continually improved to increase scalability, decrease cost and time investments, and reduce the amount of specialized expertise required. Specific antibody detection methods used for coronaviruses are outlined in "Virus-Specific Antibody Detection." While serology surveillance systems cannot detect active infections, they can identify additional species susceptible to specific coronavirus infections.

Both viral RNA and antibody strategies have inherent advantages and disadvantages. Sample type availability often dictates which detection methods are possible; serology tests require blood samples, which are more difficult to collect, especially from wild animals, than oral, nasal, or fecal samples that can be used for viral RNA detection. Detection of viral RNA indicates active infection, and although seropositivity is usually not used to determine infection timing, some researchers have quantified antibody levels to roughly estimate infection timing in humans and animals.[625,626] Overall, because viral infections usually resolve after a period of time, the rate of active infections of a coronavirus is much lower than the rate of seropositivity for the same coronavirus. Therefore, larger sample sizes are likely required to detect active infections compared to sample sizes used for the detection of coronavirus antibodies.

Sample Types and Sizes

Biological Sample Types

Different biological sample types are required for the detection of different assay readouts. Oral, nasal, respiratory, anal, and fecal swabs are used to detect coronavirus viral RNA, while blood samples are required for detection of coronavirus antibodies. Different types of samples require different levels of contact between researchers and animal subjects. Blood sampling requires close, prolonged contact with animals and may not be feasible for certain wildlife species. Oral, nasal, and rectal swabs require more limited animal contact, and fecal samples can be safely collected well after excretion with minimal to no animal contact. Notably,

reported RNA extraction methods used different pre-extraction steps for fecal samples versus swab samples, but extraction kits were used across sample types.

Interestingly, scientists created a device called the SnotBot® to collect blowhole samples from cetaceans with minimal invasiveness, which could be used to detect respiratory pathogens such as coronaviruses.[627]

Pooled Sampling Strategies

Pooled sampling methods are currently used to reduce the cost of SARS-CoV-2 surveillance in humans at airports. The Centers for Disease Control and Prevention has deployed at select airports pooled surveillance strategies using nasal samples from voluntary travelers as well as samples collected from aircraft wastewater tanks.[628,629] Similar pooled sampling strategies could be applied to various animal species for initial detection of coronaviruses in oral, nasal, rectal, or fecal samples. However, sequence analysis of pooled samples requires robust bioinformatic pipelines capable of data deconvolution to assess the presence of multiple mutations within the same viral genome.[630] Further streamlining of these pipelines as well as adaptation for use in animal species will enable successful pooled sample surveillance systems for zoonotic coronaviruses.

Environmental Sample Types

Environmental sampling requires less invasive procedures compared to more conventional biological sampling methods and may represent many different animals, even from different species. Sample types include fomites, air, and wastewater. Notably, the lack of detection of coronaviruses in air, fomites, or wastewater does not eliminate the possibility of water droplet and airborne transmission-dependent coronavirus outbreaks. However, detection of coronavirus RNA in environmental samples can identify potential modes of transmission threatening certain animal species. Environmental samples positive for coronavirus RNA should also be tested for live, infectious virus to further determine the level of risk of transmission. Infectious virus can be determined based on successful viral culture and isolation, but these methods are very difficult. As an alternative, a research group in Portugal collected air samples and pretreated them with RNAse A to destroy any non-encapsulated coronavirus RNA, leaving only RNA within intact viral particles.[631]

Various sampling devices have been described previously for air sample collection and surveillance: Coriolis Compact (Bertin Instruments),[631] AerosolSense™ (Thermo Fisher Scientific),[632] and various in-house sampling devices.[633–636] Notably, an air-to-liquid device collected air samples in 1-2 minutes, compared to several hours required from traditional air sampling devices.[633]

Efforts across the globe are using sewershed samples to monitor SARS-CoV-2 RNA in wastewater, and this monitoring has proven useful and less expensive than clinical sampling. However, multiple published articles detail concerns about discharge of SARS-CoV-2-contaminated wastewater into bodies of water.[161,162] To detect SARS-CoV-2 RNA in

contaminated coastal waters, researchers may need to concentrate SARS-CoV-2 and other coronaviruses from water samples. One peer-reviewed publication compared five different methods for concentrating SARS-CoV-2 in wastewater and found that the elution with beef extract and polyethylene glycol precipitation method as well as Amicon filtration robustly concentrated SARS-CoV-2 in real-world wastewater samples.[637] Notably, although coastal water samples may contain coronavirus RNA, this does not necessarily indicate the presence of live, infectious virus. In addition, the viral concentration may not be sufficient for transmission to cetaceans and other animals.

Surveillance using fomite samples can provide indications of viral shedding as well as risk to nearby animal populations. For example, bedding belonging to a SARS-CoV-2-positive *F. catus* tested positive for SARS-CoV-2 RNA in Switzerland.[425] However, the utility of fomite-based surveillance depends on coronavirus stability on fomite surfaces (see "Fomites").

Sample Size

Surveillance systems should also use adequate sample sizes for coronavirus detection. Various computational models can be used to estimate sufficient sample sizes for future surveillance efforts.[638–640] Notably, sample size calculations depend on various factors, including the key question for the surveillance project (e.g., virus detection versus variant detection versus variant frequencies) and intended sampling frequency (e.g., cross-sectional versus periodic surveillance).[639]

Types of Surveillance Systems

Passive Versus Active Surveillance

Passive surveillance strategies rely on case reports; for example, the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) collects animal cases of SARS-CoV-2 in the United States.[328] The USDA National Animal Health Reporting System (NAHRS) is another voluntary disease reporting program for animal diseases.[641] An ideal passive surveillance strategy, however, would consist of a global effort of animal case reporting to a central entity. For example, the World Organisation for Animal Health (WOAH) collects voluntary SARS-CoV-2 case reports in animals across the globe.[642] Conversely, active surveillance systems are more targeted and designed to surveil for a specific pathogen in a specific species in a particular area. Many articles cited within this review containing coronavirus incidence or prevalence are examples of active surveillance projects. Their diagnostic methods (see "Diagnostics") were scalable and appropriate for use in animals; these methods could inform the design of a global, active surveillance program of emerging coronaviruses.

Syndromic Versus Laboratory-Based Surveillance

Syndromic surveillance strategies use symptom data to identify potential cases, while laboratory-based surveillance uses specific assays to identify a causative pathogen (see "Assays"). Although syndromic surveillance does not involve the identification of a pathogen, it does enable non-experts to identify syndromic cases. In a passive surveillance system, everyday

citizens can report symptomatic or deceased animals; such a system is used for highly pathogenic avian influenza (HPAI) surveillance in the United States, and another for SARS-CoV-2 is already in development. Individuals can contact local or state wildlife and animal health agencies as well as local veterinarians to report deceased birds, while mass die-offs and other unusual observations should be reported to the USDA directly via their hotline.[643] In contrast, laboratory-based surveillance programs are more resource-intensive and require careful planning to maximize useful information and minimize costs.

Sentinel and Targeted Active Versus Whole Population Surveillance

Sentinel surveillance focuses on testing fewer animals as representatives of larger animal populations. Sentinel strategies can provide critical surveillance information in a shorter period of time with a smaller financial investment.[644] Sentinel animals are sometimes used as a surveillance tool to monitor viruses present in species that are more difficult to sample. For example, analysis of potential surveillance strategies for HPAI utilized mute swans (*Cygnus olor*) and *A. platyrhynchos* as sentinel animals for wild birds. Because of H5N1's high mortality in birds, researchers designed a targeted active surveillance strategy to test dead birds, which was more cost-effective than capturing live birds.[645] Selection of appropriate sentinel species is critical for designing a sentinel surveillance system that can adequately answer surveillance questions.

Diagnostics

Viral RNA Detection

Viral RNA detection is used as a proxy for active infection, but the presence of genetic material is not always indicative of live virus. One RNA isolation protocol—viral particle-protected nucleic acid extraction—uses RNAses to destroy non-encapsulated RNA to subsequently isolate genetic material from intact viral particles, but this protocol has not been in widespread use for coronavirus surveillance applications.[631,646–648] Various methods used for surveillance of SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are summarized below.

Next-Generation Sequencing

Next-generation sequencing strategies are often used to screen samples for a variety of viruses. Metagenomics techniques screen for pathogenic nucleic acids and have been applied to Chiropterans and *M. javanica*.[29,51,649] Targeted sequencing methods characterize RT-PCR-amplified conserved regions of closely related viruses. Targeted sequencing of RdRp is commonly used to identify coronaviruses, as summarized in Table 13.

Table 13: Conserved Coronavirus RT-PCR Strategies

Target	Viral RNA Detection Method	Primers	Validated Species
RdRp	nested RT-PCR[650]	PCR 1: TTATGGGTTGGGATTATC + TGATGGGATGGGACTATC; PCR 2: CTTATGGGTTGGGATTATCCTAAGTGTGA + CTTATGGGTTGGGATTATCCCAAATGTGA	Various Chiropterans,[24,26,34,43,50,155] *Camelus dromedarius*[651]

Target	Viral RNA Detection Method	Primers	Validated Species
	RT-PCR[652]	TCCTAAGTGTGATAGAGCTATGCC + GTGCACACTCATTTGCTAACCG	Various Chiropterans,[38,44,49] *Manis javanica*[38,475]
	Nested RT-PCR with degenerative primers[37]	PCR 1: CGTTGGIACWAAYBTVCCWYTICARBTRGG + GGTCATKATAGCRTCAVMASWWGCNACNACATG; PCR 2: GGCWCCWCCHGGNGARCAATT + GGWAWCCCCAYTGYTGWAYRTC	Various Chiropterans,[25,38,44] *Manis javanica*[38,475]
	RT-PCR[653]	AYAACCAAGATCTTAATGG + TGCTTAGAACCCAAAATCAT	Various Chiropterans,[22] *Felis catus*[354]
	Heminested RT-PCR[654]	PCR 1: GARTTYGATTGGRCKCGKTAYGA/GARTTYGATTGGRCKAGGTAYGA + GGYTTKACCCACATNCCRAA; PCR 2: CGKTAYGATGGKACKATHCC/AGGTAYGATGGKACKATHCC + GGYTTKACCCACATNCCRAA	Various Chiropterans[654]
	RT-PCR[655]	GGTTGGGACTATCCTAAGTGTGA + CCATCATCAGATAGAATCATCATA	Various Chiropterans[30,31]
	Nested RT-PCR[656]	PCR 1: CARATGAATYTIAARTAYGC + reverse TGYTGWGARCAAAAYTCRTG; PCR 2: ATGGGWTGGGAYTAYCCIAARTG-3'+ reverse ACRTTRTTYTGRWARTA	Various Chiropterans[30,31]
	RT-PCR[657]	PCR 1: ATGGGITGGGAYTATCCWAARTGTG + AATTAT ARCAIACAACISYRTCRTCA; PCR 2: ATGGGITGGGAYTATCCWAARTGTG + CTAGTICCACCIGGYTTWANRTA	Various Chiropterans[48]
	RT-PCR to conserved RdRp[658]	GGTTGGGACTATCCTAAGTGTGA + CCATCATCAGATAGAATCATCATA	Various Chiropterans,[33,42]
	RT-PCR[659]	ACWCARHTVAAYYTNAARTAYGC + TCRCAYTTDGGRTA RTCCCA	Various Chiropterans[47,660]
RBD	RT-PCR[155]	PCR 1: VWGADGTTGTKAGRTTYCCT + TAARACAVCCWGCYTGWGT; PCR 2: TGTKAGRTTYCCTAAYATTAC + ACATCYTGATANARAACAGC	Various Chiropterans[26]
S	RT-PCR[661]	TGGCWTATAGGTTYAATGGYATTGGAG + CCGTCGATTGTGTGWATTTGSACAT	*Manis javanica*[661]

RT-PCR

Large numbers of samples are readily screened for coronavirus pathogens using various RT-PCR amplification methods specific to a coronavirus of interest. Primers selected for RT-PCR should be specific and not produce off-target RT-PCR products from host species or other pathogens. Nested and heminested RT-PCR strategies improve both sensitivity and specificity to the viral gene of interest by using multiple primer pairs.

SARS-CoV-1

SARS-CoV-1 viral RNA has been isolated from *P. larvata* using three different RT-PCR strategies, summarized in Table 14.

Table 14: SARS-CoV-1 RT-PCR Strategies

Target	Viral RNA Detection Method	Primers	Validated Species
P and N	RT-PCR kit from Qiagen[662]	Not specified	*Paguma larvata*[77]
ORF1ab and N	RT-PCR and nested RT-PCR[663]	Nested N PCR 1: ATGAATTACCAAGTCAATGGTTAC + CATAACCAGTCGGTACAGCTAC Nested N PCR 2: GAAGCTATTCGTCACGTTCG + CTGTAGAAAATCCTAGCTGGAG ORF1ab PCR: TACACACCTCAGCGTTG + CACGAACGTGACGAAT	*Paguma larvata*[73]
N, M, S	RT-PCR[71]	PCR N: ATGTCTGATAATGGACCCCAAT + TTATGCCTGAGTTGAATCAG PCR M: ATGGCAGACAACGGTACTATT + CTTACTGTACTAGCAAAGCAAT PCR S: ATGTTTATTTTCTTATTATTTC + GTCGACATGCTCAGCTCCTAT	*Paguma larvata*[71]

MERS-CoV

MERS-CoV viral RNA has been isolated mainly from Chiropterans and *C. dromedarius*, as well as other livestock species. Amplification strategies are summarized in Table 15.

Table 15: MERS-CoV RT-PCR Strategies

Target	Viral RNA Detection Method	Primers	Validated Species
UpE	RT-PCR[664]	GCAACGCGCGATTCAGTT + GCCTCTACACGGGACCCATA	*Camelus dromedarius*;[53,59,60,62,63,66–70] *Llama glama*;[369] *Vicugna pacos*;[369,370] *Ovis aries*;[361] *Capra hircus*;[361] *Bos taurus*;[361] *Equus asinus*;[361] various Chiropterans[44]
N2 & N3	RT-PCR[665]	GGCACTGAGGACCCACGTT + TTGCGACATACCCATAAAAGCA; GGGTGTACCTCTTAATGCCAATTC + TCTGTCCTGTCTCCGCCAAT	*Camelus dromedarius*[61,67]

Target	Viral RNA Detection Method	Primers	Validated Species
N	nested RT-PCR followed by sequencing[666]	PCR 1: CCTTCGGTACAGTGGAGCCA + GATGGGGTTGCCAAACACAAAC; PCR 2: TGACCCAAAGAATCCCAACTAC; GATGGGGTTGCCAAACACAAAC	*Camelus dromedarius*;[53,60,63] *Ovis aries*;[361] *Capra hircus*;[361] *Bos taurus*;[361] *Equus asinus*;[361] various Chiropterans[44]
ORF1b	RT-PCR[664]	TTCGATGTTGAGGGTGCTCAT + TCACACCAGTTGAAAATCCTAATTG	various Chiropterans[44]
ORF1a	RT-PCR[666]	CCACTACTCCCATTTCGTCAG + CAGTATGTGTAGTGCGCATATAAGCA	*Camelus dromedarius*;[53,59,60,62,66,68,69] *Ovis aries*;[361] *Capra hircus*;[361] *Bos taurus*;[361] *Equus asinus*;[361] various Chiropterans[44]
RdRp	RT-PCR followed by sequencing[666]	TGCTATWAGTGCTAAGAATAGRGC + GCATWGCNCWGTCACACTTAGG	*Camelus dromedarius*;[53,60,65] various Chiropterans[44]

SARS-CoV-2

SARS-CoV-2 viral RNA has been isolated mainly from *F. catus*, *C. lupus familiaris*, and Chiropterans, as well as other feline species, small carnivores, and *O. virginianus*. These amplification strategies are outlined in Table 16.

Table 16: SARS-CoV-2 RT-PCR Strategies

Target	Viral RNA Detection Method	Primers	Validated Species
E	RT-PCR[667]	ACAGGTACGTTAATAGTTAATAGCGT + ATATTGCAGCAGTACGCACACA	Various Chiropterans;[25] *Felis catus*;[126,399,404,405,420,421,425,434,438] *Manis javanica*;[475] *Canis lupus familiaris*;[126,387,399,403–405] *Neogale vison*;[118]
	COVISure (Genetix)	Not provided	*Panthera pardus*[474]
	Bio-T kit TRISTAR COVID-19 (Biosellal)	Not provided	*Felis catus*[409]
	Allplex 2019-nCoV Assay (SeeGene) for RdRp, N, E	Not provided	*Canis lupus familiaris*;[430] *Puma concolor*[468]
	RT-PCR E/RP kit (Bio Manguinhos)	Not provided	*Felis catus*;[431] *Canis lupus familiaris*[431]

Target	Viral RNA Detection Method	Primers	Validated Species
	modular RT-PCR kits for multiplexing (TIB MOLBIOL)	Not specified	*Felis catus*;[415,421,442] *Panthera leo*[468]
M	Genesig COVID-19 kit	Not provided	*Felis catus*[436]
	RT-PCR[668]	GGYTCTAARTCACCCATTCA + TGATACTCTARAAAGTCTTCATA	*Felis catus*;[415] *Canis lupus familiaris*[387]
N	RT-PCR[667]	CACATTGGCACCCGCAATC + GAGGAACGAGAAGAGGCTTG	Various Chiropterans;[25] *Felis catus*;[399,405,420,421] *Canis lupus familiaris*[399,405]
	Viasure RT-PCR kit (certest)	Not provided	*Lutra lutra*;[130] *Neogale vison*[669]
	Allplex 2019-nCoV Assay (SeeGene) for RdRp, N, E	Not provided	*Canis lupus familiaris*;[430] *Puma concolor*[468]
	EURORealTime SARS-CoV-2 (EUROIMMUN) to ORF1ab and N	Not provided	*Canis lupus familiaris*[430]
	RT-PCR E/RP kit (Bio Manguinhos)	Not provided	*Felis catus*;[431] *Canis lupus familiaris*[431]
	OPTI SARS-CoV-2 RT-PCR (Thermo Fisher Scientific)	Not provided	*Odocoileus virginianus*;[349,350] *Felis catus*[441]
	IDT Primer & Probe Kit (IDT)	Not specified	*Felis catus*[440]
	modular RT-PCR kits for multiplexing (TIB MOLBIOL)	Not specified	*Felis catus*;[415,421,442] *Panthera leo*[468]
nsp16	RT-PCR[668]	GGWCAAATCAATGATATGATTTT + GTTGTTAACAAGAACATCACTAGA	*Felis catus*;[415] *Canis lupus familiaris*[387]
ORF1ab	Genesig COVID-19 kit	Not provided	*Felis catus*[436]
	Viasure RT-PCR kit (certest)	Not provided	*Lutra lutra*;[130] *Neogale vison*[669]
	EURORealTime SARS-CoV-2 (EUROIMMUN) to ORF1ab and N	Not provided	*Canis lupus familiaris*[430]
ORF1b	RT-PCR[670]	TGGGGYTTTACRGGTAACCT + AACRCGCTTAACAAAGCACTC	*Felis catus*[435]
RdRp	RT-PCR[667]	GTGARATGGTCATGTGTGGCGG + CARATGTTAAASACACTATTAGCATA	various Chiropterans;[25] *Felis catus*;[405,421,425] *Canis lupus familiaris*;[387,403,405] *Manis javanica*;[475] *Odocoileus virginianus*[351,355,356]
	COVISure (Genetix)	Not provided	*Panthera pardus*[474]

Target	Viral RNA Detection Method	Primers	Validated Species
	Bio-T kit TRISTAR COVID-19 (Biosellal)	Not provided	*Felis catus*[409]
	Allplex 2019-nCoV Assay (SeeGene) for RdRp, N, E	Not provided	*Canis lupus familiaris*;[430] *Puma concolor*[468]
	RT-PCR E/RP kit (Bio Manguinhos)	Not provided	*Felis catus*;[431] *Canis lupus familiaris*[431]
	IDT Primer & Probe Kit (IDT)	Not specified	*Felis catus*[440]
	modular RT-PCR kits for multiplexing (TIB MOLBIOL)	Not specified	*Felis catus*;[415,421,442] *Panthera leo*[468]
S	Genesig COVID-19 kit	Not provided	*Felis catus*[436]

Sample Types

Coronavirus RNA is mainly detected in oral and nasal samples as well as fecal samples. Viral detection via fecal samples is only appropriate when the species of interest sheds a virus of interest in its fecal matter, which does not always occur for respiratory viruses. Reported RNA extraction methods of SARS-CoV-1, MERS-CoV, and SARS-CoV-2 used different pre-extraction steps for fecal samples versus swab samples, but extraction kits were used across sample types.

Virus-Specific Antibody Detection

Antibody detection and neutralization methods are frequently used to assess individuals for previous exposure to coronaviruses of interest. Because coronaviruses contain similar S proteins, antibodies to one coronavirus may cross-react and provide protection against subsequent infection with another coronavirus. Therefore, antibody detection and neutralization assays should be assessed for specificity to the coronavirus of interest, especially when used in various animal species vulnerable to infection with other coronaviruses. For example, researchers studying SARS-CoV-2 prevalence in *F. catus* previously demonstrated that feline sera with hyperimmunity to feline infectious peritonitis—a feline coronavirus—did not cross-react with SARS-CoV-2 enzyme-linked immunosorbent assays (ELISAs) and microneutralization assays (MNAs); this increased confidence that positive results were due to previous exposure to SARS-CoV-2, not another coronavirus.[671] Various antibody detection and neutralization assays are summarized below.

Antibody Binding Detection

Various serological technologies have been adapted to detect binding antibodies for coronaviruses as strategies for discerning prior exposure to specific viruses. Antibody binding assays have been developed for SARS-CoV-1, MERS-CoV, and SARS-CoV-2. Although these strategies are readily scalable, results are less biologically relevant compared to virus neutralization assays. Samples positive for binding antibodies do not always contain neutralizing antibodies that confer protection against reinfection with the same virus;

therefore, antibody detection methods should be selected based on correlation with neutralization assays.

Enzyme-linked Immunosorbent Assay

ELISAs are scalable tests used for detecting various peptides, including antibodies. ELISA strategies for coronavirus antibody detection use spike or RBD antigens and indirect and double-antigen sandwich strategies. Notably, although the indirect method can be highly sensitive, it uses a secondary antibody for binding detection, which may result in nonspecific signals. The double-antigen sandwich ELISA retains similar sensitivity while increasing specificity at the detection step; rather than a secondary antibody for detection, the bound antigen-antibody complex is incubated with tagged (e.g., fluorescence, luminescence, HRP) antigen. In addition, previous evidence suggests that for coronaviruses, ELISAs targeted to S and RBD are more virus-specific but less sensitive compared to N-targeted ELISAs.[651,672,673] Therefore, prior to selecting an ELISA for use in animals, the method should be tested for cross-reactivity with antibodies to other common pathogens in the species of interest to verify assay specificity.

Both in-house laboratory and commercial indirect ELISA protocols can detect MERS-CoV-binding antibodies (Table 17). Euroimmun manufactures an indirect ELISA kit for MERS-CoV S1 protein antibodies that has been used successfully in *C. dromedarius, V. pacos,* and *L. glama*,[69,369] while in-house strategies have only detected MERS-CoV antibodies in *C. dromedarius*.[61,68,70,674]

Table 17: ELISAs for MERS-CoV Antibodies

Target	ELISA Type	Laboratory/Company	Species with Successful Antibody Detection
S1	Indirect	Euroimmun	*Camelus dromedarius*[69,369]
			Vicugna pacos[369]
			Llama glama[369]
		Alexandersen (National Centres for Animal Disease, Canada)	*Camelus dromedarius*[674]
		Drosten (University of Bonn, Germany)	*Camelus dromedarius*[70]
S	Indirect	Alagaili (King Saud University, Saudi Arabia) and Briese (Columbia University, United States)	*Camelus dromedarius*[68]
		Agwanda (National Museums of Kenya, Kenya)	*Camelus dromedarius*[61]
N	Indirect	Alagaili (King Saud University, Saudi Arabia) and Briese (Columbia University, United States)	*Camelus dromedarius*[68]

Indirect ELISA methods for SARS-CoV-1 have been mostly used for antibody detection in human samples, although an indirect S ELISA has successfully detected SARS-CoV-1 antibodies in palm civet.[73] Studies in patient samples have demonstrated that an ELISA for N-binding antibodies was more sensitive than an ELISA for S-binding antibodies.[675,676]

Both indirect and double-antigen sandwich ELISAs have successfully detected SARS-CoV-2-binding antibodies in some animal species and are summarized in Table 17. Numerous in-house ELISAs tested in animals have been used in seroprevalence studies of *F. catus* and *C. lupus familiaris*. Developers of some methods have previously published cross-reactivity data for other coronaviruses. Because of sequence similarities, SARS-CoV-2 ELISAs may cross-react with antibodies to SARS-CoV-1 and MERS-CoV. For example, the Sparer laboratory's indirect RBD ELISA cross-reacts with SARS-CoV-1, and Biorad's SARS-CoV-2 ELISA may cross-react with MERS-CoV.

Developers of other ELISAs have assessed their methods for cross-reactivity with other, less similar coronaviruses. The Segalés and Vergara-Alert laboratories tested lions using their double-antigen sandwich method for N that was non-reactive with OC43 and Bov-CoV.[465] In addition, the Sparer laboratory's indirect RBD ELISA did not cross-react with antibodies for turkey coronavirus, porcine respiratory coronavirus, canine coronavirus, feline coronavirus, and Bov-CoV.[354] Another ELISA for RBD from the laboratories of Zou, Shi, and Jin demonstrated no cross-reactivity for feline coronavirus, making it a suitable method for feline coronavirus seroprevalence studies.[671] Vircell's indirect S and N ELISAs show no cross-reactivity to cCoV and CRCoV, indicating suitability for canine seroprevalence studies.[393] Biorad's double-antigen ELISA for N does not cross-react with antibodies for CoV 229E, CoV NL63, CoV HKU1, and CoV OC43, but data on other animal coronaviruses were not found before the completion of this literature review.[677]

Notably, Innovation Diagnostics' (ID's) double-antigen ELISA for N (i.e., IDScreen®) has successfully detected SARS-CoV-2 antibodies in numerous species including *O. aries,*[359] *C. hircus*[359]*, F. catus*[401,405,406,416,421,435,437,438]*, S. scrofa,*[326,360] *V. vulpes,*[360] *Canis aureus moreoticus,*[360] *M. martes,*[131] *M. meles,*[131] *C. lupus familiaris,*[326,401,427] and *Allochrocebus solatus.*[326] However, cross-reactivity has only been officially demonstrated for avian and porcine coronaviruses. ID stated in assay documentation that the lack of conservation of N protein sequences across other coronaviruses suggests that cross-reactivity with other animal coronaviruses would be minimal.[678] Because of the wide applicability of IDScreen to various species, this ELISA may become an important tool for seroprevalence studies in other species identified as susceptible to SARS-CoV-2 infection.

Table 18: ELISAs for SARS-CoV-2 Antibodies

Target	ELISA Type	Laboratory/Company	Species with Successful Antibody Detection
RBD	Indirect	Gamarnik (Fundación Instituto Leloir-CONICET, Buenos Aires, Argentina)	*Felis catus*[436,679]
		Egberink (Utrecht University, Utrecht, Netherlands)	*Felis catus*, *Canis lupus familiaris*[680]
		Raybiotech	*Felis catus*[403]
		Stevanovic, Tabain, Vilibic-Cavlek (University of Zagreb and Croatian Institute of Public Health, Croatia)	*Canis lupus familiaris*[393,394]

Target	ELISA Type	Laboratory/Company	Species with Successful Antibody Detection
		Sparer (University of Tennessee, United States)	*Felis catus, Odocoileus virginianus*[354]
		Zou (Huazhong Agricultural University, China), Shi (Wuhan Institute of Virology, China), and Jin (Huazhong Agricultural University and Ministry of Agriculture, China)	*Felis catus*[671]
		Beer (Friedrich-Loeffler-Institut, Germany)	*Felis catus*[417,418,681]
		Fernández (University of Zaragoza, Spain)	*Felis catus,*[423,424] *Mustela putorius furo*[132]
		Ly and Liang (University of Minnesota, United States)	*Felis catus, Canis lupus familiaris*[408]
		Klaus (University of Zurich, Switzerland)	*Felis catus,*[400,425] *Canis lupus familiaris*[400]
		Yilmaz (Istanbul University-Cerrahpasa, Turkey)	*Felis catus*[426]
S	Indirect	Egberink (Utrecht University, Netherlands)	*Felis catus*, *Canis lupus familiaris*[680]
		Huergo (Federal University of Paraná, Brazil)	*Canis lupus familiaris*[430]
		Beer (Friedrich-Loeffler-Institut, Germany)	*Felis catus*[417,418,681]
		Vircell	*Felis catus, Canis lupus familiaris*[393]
N	Double antigen	Innovation Diagnostics	*Ovis aries,*[359] *Capra hircus,*[359] *Felis catus*[401,405,406,416,421,435,437,438], *Sus scrofa,*[326,360] *Vulpes vulpes,*[360] *Canis aureus moreoticus,*[360] *Martes martes,*[131] *Meles meles,*[131] *Canis lupus familiaris,*[326,401,427] *Allochrocebus solatus*[326]
		Segalés and Vergara-Alert (Universitat Autónoma de Barcelona, Spain)	*Panthera leo*[465]
		Biorad	*Felis catus*[441]
	Indirect	Egberink (Utrecht University, Netherlands)	*Felis catus*[680]
		Huergo (Federal University of Paraná, Brazil)	*Canis lupus familiaris*[430]
		Ly and Liang (University of Minnesota, United States)	*Felis catus, Canis lupus familiaris*[408]
		Vircell	*Felis catus, Canis lupus familiaris*[393]

Protein Microarray

Protein microarrays enable multiplexing for multiple antigens, require smaller sample amounts, and often provide higher sensitivity compared to ELISAs.[682] A quantitative SARS-CoV-2 antibody microarray originally used to assess vaccines[683] has successfully detected antibodies to S, RBD, and N in cats.[420] Another protein microarray for multiple coronaviruses (i.e., 229E, NL63, OC43, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2) developed for use in humans[684] detected SARS-CoV-2 antibodies in one beech marten (*Martens martens*).[118] Although protein microarrays can

potentially provide multiplexed solutions for screening animals for multiple coronaviruses, further development is needed to identify applicable species.

Microsphere Immunoassay

Microsphere immunoassays (MIAs) couple antigens of interest to microspheres instead of plates used in ELISAs and microarrays. These coated microspheres are then incubated with serum to capture antibodies of interest, followed by a secondary antibody for detection via flow cytometry. This immunoassay type can also be used for multiplex assay design with a reported higher reproducibility and dynamic range as well as lower preparation time compared to ELISAs.[685]

MIAs have been used to detect SARS-CoV-2 antibodies in select animal species. One multiplex MIA for N and two S regions successfully detected SARS-CoV-2 antibodies in *F. catus* and *C. lupus familiaris*,[428] while another multiplex MIA for N and S was established in cats.[416] Microspheres coated with RBD and trimeric S also successfully detected SARS-CoV-2 antibodies in *C. lupus familiaris* and *F. catus* as well as *O. cuniculus*.[409,686–688]

Luciferase Immunoprecipitation System

The luciferase immunoprecipitation system (LIPS) uses antigen of interest fused to luciferase, and after incubation with a serum sample, serum antibodies are immunoprecipitated and luciferase activity is used as a readout of antibodies bound to the antigen of interest. LIPS requires less time for completion and can be adapted to various formats according to specific needs.[689] The Eloit and Schwartz laboratories at the Pasteur Institute developed a LIPS assay for multiple SARS-CoV-2 S regions[690] that was subsequently used to detect SARS-CoV-2 in cats.[441]

Antibody Neutralization Assays

Importantly, not all binding antibodies can successfully neutralize a target virus. Neutralization assays provide information on the activity of antibodies present in serum and their effects on virus replication. Live virus neutralization assays consist of plaque reduction neutralization tests (PRNTs) and MNAs to test neutralization activity against a live, intact virus. Because of the transmissibility and virulence of emerging coronaviruses, experiments using live viruses require biosafety level 3 (BSL-3) laboratory certification and compliance. A report released in August 2022 identified only 381 BSL-3 laboratories worldwide, with 148 of those located in the United States.[691] Therefore, laboratories without BSL-3 certification test neutralization of antibodies to coronaviruses using either pseudoparticle neutralization tests (ppNTs) or surrogate virus neutralization tests (sVNTs) that do not use intact, infectious coronavirus. Antibody neutralization methods are described below.

Live Virus Neutralization

PRNTs have been long regarded as a gold standard for detection of neutralizing antibodies and are more sensitive than other neutralization assays, but also less scalable. However, these assays require advanced expertise and longer timeframes, compared to other neutralization

assays.[692] PRNTs have been used to assess neutralization activity against MERS-CoV[693] and SARS-CoV-2.[399,668,694–696] These assays have detected MERS-CoV neutralizing antibodies in *C. dromedarius* and *Vicugna pacos*,[370] as well as *C. hircus*, *B. taurus*, and *E. asinus*.[361] PRNTs have also successfully detected SARS-CoV-2 neutralizing antibodies in *F. catus*,[399,431] *C. lupus familiaris*,[399,431,668] and *O. virginianus*.[355,356]

MNAs enable scalable testing of neutralization activity—albeit at lower sensitivity than PRNTs—compared to conventional virus neutralization tests (cVNTs) by incubating live virus with serum, followed by inoculation of a permissive cell line in a 96-well format. After an incubation period, wells are analyzed using infection readouts, most often cytopathic effect (CPE).[692] MNAs have been used to assess neutralization activity against MERS-CoV,[297,363,651,693,697,698] SARS-CoV-1,[75,154] and SARS-CoV-2.[393,401,417,437,699] MERS-CoV MNAs have successfully detected neutralizing antibodies in *C. dromedarius,*[53,60,61,63,362,369,651,674] *C. bactrianus,*[365] *V. pacos,*[369] llamas (*Llama glama*),[369] *O. aries*,[60,361] *C. hircus*,[361] *B. taurus*,[361] *E. caballus*,[361] and *E. asinus*.[361] SARS-CoV-1 MNAs have detected neutralizing antibodies in *S. scrofa*,[154] *P. larvata*, *M. moschata*, and *N. procyonoides*.[75] SARS-CoV-2 MNAs have been used to detect neutralizing antibodies in *O. aries*,[359] *F. catus*,[126,393,398,401,407,414,417,418,423,437,441,671] *C. lupus familiaris*,[126,393,394,398,403,407,432,471] ferrets (*M. putorius furo*),[699] *N. vison*,[121] *M. lutreola*,[116] *P. leo*,[466] and *P. tigris*.[463,464]

Pseudovirus Neutralization

ppNTs have increased accessibility of laboratories to neutralization tests, but importantly, not all aspects of pseudoviruses recapitulate their corresponding, naturally-occurring viruses. A typical ppNT uses a recombinant pseudovirus engineered to block expression of native surface proteins, resulting in a virus unable to replicate beyond a single round. Because of their reduced virulence, pseudoviruses can be handled in biosafety level 2 (BSL-2) laboratories. These pseudoviruses are then used to package surface protein(s) of interest. Common pseudovirus packaging systems include the human immunodeficiency virus (HIV)-1-derived lentiviral system, murine leukemia virus (MLV)-based system, and vesicular stomatitis virus (VSV)-derived system.[692]

SARS-CoV-2 pseudoviruses have been developed using HIV-1-derived lentiviral system,[36] MLV-based system,[428] and VSV-derived system,[700,701] while MERS-CoV pseudovirus has been developed using the HIV-1-derived lentiviral system.[297,298] SARS-CoV-2 VSV and MLV pseudovirus systems have been successfully used with serum from *C. lupus familiaris*[408,428] and *F. catus*,[408,409,428,436] and the newly-created SARS-CoV-2 lentiviral system has not been used with non-human serum. The MERS-CoV lentivirus system has only detected neutralizing antibodies in camels.[54,62,66,297,702]

Surrogate Virus Neutralization

Genscript has developed the cPass kit, which serves as an sVNT by recreating ACE2-SARS-CoV-2 interactions under cell-free conditions, eliminating handling of any live virus or pseudovirus. When kit contents are incubated with neutralizing antibodies, HRP-conjugated SARS-CoV-2 RBD cannot bind to ACE2, resulting in HRP detection.[672,703] This sVNT is highly specific to SARS-CoV-2

and strongly correlates with MNA and ppNT assay results. In addition, researchers have demonstrated that Genscript's cPass kit is more sensitive than live virus neutralization assays and has the potential for further modifications to increase sensitivity.[672]

Vaccines

Different inactivated and attenuated vaccines, as well as recombinant protein, mRNA, and DNA vaccines, have been developed over the past several years to provide protection against infection from SARS-CoV-1, MERS-CoV, and more recently SARS-CoV-2.[704–706] Numerous studies have demonstrated that the S protein on the surface of these three coronaviruses is particularly immunogenic and therefore this protein has been targeted in many vaccine formulations. Many of the resulting vaccine strategies result in reduced viral titers and some degree of protection against severe disease, with less morbidity and mortality when compared to lack of vaccination. However, just as many studies to date have shown that infections with SARS-CoV-1, MERS-CoV-1, and SARS-CoV-2 do not provide a long-lasting antibody response and wane post infection,[707,708] several vaccines developed against SARS-CoV-1 also produced only short-term protection against subsequent viral challenges.[709,710] These vaccines also caused complications related to inflammatory disease and, for vaccines targeting the SARS-CoV-1 S protein, triggered antibody-dependent enhancement (ADE).[710,711] Inactivated viral vaccines have also produced non-neutralizing antibodies that exhibit ADE functions that promote inflammation and tissue destruction, with the activation of myeloid cells via Fc receptors.[712] Therefore, it remains imperative to develop a vaccine with minimal risk of ADE that can provide long-lasting protection against potentially emerging variants of the coronavirus. Vaccines that confer long-term protection should elicit both the production of virus-specific neutralizing antibodies as well as antigen-specific T and B cell adaptive immune responses. Select vaccines tested in animals are summarized in Table 18, and notable vaccines are discussed below.

Research Tools for Vaccine Development

A good deal of data on coronavirus vaccines in animals comes from animal model testing of vaccines intended for human use. Initial in vivo tests of vaccine candidates typically involve mice, rats, and other small animals that may not be susceptible to infection with SARS-CoV-1, MERS-CoV, or SARS-CoV-2. These initial tests are used to determine whether a candidate antigen is immunogenic and elicits a humoral response generating antibodies. This approach has been used for recombinant protein antigens and inactivated SARS-CoV-2 virus, which can generate neutralizing antibodies in mice and rats.[713,714]

However, species not susceptible to SARS-CoV-1, MERS-CoV, or SARS-CoV-2 cannot be used in follow-up challenge experiments to determine whether these immune responses are protective. Therefore, transgenic[715] and adenovirus transduced mouse strains[566,567] expressing human ACE2, as well as wildtype species susceptible to infection (e.g., *M. auratus*, *M. putorius furo*, NHPs) are used to test vaccines' protective effects. Although these particular models cannot shed light on animal protection, mouse-adapted strains of SARS-CoV-2 have also been used for vaccine development, and challenge experiments can be conducted on these mouse models to determine the degree of protection afforded by a given vaccine. Several subunit vaccines have been tested via this method and demonstrate efficacy, with some protection

obtained and infected mice developing less severe disease.[561] Another vaccine consisting of a virus-like particle was tested and also demonstrated efficacy, with lower viral titers observed in infected mice.[587]

Large animal models such as NHPs have been used extensively to evaluate vaccine candidates prior to testing in humans due to their closely related immune systems. These animals have helped explore the decrease in neutralizing antibody titers and T cell responses over time that has been one of the major challenges in developing a vaccine against SARS-CoV-2, with multiple studies showing a decrease to low levels within 2-3 years post infection in humans.[707,708] Both rhesus macaques and pigtailed macaques have been the most widely used NHPs due to their availability and presence of reagents that can be used to evaluate the immune response and correlates of protection.

Vaccines Tested in Susceptible Animals

During the past two years, vaccine development for SARS-CoV-2 has focused on novel mRNA vaccines that can be rapidly produced. The two major novel mRNA vaccines used in the U.S., both encoding the S protein of SARS-CoV-2, were largely developed and evaluated in rhesus macaques prior to human clinical trials. Both achieved 95% efficacy in clinical trials and are in use today.[716,717]

ChAdOx1, an adenovirus vector-based vaccine, has been used in vaccines against MERS-CoV and SARS-CoV-2.[141,718] The MERS ChAdOx1 spike vaccine induced neutralizing antibodies and protected *C. dromedarius* from future MERS-CoV infections,[719] while the SARS-CoV-2 spike-based vaccine elicited a strong humoral as well as cell-mediated responses in the same species. The vaccine was then tested in rhesus macaques and was demonstrated to provide protection against infection in challenge experiments prior to being evaluated in clinical trials.[718]

PiccoVac, an inactivated SARS-CoV-2 vaccine, elicited a strong immune response involving both the production of neutralizing antibodies as well as T cell responses in *M. mulatta.*[714]

Like other RNA viruses, SARS-CoV-2 is prone to genetic evolution and acquires mutations that allow the virus to adapt to new hosts and/or evade the host's immune response. Over the past 3 years, multiple variants of concern have arisen and many are able to escape the immunological response afforded by current vaccines, necessitating the development of new formulations. However, although the vaccines may not afford complete protection from infection by the newly emerging variants, they do appear to protect animals and humans against more severe disease.[720] Current vaccine development efforts focus on generating vaccines with broader protection against multiple variants.

Vaccines Designed for Veterinary Use

Animal protection strategies against SARS-CoV-1, MERS-CoV-1, and SARS-CoV-2 require effective vaccines in various animal species, including companion, farmed, captive, and wild animals. Several of the FDA-approved and emergency use vaccines that have been approved for humans may not be useful in the immunization of animal species due to their expense and logistically difficult administration (e.g., temperature requirements). Therefore, the

development of specific veterinary vaccine formulations that can be disseminated rapidly during a pandemic outbreak are still needed.

Multiple veterinary vaccines are being formulated and tested in animals for this purpose. An experimental SARS-CoV-2 S protein vaccine developed by Zoetis was reported to elicit a strong immune response in domesticated cats.[3] Neutralizing antibody titers were present in cat serum of vaccinated animals after the first and second vaccinations with the recombinantly produced S protein trimer, and robust levels of these neutralizing antibody titers were induced for both the wildtype Wuhan strain and the more severe delta variant. This experimental vaccine has been provided to zoos and mink farms to immunize animals at risk for contracting the virus and transmitting it to other animals as well as humans. Captive orangutans housed at the San Diego Zoo were one of the first NHPs to receive this experimental vaccine and since that time other species housed at zoos have been immunized.

The LinearDNA COVID-19 vaccine is also currently being developed by Applied DNA Sciences and EvviVax for use in domestic cats. This vaccine has received regulatory approval from the United States Department of Agriculture and is currently undergoing clinical trials to evaluate its immunogenicity and efficacy. According to preliminary results reported by the company, the vaccine is well tolerated and generates high levels of neutralizing antibodies with a single dose in domesticated cats.[721] Immunization of cats with this vaccine could prevent SARS-CoV-2 infections and prevent transmission not only to feral cats that could subsequently transmit the virus to wildlife but also to humans.[615]

An inactivated SARS-CoV-2 vaccine, Carnivac-CoV, has been recently registered as a vaccine formulation for animals based on testing in dogs, cats, foxes, and mink. Clinical trial data indicate that protective immunity is achieved and has a duration of at least six months after vaccination.[722]

Table 19: Summary of select vaccine studies in animal species

Species	Virus	Vaccine Type	Target	Vaccine Name	Administration Route	Induction of neutralizing antibodies	T cell responses	Protection against infection
Camelus bactrianus	MERS-CoV	recombinant, nonvirulent Newcastle disease virus	S	rLa-MERS-S[723]	intramuscular	Yes	N/A	N/A
Camelus dromedarius	MERS-CoV	adenovirus vector	S	ChAdOx1 MERS[719]	intramuscular	Yes	N/A	Yes
		DNA	S	not specified[724]	intramuscular	Yes	N/A	N/A

		modified vaccinia virus Ankara vectored DNA	S	not specified[725]	intranasal	Yes	N/A	Yes
Cavia porcellus	SARS-CoV-2	inactivated virus	whole virus	BBIBP-CorV[726]	intramuscular	Yes	N/A	N/A
Felis catus	SARS-CoV-2	Protein	S	not specified[727]	intramuscular	Yes	N/A	Yes
			S, trimer	Zoetis vaccine[3]	not specified	Yes	N/A	N/A
Llama glama	SARS-CoV-2	Protein	S1	not specified[728]	intramuscular	Yes	N/A	Yes
Macaca fascicularis	SARS-CoV-2	DNA	S	GX-19[729]	intramuscular	Yes	Yes	Yes
		Sendai virus vector	N, M, E	SeV-NME[730]	intramuscular; intranasal	Yes	Yes	Yes
		fusion protein	S	S1-Fc[731]	unknown	Yes	N/A	N/A
		inactivated virus	whole virus	BBIBP-CorV[726]	intramuscular	Yes	N/A	N/A
		mRNA	S, pre-fusion stabilization mutation and furin cleavage site mutation	MRT5500[732]	intramuscular	Yes	N/A	N/A
		nanoparticle-encapsulated mRNA	RBD	ARCoV[733]	intramuscular	Yes	Yes	N/A

		Protein	S	NVX-CoV2373[734]	intramuscular	Yes	N/A	Yes
Macaca mulatta	MERS-CoV	DNA	S	not specified[724]	intramuscular	Yes	Yes	Yes
		DNA priming + protein boost	S DNA prime + S1 protein boost	not specified[735]	intramuscular	Yes	N/A	Yes
		Protein	RBD	not specified[736]	intramuscular	Yes	Yes	Yes
		recombinant VSV vector	S	VSVΔG-MERS[737]	intramuscular; intranasal	Yes	Yes	N/A
		virus-like particle	whole virus	MERS-CoV VLP[738]	intramuscular	Yes	Yes	N/A
	SARS-CoV-1	adenovirus vector	S1	Ad5-SARS-CoV[739]	intramuscular	Yes	N/A	N/A
		human adenovirus vector; chimpanzee adenovirus vector	S	not specified[740]	intramuscular	Yes	Yes	N/A
	SARS-CoV-2	adenovirus vector	S	ChAdOx-1nCoV-19[718]	intramuscular	Yes	Yes	Yes
			S	Sad23L-nCoV-S & Ad49L-nCoV-S[741]	intramuscular	Yes	Yes	N/A

			S	Ad5-nCoV[742]	nebulization inhalation	Yes	Yes	N/A
			S	not specified[743]	intramuscular	Yes	Yes	Yes
		DNA	S	not specified[744]	intramuscular	Yes	N/A	Yes
			S	INO-4800[745]	intradermal	Yes	Yes	Yes
		inactivated virus	whole virus	BBV152[746]	intramuscular	Yes	N/A	Yes
			whole virus	PiCoVacc[714]	intramuscular	Yes	N/A	N/A
			whole virus	BBIBP-CorV[726]	intramuscular	Yes	N/A	Yes
			whole virus	not specified[747]	intramuscular	Yes	Yes	Yes
		modified vaccinia virus Ankara vector	S (membrane anchored, pre-fusion stabilized)	not specified[748]	intramuscular	Yes	Yes	Yes
		mRNA	S	mRNA-1273[716]	intramuscular	Yes	Yes	Yes
			S	BNT162b2[749]	intramuscular	Yes	N/A	Yes

		Protein	RBD	not specified[750]	intramuscular	Yes	Yes	Yes
			S, trimer	S-Trimer[751]	intramuscular	Yes	N/A	Yes
		VSV vector	S	not specified[752]	intramuscular; intranasal	Yes (both routes)	Yes (intramuscular only)	Yes (intramuscular only)
Macaca nemestrina	SARS-CoV-2	mRNA	S	repRNA-CoV2S[753]	intramuscular	Yes	Yes	N/A
		nanoparticle + mRNA	S	LION/repRNA-CoV2S[754]	intramuscular	Yes	Yes	N/A
Mesocricetus auratus	SARS-CoV-2	inactivated rabies viral DNA vector plus MPLA-AddaVax (TRL4 agonist)	S1	CORAVAX[755]	intramuscular	Yes	N/A	Yes
		mRNA	S	Moderna vaccine[756]	intramuscular	N/A	N/A	Yes
			S (pre-fusion stabilization mutation and furin cleavage site mutation)	MRT5500[732]	intramuscular	Yes	N/A	Yes
		VSV vector	S	not specified[757]	intramuscular; intranasal	N/A	N/A	Yes

Mus musculus	SARS-CoV-1	adenovirus vector	N, S	not specified[758]	intramuscular; intranasal	Yes	Yes	Yes
		protein (RBD-Fc); AAV (RBD)	RBD	RBD-Fc; RBD-rAAV[759]	intramuscular; intramuscular or intranasal	Yes	Yes	N/A
		Venezualan equine encephalitis virus replicon particles	S	VRP-S[760]	not specified	Yes	N/A	Yes
		virus like particles	whole virus	not specified[761]	intramuscular; intranasal	Yes	N/A	Yes
			whole virus	not specified[762]	subcutaneous	Yes	Yes	N/A
		whole killed virus	whole virus	not specified[758]	subcutaneous	Yes	Yes	Yes
Mus musculus (adapted SARS-CoV-2 virus)	SARS-CoV-2	virus-like nanoparticle (contains 120 RBD copies)	RBD	VLP-RBD[763]	intramuscular	Yes	N/A	Yes
Mustela putorius furo	SARS-CoV-1	adenovirus vector	N, S	not specified[764]	intramuscular	Yes	N/A	Yes, incomplete
		human adenovirus vector (prime); chimpanzee adenovirus vector (boost)	S	not specified[740]	intramuscular	Yes	Yes	Yes
		modified vaccinia Ankara vector	N, S	not specified[765]	subcutaneous	Yes (spike only)	No	No, caused inflammatory response

		whole killed virus	whole virus	not specified[764]	intramuscular	Yes	N/A	Yes, incomplete
	SARS-CoV-2	adenovirus vector	S	Ad5-nCoV[766]	intramuscular; oral + intranasal	Yes	N/A	Yes
		DNA	S	INO-4800[2]	intramuscular	Yes	N/A	N/A
Oryctolagus cuniculus	SARS-CoV-2	inactivated virus	whole virus	BBIBP-CorV[726]	intramuscular	Yes	N/A	N/A
Papio anubis	SARS-CoV-2	Protein	S	NVX-CoV2373[767]	intramuscular	Yes	Yes	N/A

Therapeutics

Similar to vaccines, some data on therapeutic efficacy against animal coronaviruses come from studies in animal model conducted for human drugs. Wherever wildtype animals are employed in these studies, they can provide helpful information relevant to controlling coronavirus in animal species, with implications not only for treating animal infection but also for reducing risk of subsequent transmission. This summary surveys the mechanisms of action and impacts of the predominant drug classes of antivirals and biologics, as well as of a broader class of FDA-approved agents includes microbicides, polyphenols, anti-inflammatory, and other agents with varying degrees of efficacy across animal species.

FDA-Approved Antivirals

Antivirals Targeting RNA-Dependent RNA Polymerase

Rhesus macaques

Remdesivir is an FDA-approved antiviral that, as an adenosine nucleoside triphosphate analog, is incorporated during replication by RdRp, which results in chain termination and halting of replication in SARS-CoV-1, MERS-CoV, and SARS-CoV-2.[768–771] A novel subcutaneous formulation was studied using SARS-CoV-2-infected *M. mulatta*. *M. mulatta* treated with 10 milligrams per kilogram (mg/kg) of remdesivir for 6 days showed no signs of interstitial pneumonia starting at 12 hours post-infection.[772] Additionally, there were reduced signs of respiratory disease and virus replication in the lower respiratory tract compared to untreated animals.[772]

Mice

Remdesivir in mice has relatively poor plasma stability. However, GS-441524, the parent nucleoside and metabolite of remdesivir, inhibits SARS-CoV-2 with a mechanism similar to remdesivir in vivo.[773] An intraperitoneal dose of 25 mg/kg GS-441524 24 hours prior to infection resulted in significant viral clearance in the lungs at 2 dpi and no weight loss in *M. musculus*.[2] An additional study tested molnupiravir, which is an analogue that increases the frequency of mutations resistant to the proofreading exoribonuclease encoded by coronavirus. Severe combined immunodeficient (SCID) mice infected with the beta variant of SARS-CoV-2 were treated with 200 mg/kg of molnupiravir orally and experienced reduced viral RNA loads. Fifty percent of the treated animals showed no infectious viral titers and demonstrated improved lung histology scores.[774]

Ferrets

GS-621762 is an oral prodrug of parent nucleoside GS-441525 and is effective at controlling SARS-CoV-2 infection in ferrets that received treatment twice daily for 4 dpi.[775] Compared to the vehicle control group, virus was undetectable in nasal turbinates and RNA copy numbers were lower in treated ferrets.[775] Molnupiravir has also been tested in ferrets.[776] Ferrets treated with molnupiravir twice daily demonstrated reduced viral load in the upper respiratory tract, which subsequently prevented spread to other animals.[776]

Syrian Hamsters

Early treatment of SARS-CoV-2 with galidesivir, molnupiravir, or favipiravir reduced weight loss, viral titers, and viral burden in Syrian hamsters.[774,777,778] Molnupiravir may be particularly advantageous in the treatment of animals because it can be administered orally. Combination treatment with molnupiravir and favipiravir for early treatment of SARS-CoV-2 in hamsters resulted in higher overall potency, with undetectable virus titers in the lungs of greater than 60 percent of animals.[779] This effect was lessened when treatment was delayed by 1 day.[779] Favipiravir increases antiviral activity because it increases the number of mutations in the SARS-CoV-2 genome, ultimately decreasing viral infectivity.[778,779]

Hamsters treated with remdesivir and methylprednisolone demonstrated reduced weight loss and inflammation.[780] The combinatorial effect reduced viral protein expression and viral loads. In the presence of remdesivir, methylprednisolone suppressed antibody activity.[780]

Reverse Transcriptase Inhibitor

Ferrets

Emtricitabine-tenofovir is used for the treatment of HIV and has been tested for the treatment of SARS-CoV-2.[306] Emtricitabine-tenofovir-treated ferrets had reduced virus titers compared to untreated animals, but this effect was eliminated when the immune system was compromised with exposure to azathioprine.[306]

Protease Inhibitors

BALB/cAJcl and K18-hACE2 mice

Ensitrelvir, which targets the 3C-like protease (3cLpro) of SARS-CoV-2, was shown to be effective against the SARS-CoV-2 gamma strain in mice treated twice daily for 5 dpi. Ensitrelvir reduced viral loads and body weight loss in mice compared to the vehicle control group.[781] Post-infection treatment of SARS-CoV-2 with GC376 (i.e., 3cLpro inhibitor) increased survival and decreased lung viral titers in mice.[17]

Syrian Golden Hamsters

Nirmatrelvir, an orally administered inhibitor of viral 3cLpro, has antiviral activity against four SARS-CoV-2 variants of concern in vitro.[782] PF-332 administered orally twice daily to hamsters conferred complete protection against SARS-CoV-2 beta and delta variants and prevented transmission.[782]

Ferrets

Although lopinavir-ritonavir treatment has shown efficacy against SARS-CoV-1, MERS-CoV, and SARS-CoV-2 in vitro, those results have not translated to in vivo effectiveness. The antiviral efficacy of lopinavir-ritonavir was assessed in a ferret infection immunosuppressive model, in which ferrets were administered 16 mg/kg daily post-infection via oral gavage for 14 days. The lopinavir-ritonavir-treated group demonstrated clinical symptoms (cough, rhinorrhea, and reduced activity) comparable to the placebo group.[306]

Biologics

Antibodies

Marmosets

Two human neutralizing MERS-CoV antibodies, REGN3051 and REGN3048, have shown prophylactic and therapeutic efficacy in a murine MERS model. MERS-infected marmosets treated with either one or both antibodies also had less severe respiratory disease and lung lesions and reduced viral loads in the lungs compared to untreated animals.[783] de Wit et al. demonstrated that the combination antibody treatment was *most* effective in MERS-CoV-infected marmosets, with reduced virus replication when the combination was administered prophylactically 24 h before infection compared to a single neutralizing antibody.[783]

Hamster

NIH-CoVnb-112, a neutralizing nanobody, binds to SARS-CoV-2 RBD in the spike trimer in the "up" conformation.[784] SARS-CoV-2-infected hamsters treated with NIH-CoVnb-112 demonstrated a reduction in viral lung burden and weight loss compared to untreated animals.[784] The ZRC3308 monoclonal antibody cocktail contains two humanized monoclonal

antibodies (mAbs), ZRC3308-A7 and ZRC3308-B10, that bind to SARS-CoV-2 RBD. Hamsters treated with this antibody cocktail prophylactically did not develop pneumonia and had reduced viral loads compared to untreated controls.[785] Polyclonal immune sera from previously challenged rhesus macaques were also used to treat SARS-CoV-2 infected hamsters, resulting in protection against the virus.[786] Hamsters had reduced weight loss and macrophage infiltrates in the lungs, but the number of innate and adaptive immune cells present was not affected by immune sera treatment.[786]

Cas Proteins

Hamsters

Certain bacteria use Cas13a as part of an immune system to degrade foreign RNAs. Blanchard et al. designed clustered regularly interspaced short palindromic repeats (CRISPR) RNA with conserved regions for influenza virus and replicase and nucleocapsid genes of SARS-CoV-2 as targets to create a polymer-formulated Cas13a mRNA.[787] SARS-CoV-2-infected hamsters were treated pre- and post- infection using a nebulizer, and Cas13a degraded influenza RNA in lung tissue and reduced viral load.[787]

Other FDA-Approved Drugs

Anti-virals

Rhesus macaques

IFNs are antiviral factors secreted from infected cells that induce cytokine production. The combined treatment effect of IFN and ribavirin was assessed in MERS-CoV-infected rhesus macaques. The antiviral effect of IFN-a2b against MERS-CoV was enhanced by the concomitant use of ribavirin 8 h post infection. Interestingly, elevated levels of IL-6, IFN-γ, and MCP-1 in homogenates of lung tissue three dpi indicated a tissue-specific host response to infection that can be moderated by treatment.[788]

Microbicides

Mice

Astodrimer sodium is a polyanionic, polysulfonate compound that is currently used to treat bacterial vaginosis. It effectively prevents the formation of biofilms and its polyanionic surface attaches to targets on viruses, thereby blocking viral entry into host cells. Some evidence suggests that astodrimer sodium can prevent SARS-CoV-2 infection and reduce the severity of COVID-19.[789] SARS-CoV-2-infected mice treated with astrodrimer sodium 1% nasal spray exhibited reduced viral genome copies and virus in the lung, trachea, and nasal cavity.[789] Astodrimer sodium 1% also reduces pro-inflammatory cytokines interleukin (IL)-6, IL-1β, IL-1α, tumor necrosis factor alpha (TNFα), transforming growth factor beta (TGFβ), and MCP-1 in the serum, lung, and trachea.[789]

Antidepressants

K18-hACE2 Mice

Fluoxetine has potential as a SARS-CoV-2 treatment due to its inhibitory effect on acid sphingomyelinase/ceramidase.[790] Acid ceramidase, which is considered a host factor, is an essential component involved in viral replication, and inhibiting acid ceramidase increases the ceramide content in SARS-CoV-2-infected cells. Fluoxetine treatment demonstrated antiviral properties in mice infected with different SARS-CoV-2 variants (i.e., delta, omicron, alpha, and gamma).[790] Fluoxetine significantly reduced both lung tissue viral titers and pro-inflammatory expression markers in serum, such as IL-6, TNFα, chemokine ligand 2 (CCL2), and C-X-C motif chemokine ligand 10 (CXCL10).[790]

Polyphenols

C57BL/6 Mice

Catechin is a phenolic compound found in green tea polyphenols that helps protect cells against damage from free radicals. This potent antioxidant molecule is also abundant in cocoa and berries.[791] A previous study demonstrated that epigallocatechin gallate (EGCG), a green tea polyphenol, inhibited coronavirus replication in vitro.[792] Park et al (2021) demonstrated that EGCG incorporated into regular drinking water for two weeks post infection in mice infected with human coronavirus OC43 reduced coronavirus RNA and protein levels in mouse lungs.[792]

Aldehyde dehydrogenase inhibitors

Golden Hamsters

Disulfiram is an FDA-approved drug for the treatment of alcohol use disorder, and its ability to block gasdermin D-dependent neutrophil extracellular trap (NET) formation in macrophages suggests it may be useful as a SARS-CoV-2 treatment. Upon lung injury caused by SARS-CoV-2 and other insults, neutrophil infiltrates form NETs that trigger additional damage and an increased immune response. Adrover et al. demonstrated that disulfiram's ability to inhibit NET formation provided protection against SARS-CoV-2 in hamsters. SARS-CoV-2-infected hamsters that were treated with 50 mg/kg of disulfiram had reduced NETs and less perivascular fibrosis in their lungs. Additionally, disulfiram treatment downregulated innate immune and complement/coagulation pathways.[793]

Non-structural protein targets

Syrian hamsters

Ranitidine bismuth citrate is a metal compound previously used as an effective treatment for *Helicobacter pylori* infection.[794] Ranitidine bismuth citrate targets helicase, which inhibits DNA unwinding activity, and quantitative reverse transcriptase PCR experiments indicated that the compound inhibits a late-stage process in the SARS coronavirus replication cycle.[795] Yuan et al.

investigated whether this antimicrobial drug also impedes viral helicase in Syrian hamsters infected with SARS-CoV-2. Hamsters treated with ranitidine bismuth citrate had decreased viral loads in the upper and lower respiratory tracts and no lung damage or cell infiltration in the alveolar space.[794]

Fatty Acid Synthesis

Mice

Chu et al. demonstrated that SARS-CoV-2 requires fatty acid synthesis to establish and maintain infection in the lungs.[796] Mice treated with orlistat (fatty acid synthase inhibitor) pre- and post-infection exhibited lower SARS-CoV-2 viral levels in the lung, reduced lung pathology, and increased survival.[796]

Statins

Mice

Simvastatin reduces physiological inflammatory response triggered by SARS-CoV-2.[797] Mice treated orally with simvastatin (20 mg/kg) 24 hours prior to infection demonstrated reduced virus replication and lung damage, as well as increased survival time compared to vehicle-treated groups.[797] Interestingly, there was no difference in mortality between treated mice and the vehicle control group.[797] Teixeira et al. demonstrated that simvastatin disrupts SARS-CoV-2 cell entry by shifting host ACE2 on cell membrane rafts.[797]

Anti-inflammatory

Rhesus macaques

The duration and severity of inflammation influences the burden of disease associated with SARS-CoV-2 infection. Baricitinib is a janus kinase (JAK) inhibitor and anti-inflammatory drug used to treat moderate to severe rheumatoid arthritis that has also shown potential against SARS-CoV-2-related inflammation.[798] SARS-CoV-2-infected Rhesus macaques that were orally administered 4 mg of baricitinib demonstrated reduced neutrophil degranulation in BALs.[798] Further analyses showed that baricitinib treatment inhibited inflammatory cytokines and neutrophil chemoattractant expression in BALs. Barcitinib treatment also decreased T cell proliferation and activation.[798]

Rodents

Loratidine (antihistamine) stabilizes mast cells and prevents the influx of pro-inflammatory cytokines. Hamsters that received a combination dose of loratidine and remdesivir demonstrated a reduction in not only SARS-CoV-2 replication but also inflammation, which protected against lung injury.[799]

Glucocorticoids (steroid hormones), such as dexamethasone, are also used to treat inflammation, as well as autoimmune disorders, asthma, and organ transplant. Dexamethasone has been evaluated against a particular type of pulmonary inflammation observed in SARS-CoV-1, in which the nucleocapsid protein (N-protein) triggers a poorly regulated influx of pro-inflammatory and anti-inflammatory cytokines. The effect of dexamethasone was evaluated in a rat model of SARS-CoV with pulmonary inflammatory reaction induced by .2 mg/kg of N-protein of SARS-CoV. Levels of IL-6, IL-10, and TGF-beta1 were significantly lower in rats administered dexamethasone compared to the untreated group. This result suggests that glucocorticoids can be used to mitigate the pulmonary inflammatory reaction induced by the N-protein of SARS-COV.[800]

Anti-parasitic

Rhesus macaques

Hydroxychloroquine (HCQ) and chloroquine are antimalarial drugs that are also used as immunosuppressives and DMARDs (disease-modifying antirheumatic drugs). Both drugs demonstrated an inhibitory effect on SARS-CoV-2 replication in vitro both alone and in combination with macrolide antibiotic azithromycin. However, these results did not translate to an in vivo context. A SARS-CoV-2 Rhesus macaques model was administered 6.5 mg/kg of HCQ prophylactically (weekly) and as a treatment (daily). There was no effect on replication and no impact on viral shedding or signs of disease progression.[801]

Hamsters

The administration of HCQ alone was not beneficial either as a treatment (50 mg/kg daily) or prophylactically (50 mg/weekly). Therefore, Cochin et al tested the combination of azithromycin (20 mg/ml) and (200 mg/ml) HCQ and (200 mg/ml) HCQ alone in a SARS-CoV-2 hamster model at the time of infection. Neither course of treatment inhibited SARS-CoV-2 replication, or impeded lung impairments in the hamster model. Hydroxychloroquine and chloroquine are not effective treatments for SARS-CoV-2.[802]

Biosecurity

Although this review focuses on coronaviruses in animal populations, humans play a necessary role in implementing biosecurity measures. Because humans are a primary reservoir of coronaviruses, with the potential for zoonosis (i.e., animal to human transmission) and subsequent reverse zoonosis (i.e., human to animal transmission),[803,804] human infection prevention must be considered part of animal biosecurity. In addition, many species-specific coronaviruses, such as Bov-CoV, cause respiratory disease and are transmitted through droplets and/or aerosol, similar to SARS-CoV-1, MERS-CoV, and SARS-CoV-2.[805] Therefore, animal biosecurity measures implemented for these species-specific coronaviruses should be considered as relevant strategies for SARS-CoV-1, MERS-CoV, and SARS-CoV-2. Outlined below are considerations for areas of biosecurity measures against coronaviruses, accounting for the protection of animals and humans, to prevent viral transmission to animals.

Vaccines

Vaccines are one of the most effective methods for controlling the spread of infectious diseases such as SARS-CoV-1, MERS-CoV, and SARS-CoV-2. Furthermore, vaccinating animals that are susceptible to these coronaviruses can substantially reduce animal-to-animal and animal-to-human transmission, which in turn reduces the emergence of novel variants.[806] Viral transmission is reduced from vaccination through population immunity (i.e., herd immunity), in which an entire population is protected from viral infection once a specified percentage of the population produces sufficient immune responses from vaccination. In veterinary medicine, population immunity has been successfully reached to completely eradicate animal diseases.[805] Therefore, vaccination of susceptible animal populations should be a top measure of biosecurity (see "Biosecurity"). However, with vaccines only being approved for use in humans[807] and tested on a limited quantity of species, such as companion and zoo animals,[1–4] other biosecurity measures should also be considered.

Surveillance

During the 2003 SARS-CoV-1 pandemic, non-specific symptoms of SARS-CoV-1 infection prevented accurate clinical diagnosis, and thus enhanced disease transmission to local communities through delayed implementation of biosecurity measures. Surveillance of coronaviruses within animal populations is necessary for early viral detection and diagnosis, which can facilitate implementation of biosecurity measures, such as quarantine of infected animals.[808] Under a One Health approach that aims to "balance and optimize the health of people, animals, and ecosystems,"[809] coordinated surveillance should be established for wildlife, farmed wildlife, domestic animals, and individuals who have frequent contact with animals.[810] When animals test positive, diagnostic and clinical factors should be evaluated to determine potential viral sources and other animals that were potentially exposed.[804] To rapidly identify potential viral sources, the Norwegian BRSV [bovine respiratory syncytial virus] and Bov-CoV Control Program implemented a hotline through which farmers can report potential respiratory disease outbreaks.[811] In addition, samples from known wildlife viral reservoirs can be collected for genome sequencing, which can identify novel variants that can potentially evade coronavirus immune responses from vaccines or prior infections (see "Surveillance" for more details).[614,810]

Disinfection, Decontamination, and Personal Protective Equipment

USDA APHIS provides guidance for *N. vison* and *O. virginianus* farmers on proper disinfection, decontamination, and personal protective equipment (PPE) usage in "One Health: Keeping Animal and People Safe from SARS-CoV-2." When working around healthy animals, workers should wear a cloth face covering and wash or sanitize their hands regularly. However, when working around animals that are suspected of having illness, workers should use appropriate PPE, such as gloves, face masks, and goggles or face shields. In addition, workers should immediately wash their hands for at least 20 seconds after coming in direct contact with sick animals, their food, water supply, or waste.[812] Takahashi et al. (2020) also found that when workers exchanged boots and used footbaths at the entrance of calf sheds on bovine farms, detection of Bov-CoV and calf mortality rates were significantly reduced.[813]

According to the USDA APHIS guidance, surfaces frequently used by workers or animals should be cleaned and disinfected regularly.[812] Some conventional disinfection measures include liquid spray, ultraviolet light, and heat treatment.[814] Workers should avoid using disinfection methods that could spray infectious material into the air, such as compressed air or pressurized water.[812] Air filtration or treatment systems are another effective means of removing infectious material from animal housing areas, especially when negative-pressure ventilation is implemented, which effectively reduces viral load.[805]

Lastly, animal carcasses that are positive, or suspected to be positive, for a coronavirus should be properly disposed to prevent contamination and viral transmission. Carcasses should be carefully transported to an approved disposal site or disposed of using onsite composting, burial, incineration, landfill, and/or rendering. During any carcass transport, measures should be implemented to prevent the escape of contaminated material and vehicles should be disinfected after each use.[812] In addition, farmers should use caution when handling products (e.g., dairy and meat products) from animals that are positive, or suspected to be positive, for a coronavirus. Although heat treatment can reduce or eliminate viral detection of SARS-CoV-2,[815] MERS-CoV can survive in milk for prolonged periods of time.[816] Thus, farmers should consider appropriately disposing of any raw animal products from positive animals to prevent further contamination and viral transmission.

Reducing Animal Contact

Reducing contact between animals and humans and other animals as a biosecurity measure should be highly prioritized because physical separation and distancing substantially reduces exposures to coronaviruses. CDC provides guidance on reducing wildlife animal contact in "Reducing the Risk of SARS-CoV-2 Spreading between People and Wildlife," stating that wildlife researchers should substitute animal capture with remote monitoring methods, which can reduce human contact and animal transport to other locations where virus can spread. When onsite field research is necessary, the minimum number of personnel should be deployed to safely complete tasks. Wildlife rehabilitation facilities should also consider suspending rehabilitation of wildlife species that are highly susceptible to SARS-CoV-2 infection.[817]

USDA APHIS also provides guidance on reducing animal contact on *N. vison* and *O. virginianus* farms in "One Health: Keeping Animals and People Safe from SARS-CoV-2." Workers should stagger work hours, maintain social distancing between each other, and implement physical partitions between each other and animals when possible.[812] Similar methods are used when controlling Bov-CoV transmission on bovine farms, in which farmers have separate pastures and use separate transport vehicles for sero-positive and -negative herds.[811] In addition, to reduce the risk of externally introducing virus to a farm, access to animal housing facilities should be restricted, and workers should stay home if they feel sick.[811,812]

According to CDC's guidance on reducing the risk of viral transmission among pets in "What You Should Know about COVID-19 and Pets," pet owners should avoid contact with pets that test positive for SARS-CoV-2, and depending on veterinarian recommendation, should isolate their infected pets. In addition, pet owners should not allow pets that test positive to roam outside,

in order to reduce viral transmission to other pets and wildlife.[818] Omrani et al. (2015) provides similar recommendations for reducing the transmission of MERS-CoV from camels, such as strict regulation of camel movements and isolation of camels that test positive for MERS-CoV.[819]

Land Use Changes

Land use changes, such as logging, mining, and railroad building, can alter the movement of wildlife and create new habitats for species, allowing for contact between previously isolated species, and thus increasing the risk of recombinant viral mutations.[810] Reducing land use changes can help keep ecosystems with high species diversity intact, which enhances the ecological conditions that maintain and strengthen wildlife immune function and prevents conditions that lead to high viral prevalence and transmission.[820] In accordance with a One Health approach,[809] wildlife conservation and distancing measures are needed to maintain these optimal ecological conditions and reduce viral transmission among wildlife.[820]

Policy on Markets and Trade

The origins of SARS-CoV-2 can be traced back to a live animal market in Wuhan, China,[168] in which intermediate animal hosts may have contributed to the eventual outbreak of the COVID-19 pandemic.[808] Due to the nature of live animal markets in the United States and worldwide, where multiple species that are otherwise isolated from each are in close proximity, there is high likelihood of viral transmission and the emergence of novel recombinant viruses.[821] Thus, biosecurity measures should be enforced in live animal markets and the trade of livestock. However, policies should aim to modify market and trade practices rather than ban them because these practices are often culturally rooted.[810] As a result of placing bans on live animal markets, illegal trade that completely lacks biosecurity may increase, as exemplified through bans enacted by the Chinese government.[808] In contrast, policy changes that can increase biosecurity within live animal markets and livestock trade include behavior change programs, risk education, effective communication, enforcement of regulations, incentives for more sustainable food production, and market rest days to allow for disinfection.[810,822]

Depopulation and Culling

Because of the susceptibility of minks to SARS-CoV-2 and the rapid transmission rates of the virus among minks, both from human-to-mink and mink-to-mink transmission, mink farms have been highly affected by the COVID-19 pandemic. Many farmers have resorted to depopulation (i.e., culling) of animals, which can quickly eliminate viral transmission within infected farm populations. However, depopulation raises ethical concerns for animal rights and welfare.[814] Therefore, other biosecurity measures should be prioritized to reduce virus transmission, especially as more coronavirus vaccinations become available for highly susceptible farm animal populations.

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