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14. ABSTRACT

Coronaviruses (CoV) are a family of viruses that result in a spectrum of infectious diseases ranging from the common cold to SARS-CoV and MERS-CoV (1). Since the emergence of SARS-CoV-2, there are now seven CoVs that can infect humans, and are collectively known as human CoV (HCoV) (2). In addition to HCoVs, there are several CoVs that can infect animals including wildlife, pets, or livestock (1, 3, 4). HCoVs are respiratory pathogens that are primarily transmitted via person-to-person transmission through airborne respiratory droplets (i.e., sneezing, coughing, breathing, and direct/indirect contact) (1, 3).
The most recently identified HCoV is SARS-CoV-2, which causes an acute respiratory disease now named COVID-19 by the WHO (1, 2). SARS-CoV-2 was identified in Wuhan, China in late 2019. SARS-CoV-2 appears to be zoonotic and was initially reported to be transmitted from animals to humans, although it has quickly spread through human-to-human contact (2, 5, 6). Animal-to-person transmission typically occurs when animals are slaughtered for consumption (2, 5, 6) or in other close contact with infected animals (7). Angiotensin-converting enzyme 2 (ACE2) mediates entry of SARS-CoV-2 into host cells (8). In humans, ACE2 is expressed in

15. SUBJECT TERMS

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Literature Review on Oral Routes of Infection for SARS-CoV-2 in Animals and Humans

Task 1 Report

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Table 1. List of Abbreviations

Abbreviations	Definitions
ACE2	Angiotensin-converting Enzyme 2
CoV	Coronavirus
CM	Cynomolgus macaque
hACE2	Human Angiotensin-converting Enzyme 2
hCoV	Human Coronavirus
GI	Gastrointestinal
MERS-CoV	Middle Eastern Respiratory Syndrome Coronavirus
NHP	Non-Human Primate
PFU	Plaque forming unit
RNA	Ribonucleic acid
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2

Section 1.
Introduction

Coronaviruses (CoV) are a family of viruses that result in a spectrum of infectious diseases ranging from the common cold to SARS-CoV and MERS-CoV (1). Since the emergence of SARS-CoV-2, there are now seven CoVs that can infect humans, and are collectively known as human CoV (HCoV) (2). In addition to HCoVs, there are several CoVs that can infect animals including wildlife, pets, or livestock (1, 3, 4). HCoVs are respiratory pathogens that are primarily transmitted via person-to-person transmission through airborne respiratory droplets (i.e., sneezing, coughing, breathing, and direct/indirect contact) (1, 3).

The most recently identified HCoV is SARS-CoV-2, which causes an acute respiratory disease now named COVID-19 by the WHO (1, 2). SARS-CoV-2 was identified in Wuhan, China in late 2019. SARS-CoV-2 appears to be zoonotic and was initially reported to be transmitted from animals to humans, although it has quickly spread through human-to-human contact (2, 5, 6). Animal-to-person transmission typically occurs when animals are slaughtered for consumption (2, 5, 6) or in other close contact with infected animals (7). Angiotensin-converting enzyme 2 (ACE2) mediates entry of SARS-CoV-2 into host cells (8). In humans, ACE2 is expressed in several tissues, including but not limited to the liver, skin, oral mucosa, colon, small intestine, lungs, kidneys, testis, bladder, adrenal glands, thyroid, and adipose tissue (9-11). Given the broad expression of ACE2, throughout the body, infection with SARS-CoV-2 may not only result in a respiratory disease, but also an enteric one. To date, most clinical research and animal models have focused on the respiratory disease resulting from SARS-CoV-2 and have largely ignored enteric symptoms and disease resulting from SARS-Cov-2.

On March 11, 2020, the worldwide human-to-human spread of SARS-CoV-2 was classified as a pandemic by the WHO (1, 12). In the United States, (as of Oct 30,2020) approximately 9.4M cases have been diagnosed with just over 230,000 deaths (13). Since the beginning of the SARS-CoV-2 outbreak, there has been an extensive effort worldwide to characterize the infectivity and life cycle of the virus (1, 14). Development of vaccines and therapeutics against the virus are a prime focus for world governments and the greater scientific community. Animal models are being used as a key approach to understanding the complexities of the infectious cycle and control of the disease known as COVID-19 (1, 14). To date, there have been numerous studies in animal models for the related viruses SARS-CoV and MERS where bats have been a key reservoir and vector for spread of the viruses (1, 15, 16). For SARS-CoV-2, transmission and life cycle studies have been conducted in bats, mice, hamsters, ferrets, domestic cats, pigs, and in NHP models including rhesus macaques, marmosets, African Green Monkeys (AGM), and cynomolgous macaques (CM) (1, 15, 16). However, there is a paucity of data on oral transmission of SARS-CoV-2 through the fecal-oral or foodborne ingestion routes of infection (8, 17-20). With viruses such as Ebola, there is a clear association of infection through the ingestion of bush meat and from contact with bodily secretions (21). Therefore, it is of interest to understand the role of the fecal-oral route of infection and transmission for SARS-CoV-2.

In this review, we will present some aspects of the role of oral-fecal and oral/ingestion routes of infection and the associated morbidity and mortality with SARS-CoV-2 in animal models and evaluate these models as potential systems for study of the oral route of infection for this virus.

Section 2.
Evidence for Human Fecal-Oral Exposure of SARS-CoV-2

It is currently accepted that the primary mode of transmission of SARS-CoV-2 is person-to-person which is intrinsically the most dangerous to humans and the driver for the current pandemic. Initial cases of SARS-CoV-2 included a cluster of elderly males that frequently visited the Huanan South Seafood market in Wuhan, China (22). This market sells seafood, in addition to live and slaughtered chicken, pheasants, bats, marmots, deer, snakes, and the organs of rabbits and other wild animals. Importantly, there were few reports of person-to-person transmission within households, that were exposed to these initial cases of SARS-CoV-2 (22). Thus, the food at the market could have been contaminated. These initial exposures could have occurred via the oral route; however, SARS-CoV-2 in these patients resulted in primarily respiratory symptoms, which would not rule out a respiratory transmission.

As previously mentioned, ACE2, the enzyme responsible for mediating entry of SARS-CoV-2 into cells is highly expressed in oral mucosa, the gastrointestinal (GI) tract, and the small intestine (9-11). SARS-CoV-2 infection of cells in the GI tract was shown to alter the expression of ACE2 in the brush border of enterocytes, resulting in microbial dysbiosis and inflammation, which could induce GI symptoms such as diarrhea (10).

To date, there are several studies suggesting there is evidence for GI involvement of SARS-CoV-2 infection in patients; thereby, bringing forth the hypothesis that SARS-CoV-2 can be transmitted via the fecal-oral route. There is evidence in the literature showing that GI involvement following HCoV infection does exist. According to Kwan et al., 76% of patients with SARS-CoV developed diarrhea within one week of illness (23). This study showed that SARS-CoV replicates within the small and large intestines, and that infectious virus could be isolated from intestinal tissue but not fecal matter (23, 24). In addition, 25% of MERS-CoV patients reported GI symptoms such as diarrhea and abdominal pain) (25). In patients, infected with MERS-CoV, GI symptoms occurred both prior to and after the appearance of respiratory symptoms (25). In addition, MERS-CoV RNA was detected in the stool of patients; however, no infectious virus was detected (26). Of note, the first reported SARS-CoV-2 case reported in the United States presented with GI symptoms (27, 28).

In a meta-analysis of over 4000 patients from Eastern Asia, over 20% of patients reported experiencing GI symptoms; viral RNA was detected in over 50% of these patient's stool samples (8). As previously observed with MERS-CoV patients, it was found that GI symptoms resulting from SARS-CoV-2 infection presented either prior to or following the appearance of respiratory symptoms (8, 29, 30). In another 200-patient study, over 50% of patients experienced GI symptoms from SARS-CoV-2 infection, and these patients had longer hospital stays as compared to patients that did not present with GI symptoms (31).

According to Wu et al, SARS-CoV-2 RNA was detected in endoscopic specimens collected from the esophagus, stomach, and rectum (30). Other studies detected SARS-CoV-2 RNA in both anal and respiratory swabs of patients; however, more anal swabs tested positive than respiratory swabs (32). In support of the potential for the oral-fecal route of transmissibility. It has been reported that the presence of SARS-CoV-2 RNA in fecal matter of infected animals is detectable much longer than SARS-CoV-2 RNA collected from respiratory samples (28, 33). It is prudent

to note, that it is unknown whether the viral RNAs detected in fecal samples were from infectious virus particles. The presence and persistence of viral RNA in the feces does provide evidence that fecal-oral transmission of SARS-CoV-2 could be a viable route of infection.

The presence of SARS-CoV-2 was identified in sewage pipes and puddles on the streets of a populated community in Guangzhou, China (20). A retrospective cohort study, studied cleaner and waste picker workers and found that they do not clean their shoes when they arrive home from work and put the dirty shoes next to their clean shoes (20). SARS-CoV-2 RNA was identified on the squat toilets and the shoes of residents inside these workers apartments (20). The apartments of community residents that tested positive in the area of the contaminated sewage were assessed for the presence of SARS-CoV-2. Viral genome sequencing found homologous viral sequences between the squat toilets, shoes, and residents who tested positive for SARS-CoV-2. Taken together, this study suggests that contaminated sewage may be a source of SARS-CoV-2 infection in communities with low hygiene standards. In addition, this sort of fecal-oral contamination would also be relevant to children and the elderly that must be cared for. If young children and non-ambulatory elderly patients are housed in childcare or nursing home facilities, respectively, then this could increase the fecal-oral exposure risk in these caregiver populations.

Zang et al. showed that SARS-CoV-2 could infect and replicate in human intestinal epithelial cells; however, SARS-CoV-2 was inactivated by human colonic fluid and infectious virus was not collected from the samples (34). Although the study did not directly support the theory that SARS-CoV-2 can be transmitted via the fecal-oral route, it did not rule out that possibility because such a small sample size may not be representative of the entire population. Currently, there are a number of publications supporting the fecal-oral route of infection for human-to-human transmission of SARS-CoV-2. Thus, developing animal models to better understand the oral routes of exposure may prove important in directing prevention and control of COVID-19. In the following sections, a brief overview of the current animal models used to study SARS-CoV-2 will be discussed, with a focus on studies that assess the fecal-oral route of transmission.

Section 3.
SARS-CoV-2 Animal Models

Bats

Evidence indicates that SARS-CoV-2 likely originated from a bat reservoir, as SARS-CoV and MERS-CoV did. Schlottau et al. Intranasally inoculated Egyptian fruit bats with SARS-CoV-2 (10^5 Median Tissue Culture Infectious Dose) (35). Importantly, no clinical signs of illness were observed in infected bats. Infectious virus was detected from the respiratory tract 2 days post inoculation, SARS-CoV-2 RNA was isolated from the respiratory tract of all bats up to 12 days post inoculation, and all bats seroconverted 21 days post inoculation (35). SARS-CoV-2 shedding was observed from individual oral swabs and pooled fecal swabs collected from all infected bats (35). Twenty-Four hours following intranasal inoculation of the fruit bats, three naïve fruit bats were exposed to the infected bats, to assess direct transmission of SARS-CoV-2 amongst fruit bats (35). Only one of the three naïve bats became infected with SARS-CoV-2. It is important to note that the naïve bat that contracted SARS-CoV-2 via direct contact was in the early stages of pregnancy, and thus, may have been more susceptible to infection due to the immunosuppression that occurs during pregnancy (35). Taken together, this study shows that Egyptian fruit bats may be a reservoir for SARS-CoV-2; however, they cannot be the original SARS-CoV-2 reservoir, as they do not exist in China. Furthermore, these data suggest that fruit bats are more likely to provide a better model for studying viral transmission rather than human SARS-CoV-2 pathogenesis.

Mouse

SARS-CoV-2 does not interact with mouse ACE2, the enzyme that mediates the entry of SARS-CoV-2 into host cells (15). Thus, humanized mouse models were created to make mice susceptible to SARS-CoV-2. Since SARS-CoV requires ACE2 to gain entry into host cells, a transgenic mouse expressing human (h)ACE2 was already generated (k18-hACE2 mouse strain) (36, 37). These mice are susceptible to SARS-CoV-2; however, the systemic inflammation and neuroinflammation was not representative the human presentation clinically (15). To improve upon this mouse model, Yang et al. developed a hACE2 transgenic mouse that utilized the endogenous mouse ACE2 promoter treated intranasally with 10^5 median tissue culture infectious dose (38). The tissue distribution of hACE2 in this mouse more closely mimicked hACE2 distribution, but the morbidity and mortality rates observed in these mice were not representative of SARS-CoV-2 morbidity and mortality rates in humans.

In addition to the aforementioned mouse models, another humanized hACE2 mouse model was generated using CRISPR/Cas9. To generate this mouse model, hACE2 linked to TdTomato, a red fluorescent protein, was inserted into the mouse ACE2 locus, producing similar tissue distribution of hACE2 as seen in the transgenic mouse produced by Yang et al (39). The CRISPR/Cas9-hACE2 mice were inoculated with SARS-CoV-2 via intragastric (oral gavage, 4×10^6 PFU) and intranasal exposure (4×10^5 PFU) (39). Data indicated that intragastric exposure produced similar viral titers as in the trachea and lungs to those observed following intranasal infection (39). Importantly, no clinical signs of infection were observed following either route of exposure in these CRISPR/Cas9-hACE2 mice (39).

Alternatively, mouse-adapted viruses have been generated to infect wild-type mice by serially passaging virus in the mouse. Gu et al. serially passaged SARS-CoV-2 in Balb/c mice for a total of 6 passages to generate mouse-adapted SARS-CoV-2 (40). Once the concentration of mouse-adapted SARS-CoV-2 was determined, mice were intranasally inoculated with (7.2×10^5 PFU) (40). Three days post-inoculation, viral RNA was detected in the lungs, trachea, heart, liver, spleen, brain and feces (40). This model mimics the human pathology but does not mimic human morbidity and mortality rates.

Taken together, these studies suggest that humanized mouse models of SARS-CoV-2 may prove useful to study SARS-CoV-2; however, these models are not ideal, as they do not accurately recapitulate human disease. In addition to the mouse models mentioned here, there are several other mouse models that have been generated. Thus, an overview of the mouse models can be reviewed here (15). The models mentioned here show that assessing fecal-oral SARS-CoV-2 exposure and/or transmission may prove useful, as studies have shown that mice are susceptible to infection through oral exposure and that viral RNA is subsequently present in their feces. One should proceed with caution if utilizing these mouse models, since the accurate recapitulation of the human disease is integral to progressing in this area of SARS-CoV-2 research.

Hamsters

Golden Syrian Hamsters have proven useful in viral research and are proving to be useful in SARS-CoV-2 research. Hamsters are a good animal species to study viral shedding and persistence (41). The structure of ACE2 in hamsters is similar to humans, making hamsters susceptible to human SARS-CoV-2 (41). Most commonly, for SARS-CoV-2 studies hamsters are intranasally inoculated to study disease pathogenesis or to test the efficacy of potential treatment options (42-44).

Following intranasal inoculation of hamsters with $8x10^4$ median tissue culture infectious dose, the highest viral titers were observed 2 days post infection. Viral titers were then found to slowly decline from days 3-7 post infection (43). At day 7, viral loads were below detection limits (43). Hamsters infected with SARS-Cov-2 spontaneously seroconverted between days 7 and 14 post infection (43). SARS-CoV-2 has been shown to have a fast transmission rate in hamsters (43). Naïve hamsters co-housed with infected hamsters all became infected with SARS-CoV-2; these hamsters could have contracted the virus via respiratory transmission or fecal-oral exposure (43). In order to tease out the transmission of SARS-CoV-2 in hamsters, naïve hamsters were placed in wire cages adjacent with infected hamsters or naïve hamsters were placed in cages that previously housed hamsters that were 0-2 days post infection from SARS-CoV-2 (43). All of the naïve hamsters stored in wire cages adjacent to infected hamsters became infected with SARS-CoV-2 one day following exposure and peak viral loads were observed 3 days following exposure, suggesting that hamsters can and do transmit SARS-CoV-2 via aerosols such as respiratory droplets (43). One out of three naïve hamsters placed in cages that previously housed infected hamsters became infected with SARS-CoV-2, these data suggest that hamsters can contract SARS-CoV-2 from fomite exposure but not as efficiently as aerosol exposure (43). In addition, viral RNA was detected in nasal washes and feces of all infected hamsters following all types of exposure; however, infectious virus was only detected from the nasal washes (43). Taken together, these studies suggest that transmission of virus amongst hamsters is primarily

though aerosol exposure, and hamsters that contracted SARS-CoV-2 from fomite exposure likely contracted SARS-CoV-2 from oral contact with an object in the cage rather than feces.

Lee et al. utilized the hamster model of SARS-CoV-2 to determine how the pathogenesis of SARS-CoV-2 differed following oral exposure in comparison to intranasal exposure (44). Hamsters were orally inoculated with the highest dose of intranasal inoculum (10^5 PFU) (44). Results indicate that oral inoculation only resulted in mild pneumonia in 67% of the animals and did not result in any clinical signs of illness (44). In addition, orally inoculated hamsters had significantly decreased lung pathology and viral load in comparison to intranasally inoculated hamsters. Over 80% of the orally inoculated hamsters shed viral RNA in oral and fecal swabs at a level similar to intranasally infected hamsters inoculated with 10^5 PFU (44). This study showed that oral exposure of hamsters to SARS-CoV-2 does actively result in SARS-CoV-2 infection, but it in a less efficient manner than the intranasal exposure route.

Ferrets

Another animal model that has been utilized to study SARS-CoV-2 is ferrets. The respiratory tract of ferrets is anatomically and physiologically similar to humans (45). In addition, ferrets were utilized to study SARS-CoV, which gains access to host cells via a similar mechanism to SARS-CoV-2 (16, 45). Shi et al. inoculated ferrets with SARS-CoV-2 intranasally with 10^5 PFU (46). SARS-CoV-2 RNA and infectious virus was isolated from the nasal turbinate, soft palate, and the tonsils, thereby suggesting that SARS-CoV-2 can replicate in the upper respiratory tract of ferrets (46). In order to determine if different strains of SARS-CoV-2 produced different symptomology in the ferrets, two groups of ferrets were inoculated intranasally with two different strains of SARS-CoV-2 (46). One strain was isolated from an environmental sample collected from the Huanan Seafood Market in Wuhan, China, while the other strain was isolated from a human sample in Wuhan, China. Disease presentation resulting from the SARS-CoV-2 was not different between the two groups of ferrets (46). In addition to assessing disease presentation, the presence of virus was assessed in both fecal and nasal swabs (46). Importantly, SARS-CoV-2 RNA was present in both fecal and nasal swabs, while infectious virus was only isolated from the nasal swabs of ferrets (46).

Kim et al. assessed the transmission SARS-CoV-2 among ferrets (47). Ferrets were exposed SARS-CoV-2 intranasally with $10^{5.5}$ median tissue culture infectious doses and then either housed in cages with permeable partitions containing naïve ferrets or cohoused with naïve ferrets, allowing for the assessment of indirect and direct SARS-CoV-2 transmission amongst ferrets (47). Naïve ferrets exposed to SARS-CoV-2 via direct contact presented with mild symptoms (increased body temperature, no weight loss, and no mortality) (47). Naïve Ferrets exposed to SARS-CoV-2 via indirect contact only experienced increased body temperature (47). SARS-CoV-2 RNA was detected in the nasal washes, feces, and urine of all ferrets (intranasal exposed, direct contact, and indirect contact) (47). Taken together, these studies suggest that the transmission of SARS-CoV-2 amongst ferrets is similar to humans; however, ferrets only present with a mild symptomology and no mortality, which is different from the human presentation of SARS-CoV-2.

Felines

Both dogs and cats live in close proximity to humans, thus their susceptibility to SARS-CoV-2 is of importance. In addition, the ability of cats and dogs to be a reservoir for SARS-CoV-2 is also important. Tigers at the Bronx Zoo in New York have contracted SARS-CoV-2 from humans (16). There are also a few reports that document human to feline SARS-CoV-2 transmission (16). Felines are susceptible to SARS-CoV, thus it is not surprising that they are susceptible to SARS-CoV-2 (48). In addition, it is known that feline ACE2 receptor interacts with SARS-CoV and SARS-CoV-2 (16, 48). Shi et al. intranasally inoculated sub adult cats with 10^5 PFU SARS-CoV-2, and SARS-CoV-2 RNA was detected in the nasal turbinate, soft palate, tonsils, trachea, and small intestine (46). In a subsequent experiment, juvenile cats were intranasally inoculated with 10^5 PFU SARS-CoV-2 then housed with naïve cats in cages containing a permeable divider (46). The naïve cats contracted SARS-CoV-2, showing that respiratory droplet transmission of SARS-CoV-2 is possible (46). Juvenile cats infected with SARS-CoV-2 presented with large viral lesions in the nasal turbinate, soft palate, trachea, and lungs, suggesting that juvenile cats are more susceptible to SARS-CoV-2 than sub-adult cats (46). All cats infected with SARS-CoV-2 were asymptomatic (46), so even though cats can contract SARS-CoV-2, they would not be a good model for human disease.

Canines

Canine ACE2 is structurally similar to hACE2 (49), thus, one would hypothesize that dogs are susceptible to SARS-CoV-2. However, dogs are not susceptible to SARS-CoV-2 (16). There are a few instances where dogs have tested positive for SARS-CoV-2 (16). A Pomeranian tested positive for SARS-CoV-2 in Hong Kong, China (16, 46). In addition, there are isolated cases of dogs that have tested positive for SARS-CoV-2 (16). Shi et al. performed a study to determine the replication and transmission of SARS-CoV-2 in beagles following intranasal inoculation with 10^5 PFU (46). Intranasally inoculated beagles were cohoused with naïve beagles (46). SARS-CoV-2 RNA was detected in the feces of exposed naïve beagles, but viral RNA was not detected in any other organ (46). The dogs that were intranasally inoculated with virus developed viral antibodies against SARS-CoV-2, while the exposed dogs remained seronegative (46).

Non-Human Primate (NHP) Models

There are several NHP models that have been utilized to study SARS-CoV-2 including cynomolgus macaques, marmosets, rhesus macaques, and African green monkeys (15, 16, 50-52). Utilizing NHPs to study human disease is important, as NHPs are closely related to humans and possess similar physiology and immunology to humans (53). Thus, assessing the relevance of different NHP models to study the pathogenesis of SARS-CoV-2 in humans will be useful in the development of treatment options for SARS-CoV-2. Each of the aforementioned NHP species are outlined below.

Cynomolgus Macaques

The cynomolgus macaque (CM) has been utilized to study SARS-CoV in the past (54). In one study, geriatric CM were intranasally exposed to SARS-CoV-2 (55). Results showed that the geriatric CM had prolonged SARS-CoV-2 RNA shedding from the upper respiratory tract than

younger CMs that were intranasally exposed to SARS-CoV-2 (55). Neither the young nor geriatric CMs showed any symptoms; however, all animals did spontaneously seroconvert beginning four days post infection (55). A study performed by Lu et al. observed increased body temperature and weight loss in CMs intranasally exposed to SARS-CoV-2 (56). Importantly, both studies identified interstitial pneumonia in the lungs of CMs (55, 56). Taken together, these studies suggest that CMs may still prove useful in studying the pathogenesis of SARS-CoV-2; however, they do not recapitulate the symptoms observed in humans following SARS-CoV-2 infection.

Common Marmosets

Marmosets are commonly used to study the pathogenesis and immunization for MERS-CoV (57); thus, it was thought that they may prove useful in studying SARS-CoV-2. Lu et al. intranasally exposed marmosets to SARS-CoV-2 (10^6 PFU), and viral RNA was detected in the blood, nasal, throat, and anal swabs until 14 days post infection (56). The marmosets did not spontaneously seroconvert (56). In addition, inoculated marmosets did not show any signs of pneumonia, nor did they have detectable levels of viral RNA in any of their organs (56). Taken together, this study indicates that marmosets are resistant to SARS-CoV-2, and do not provide a good model for studying the pathogenesis of SARS-CoV-2.

Rhesus Macaques

Rhesus macaques have most commonly been used to study reinfection potential and for vaccine design (16). Thus, they may prove useful in understanding the pathogenesis and development of new therapeutic options for SARS-CoV-2. Following intranasal inoculation, rhesus macaques show mild to moderate symptoms including decreased appetite, weight loss, fever, increased and/or irregular respiration, and hunched posture (16, 56-58). SARS-CoV-2 RNA was isolated from the respiratory and gastrointestinal tracts of infected rhesus macaques, which is similar to humans (16, 56, 58, 59). However, SARS-CoV-2 RNA has been isolated from atypical organs including the spinal cord, heart, skeletal muscle, bladder, liver, and kidney, which is dissimilar from human infection (16, 56, 57, 60). Rhesus macaques have been shown to get pulmonary edema and diffuse interstitial pneumonia, which are characteristic pathologies of human disease (16).

Research indicates that the elderly are more susceptible to contracting SARS-CoV-2 than young humans (61), thus, a few studies have assessed how age affects rhesus macaques infected with SARS-CoV-2. Importantly, geriatric (15 years old) rhesus macaques develop a more severe pneumonia and have an increased viral load in the lungs and anus than younger rhesus macaques (3-5 years old) that have been inoculated with SARS-CoV-2 (10^6 median tissue culture infectious doses per milliliter) (62). SARS-CoV-2 RNA was found to replicate in the entire lung of the geriatric rhesus macaques, while it was found to only replicate in the upper lobe of younger rhesus macaques (62). Another study assessed the transmission of SARS-CoV-2 via routes other than intranasal exposure. Rhesus macaques were inoculated with SARS-CoV-2 (10^6 median tissue culture infectious doses per milliliter) via the conjunctival and gastric route (60). Mild pneumonia and gastrointestinal infection were observed in the conjunctival route of exposure, but not the gastric route (60). Following gastric exposure, there was no detectable SARS-CoV-2 RNA in any organs (60). Taken together, these studies show that utilizing rhesus

macaques for assessing respiratory routes of exposure for SARS-CoV-2 may prove useful but this NHP model may not prove useful in studying the fecal-oral route of exposure.

African Green Monkey

African Green Monkeys (AGM) are not as frequently used in research as other NHPs such as rhesus macaques or cynomolgus macaques. AGMs have previously been used to study various pulmonary infections resulting from rift valley fever, pneumonic plague, and SARS-CoV, in addition to others (50). Thus, one would expect that AGMs could be useful in studying the pathogenesis of SARS-CoV-2.

Woolsey et al. developed a model for studying SARS-CoV-2 in AGMs (52). In this study, AGMs were inoculated with SARS-CoV-2 intranasally and intratracheally (5×10^5 PFU) (52). Over the course of the study, AGMs only developed mild symptoms (decreased appetite and increased body temperature) (52). Five days post infection, all AGMs developed pulmonary lesions and interstitial pneumonia (52). SARS-CoV-2 RNA was detected from mucosal and bronchoalveolar lavage fluid, nasal secretions, oral swabs, and rectal swabs (52). Infectious virus was detected in the oral swabs and bronchoalveolar lavage fluid from all AGMs, and the rectal swab of one AGM also had infectious virus (52). In addition, all AGMs spontaneously seroconverted beginning at day 5 post infection (52). Another study inoculated AGMs intranasally with SARS-CoV-2 and obtained similar results to the reported results of Woolsey et al. that are described above (51).

Hartman et al. performed a study to determine if different routes of inoculation in AGMs lead to a different disease presentation (50). AGMs were exposed to SARS-CoV-2 via aerosol (3.7–4.2 $\log_{10}$ PFU) or mucosal exposure (5×10^5 PFU/ml) (50). The mucosal exposure includes the (oral, nasal, and ocular mucosal membranes) (50). All AGMs developed mild symptoms (50). There was no significant difference in the SARS-CoV-2 RNA levels detected in the organs (50). It is important to note that the viral load administered to the mucosal exposed AGMs was 100-fold higher than the aerosol exposed animals (50). In both exposure groups, SARS-CoV-2 RNA was detected at much higher levels in the gastrointestinal tract than in the respiratory tract (50). Taken together, these studies show that AGMs do show symptoms of SARS-CoV-2 infection that resemble the human presentation. Also, these studies suggest that AGMs may be useful in studying SARS-CoV-2 fecal-oral route of exposure, as AGMs shed SARS-CoV-2 RNA from their gastrointestinal tract, and they are susceptible to SARS-CoV-2 exposure via the mucosal route.

Section 4.
Conclusions and Recommendations

A variety of animal models are currently being developed to describe the natural history of SARS-CoV-2 infection and attempt to match the pathology and associated morbidity and mortality seen in human cases of COVID-19. The focus of this review is to describe the knowledge base in the literature on fecal-oral transmission of SARS-CoV-2 and to provide suggestions on potential animal models. Most SARS-CoV-2 animal models utilized to study the pathogenesis of SARS-CoV-2 infection largely focuses on the respiratory exposure route via intranasal inoculation. These models have proven quite useful in studying SARS-CoV-2 pathogenesis and developing countermeasures for the treatment and/or prevention of Covid-19. In humans, the respiratory route of transmission and exposure of SARS-CoV-2 is the most well understood and characterized; thus, it is logical that animal models utilizing intranasal inoculation are the most widely studied and utilized.

As highlighted in the above sections, there is evidence in the literature supporting a role for an enteric route of SARS-CoV-2 exposure and/or transmission in humans (17, 18, 29, 31). There are several reports of GI symptoms including abdominal pain and diarrhea in humans following SARS-CoV-2 exposure (17, 18, 29, 31). These reports highlight a need for developing animal models to delineate the mechanisms by which SARS-CoV-2 is transmitted and the pathogenesis of infection following fecal-oral transmission route. A few published studies have utilized an oral exposure route in their studies, specifically, hamsters (44), AGM (50), rhesus macaques (60), and mice (39).

To date, there are few, if any studies that report or assess GI symptoms other than the detectability of infectious virus and/or viral RNA in the feces or GI tract. Lee et al. exposes Syrian golden hamsters via oral exposure (44), thereby utilizing an exclusively oral exposure model. However, the primary assessments of this oral hamster model are a comparison of symptom presentation and pathogenesis to the respiratory exposure model. Albeit, the study does indeed provide useful information to scientists, it does not report the presence or absence of GI symptoms in these animals. In some instances, animal models assessing the oral exposure route include simultaneous exposure via ocular and nasal mucosa like the study published by Hartman et al. (50). More specifically, the study by Hartman et al. does not truly examine the pathogenesis of SARS-CoV-2 infection via the oral route of exposure, as the AGMs were inoculated simultaneously via oral, nasal, and ocular mucosa. In addition, no GI symptoms were reported following the inoculation of the AGMs. Taken together, there is a clear gap in the literature to assess the pathogenesis and symptomology of SARS-CoV-2 infection following fecal-oral transmission.

Some models better mimic human infection than others, although there are still gaps in knowledge about human-to-human transmission via the fecal/oral route. As a means of organizing relevant animal models to test the fecal-oral route of infection, we outlined the various routes of infection that are utilized in the animal models we presented in this review (Table 2).

Table 2. Routes of Infection Demonstrated in Relevant Animal Models

Model	Fecal-Oral[a]	Intranasal[b]	Intratracheal[c]	Aerosol[d]
Human*	+	+	-	+
Hamster	+	+	-	+
NHP[++]	+	+	+	+
Mouse (rec)	-	+	-	-
Ferret	-	+	-	+
Felines	-	+	-	+
Canines	-	+	-	-
Bats	-	+	-	-

Presence (+) or Absence (-) of demonstrated routes of infection.

* Human included for comparison.

[++] NHP indicates that at least one species of non-human primate described in the text was published as an animal model used to demonstrate the route of infection listed in Table 2.

Key published routes of infection included:

[a] Fecal-Oral transmission demonstrated.

[b] Intranasal infection demonstrated.

[c] Intratracheal infection demonstrated.

[d] Aerosol infection demonstrated.

In order to make recommendations on relevant animal models to study fecal-oral SARS-CoV-2 transmission, we empirically quantified the following criteria: Symptoms, tissue pathology, course of infection, mortality rates, immunology, fecal-oral transmission. We did identify a major informational gap in that there is a paucity of published data on fecal-oral transmission of SARS-CoV-2 and when available, such data tended to be anecdotal or incomplete and secondary to the main studies which most often were naturally focused on respiratory disease. In Table 3, we used a compilation of various criteria including GI tract involvement and/or fecal-oral transmission, that best parallels aspects of human disease most closely.

Table 3. Ranking of animal models for potential utility in testing fecal-oral route of infection.

Model	Symptoms (General)[a]	Tissue Pathology[b]	Course of Infection[c]	Mortality Rates[d]	Immunology[e]	Fecal-Oral Transmission[f]	Totals
Human*	1	1	1	1	1	0	5
Hamster	1	1	1	0	1	1	5
NHP[++]	1	1	1	0	1	0	4
Mouse (recomb)	0	1	1	0	1	0	3
Ferret	0	1	1	0	1	0	3
Felines	0	1	1	0	0	0	2
Canines	0	0	0	0	0	0	0
Bats	0	1	1	0	0	0	2

Criteria for selecting the best model(s) are weighted based on the presence (Yes) or absence (No) of that criteria in either animal or human disease with 1 = Yes and 0 = No and ranked high to low (best to worst).

* Human included for comparison.

[++]NHP indicates that at least one species of non-human primate described in the text did or did not exhibit the criteria listed in Table 3.

Comparative criteria may include:

[a] Symptoms (General): Fever, lethargy, weight loss, cough, ruffled fur/coat, etc.

[b] Pathology (Tropism): Virus present in GI tract/stool, lungs, trachea, stool, nasopharyngeal, etc.

[c] Course of Infection: Time to onset of symptoms, recovery and immunology, etc.

[d] Mortality Rates: Death prior to recovery after corresponding symptoms of disease.

[e] Immunology: Expression of IgM and IgG in response to infection.

[f] Fecal/Oral Transmission: Focused study on F/O transmission has been conducted.

Thus, our literature review shows that hamsters and NHPs are the most probable small and large animal models, respectively, to study the transmission of SARS-CoV-2 through the fecal-oral exposure. In addition, reports from the literature reveal that both hamsters and NHPs, more specifically Syrian golden hamsters and African Green Monkeys are susceptible to oral exposure of SARS-CoV-2 and shed SARS-CoV-2 RNA in from their GI tract (44, 50). Thus, the literature suggests that hamsters and NHPs may provide viable models to mimic and study the disease progression and pathogenesis of SARS-CoV-2 in humans caused through fecal/oral routes of infection.

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