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Ferrets: A powerful model of SARS-CoV-2

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ABSTRACT

The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in recent years not only caused a global pandemic but resulted in enormous social, economic, and health burdens worldwide. Despite considerable efforts to combat coronavirus disease 2019 (COVID-19), various SARS-CoV-2 variants have emerged, and their underlying mechanisms of pathogenicity remain largely unknown. Furthermore, effective therapeutic drugs are still under development. Thus, an ideal animal model is crucial for studying the pathogenesis of COVID-19 and for the preclinical evaluation of vaccines and antivirals against SARS-CoV-2 and variant infections. Currently, several animal models, including mice, hamsters, ferrets, and non-human primates (NHPs), have been established to study COVID-19. Among them, ferrets are naturally susceptible to SARS-CoV-2 infection and are considered suitable for COVID-19 study. Here, we summarize recent developments and application of SARS-CoV-2 ferret models in studies on pathogenesis, therapeutic agents, and vaccines, and provide a perspective on the role of these models in preventing COVID-19 spread.

Keywords: SARS-CoV-2; COVID-19; Animal models; Ferret; Angiotensin-converting enzyme 2 (ACE2) receptor

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), emerged in Wuhan, Hubei Province, China, in December 2019, and spread globally thereafter (Bedford et al., 2020; Zhu et al., 2020). Today, COVID-19 remains a serious threat to human health, with over 600 million confirmed cases and 6.4 million deaths across nearly 200 countries as of 1 September 2022 (https://covid19.who.int). SARS-CoV-2 infection is characterized by respiratory disease ranging in severity from mild upper respiratory tract illness to acute respiratory distress syndrome (ARDS) and severe interstitial pneumonia, leading to potential multiple organ failure and death in severely ill patients (Wang et al., 2020a). In addition to respiratory symptoms, SARS-CoV-2 can also cause myocarditis, thromboembolism, liver dysfunction, sepsis, and anosmia (Wiersinga et al., 2020). Given the large number of mutations, multiple SARS-CoV-2 variants, including variants of interest (VOIs) and concern (VOC), have challenged global efforts to control COVID-19 due to their potential effects on viral transmissibility, epidemiology, virulence, and pathogenicity, as well as the reduced effectiveness of COVID-19 vaccines (immune escape) (Beavis et al., 2022; Sun et al., 2022a). Thus, understanding the pathogenicity of SARS-CoV-2 is necessary to develop novel antiviral drugs and therapeutic strategies against COVID-19.

Establishing an ideal animal model for COVID-19 is of great significance for clarifying the pathogenic mechanisms of disease and testing the efficacy of vaccines and antiviral agents. To date, mice, hamsters, ferrets, minks, tree shrews, and several non-human primates (NHPs) have been used to study SARS-CoV-2 infection (Ma et al., 2022; Xu et al., 2020). Among them, ferrets are naturally susceptible to SARS-CoV-2 and have been widely used in respiratory virus research (Kim et al., 2020; Wong et al., 2019). Thus, in this review, we discuss ferret models of COVID-19 and their ability to mimic natural COVID-19 infection and pathogenesis, thereby providing relevant information for further preclinical research and testing of COVID-19 vaccines and antiviral agents.

SARS-CoV-2 AND HOST CELL RECEPTORS

SARS-CoV-2 is an enveloped and pleomorphic virus belonging to the β-coronavirus genus, with round or oval viral particles about 60–140 nm in diameter (Zhu et al., 2020). The virus contains a single positive-sense RNA genome, which shares 79% and 50% sequence homology with SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), respectively (Zhou et al., 2020). The spike (S) protein is

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a vital protein located on the surface of the virus envelope and can be cleaved into S1 and S2 subunits by host cell transmembrane serine protease 2 (TMPRSS2) (Baughn et al., 2020). Subunit S1 is involved in host receptor binding, while subunit S2 participates in membrane fusion to promote viral entry into host cells by endocytosis. After entry, virus RNA is subsequently released into the cytoplasm to begin its life cycle and produce more virus particles (Li, 2016; Walls et al., 2020).

Similar to SARS-CoV, SARS-CoV-2 enters host cells primarily by targeting the human angiotensin I converting enzyme 2 (ACE2) receptor. The affinity of the S protein to ACE2 is 10–20 times stronger than that of SARS-CoV, which may be responsible for its high infectivity (Wrapp et al., 2020). Single-cell RNA sequencing has shown that ACE2 is mainly expressed in type Ⅱ alveolar epithelial cells (AT2) (Zhao et al., 2020). About 83% of cells expressing ACE2 in lung tissue are AT2 cells, suggesting that SARS-CoV-2 mainly infects the lower respiratory tract. However, in addition to respiratory epithelial cells, ACE2 is also highly expressed in cardiomyocytes, renal proximal convoluted tubule epithelial cells, bladder epithelial cells, and esophageal, ileal, and Leydig cells, making these tissues and organs permissible to SARS-COV-2 infection (Hamming et al., 2004; He et al., 2020; Liu et al., 2020).

SARS-CoV-2 ANIMAL MODELS

Animal models play a central role in COVID-19 research. SARS-CoV-2 can recognize ACE2 from transgenic mice, hamsters, rats, pigs, ferrets, and cats, suggesting their potential as animal models of SARS-CoV-2 infection. NHP models can accurately simulate the pathogenesis of human COVID-19 and are ideal models for studying SARS-CoV-2. Various species and transgenic animal models have been developed and used to facilitate the testing of vaccines and therapeutics for the prevention and treatment of COVID-19 (Lin et al., 2022) (Figure 1; Table 1).

FERRET MODEL OF SARS-CoV-2

The domestic ferret (*Mustela putorius furo*) is a popular animal model for evaluating viral pathogenesis and transmission as well as the efficacy of antiviral agents (Schiffman et al., 2022). Given their similar anatomical structure and physiology to the human respiratory system and their ability to cough and sneeze, ferrets are considered a suitable model for studying respiratory virus transmission and pathogenesis (Sun et al., 2010). Ferrets are highly susceptible to the influenza virus, and exhibit many of the clinical symptoms observed in humans following infection. As such, they are an excellent animal model for evaluating the pathogenesis and transmission of influenza viruses as well as testing countermeasure efficacy (Belser et al., 2020; Si et al., 2022). Ferrets are also valuable models for other respiratory viruses, including human respiratory syncytial virus (Prince and Porter, 1976; Stittelaar et al., 2016) and SARS-CoV (Martina et al., 2003; Van Den Brand et al., 2008). They have also been used to characterize filovirus infections, showing high susceptibility to lethal diseases caused by Ebola, Sudan, Bundibugyo, and Reston viruses and recapitulating many aspects of human filovirus disease, including systemic viral replication, coagulation abnormalities, and dysregulated immune response (Schiffman et al., 2022). In addition, their relatively small size and availability make ferrets an ideal choice for countermeasure evaluation and pathogenesis modeling of certain viruses.

Ferrets have also been shown to support SARS-CoV-2 infection and have been used extensively in COVID-19 research, including transmission, pathogenesis, and treatment.

Ferrets and transmission of SARS-CoV-2

Ferrets provide an ideal model to study SARS-CoV-2

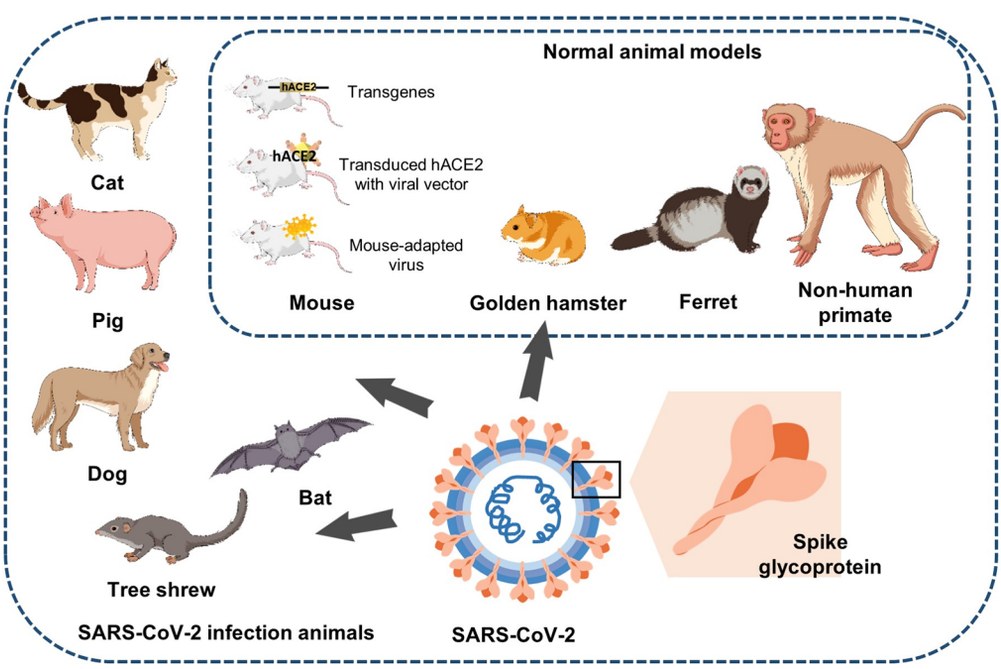

Figure 1 Animal models of SARS-CoV-2

Mice, golden hamsters, ferrets, and NHPs are often used to study pathogenesis, transmission, and countermeasure evaluation. Other animals, such as cats, pigs, dogs, and bats, can also be infected with SARS-CoV-2.

Table 1 Comparison of commonly used animal models of SARS-CoV-2

Animal model	Pathology	Application	Advantages	Disadvantages
Transgenic mouse (Bao et al., 2020a; Bao et al., 2020b; Jiang et al., 2020; Sun et al., 2020b)	Moderate interstitial pneumonia; infiltration of inflammatory cells; weight loss; immune factors can be detected	Drug screening and vaccine evaluation.	Expressing hACE2; susceptible to SARS-CoV-2.	High cost; long breeding time; development of few cases with severe symptoms or death.
Non-transgenic mouse (Israelow et al., 2020; Rathnasinghe et al., 2020; Sun et al., 2020a)	Inflammatory response develops in lungs; weight loss; immune factors can be detected.	Drug screening and vaccine evaluation	Simple to construct; easy to repeat; suitable for widespread popularization	High cost; few subjects develop severe symptoms or death
Hamster (Chan et al., 2020; Imai et al., 2020; Sia et al., 2020)	Weight loss; severe lung injury, including severe, bilateral, peripheral, multi-lobular ground glass opacity and lung consolidation.	Study of infection and transmission routes.	Low cost; susceptible to SARS-CoV-2; symptoms and manifestations are very similar to that in human infection	Few subjects develop severe symptoms or death
Ferret (Kim et al., 2020; Pulit-Penaloza et al., 2022; Ryan et al., 2021)	Acute bronchiolitis in lungs; obvious virus replication; rise in temperature.	Drug screening, vaccine evaluation, and study of immune responses	Anatomical structure of upper and lower respiratory tracts of ferrets is very similar to that of humans; susceptible to SARS-CoV-2	Showing mild clinical symptoms; virus titer in lungs is relatively low
NHPs (Rockx et al., 2020; Shan et al., 2020; Song et al., 2020; Wang et al., 2020b; Yu et al., 2020)	Typical interstitial pneumonia; diffuse alveolar damage; immune factors can be detected	Drug screening and vaccine evaluation.	Very close genetic relationship with humans, with similar anatomy, physiology, and pathology	High cost; difficult operation; small number of subjects.

transmission. Richard et al. (2020) provided the first empirical evidence of SARS-CoV-2 transmission via direct contact and respiratory droplets/aerosols among exposed ferrets, as evidenced by prolonged viral shedding and the occurrence of infectious virus in secondary recipient animals. This discovery aligns with the outcomes of other research, such as Kim et al. (2020) and Kutter et al. (2021), who documented airborne transmission of SARS-CoV-2 between ferrets over more than 1 m. However, Sawatzki et al. (2021) reported that ferrets may have a host barrier that limits natural infection and transmission after finding no evidence of infection in 29 ferrets constantly exposed to one confirmed and one suspected case of symptomatic COVID-19. Furthermore, based on genetic sequences of viruses and host, Lehtinen et al. (2022) suggested that ferrets may possess genetic factors that confer resistance to natural SARS-CoV-2 infection. Patel et al. (2021) demonstrated that ferrets are semi-permissive to SARS-CoV-2 USA-WA1/2020 isolate infection, showing efficient transmission via direct contact but poor transmission by respiratory droplets. Zhou et al. (2021) found that D614G substitution in the S protein markedly increases replication and transmissibility in hamster and ferret models of SARS-CoV-2. In addition, Ulrich et al. (2022) reported that the SARS-CoV-2 Alpha variant exhibits a clear replication advantage over wt-S614G and is easily transmitted among ferrets. Peacock et al. (2021) found that, unlike wild-type virus, SARS-CoV-2 lacking the S1/S2 furin cleavage site sheds at lower titers from infected ferrets and is not transmitted to co-housed sentinel animals. Based on the ferret model, they also revealed that the furin cleavage site is an important determinant of SARS-CoV-2 transmission.

Several inhibitors also appear to prevent SARS-CoV-2 transmission in ferret models (Cox et al., 2021b). For example, twice daily treatment of infected ferrets with MK-4482/EIDD-2801, an orally efficacious ribonucleoside analog inhibitor of influenza virus, significantly reduces SARS-CoV-2 load in the upper respiratory tract and suppresses transmission to untreated contact animals (Cox et al., 2021b; Toots et al., 2019). Oral remdesivir parent prodrug GS-441524 shows efficacious effects against SARS-CoV-2 in ferrets by blocking viral replication and preventing transmission to untreated contact animals (Cox et al., 2021a). Daily intranasal administration of lipopeptide fusion inhibitors, which block membrane fusion between the virus and host cell, appears to prevent direct contact transmission of SARS-CoV-2 during 24 h co-housing with infected animals under stringent conditions that result in the infection of 100% of untreated animals (De Vries et al., 2021). Thus, ferrets may serve as sensitive animal models to study the transmission dynamics and molecular basis of SARS-CoV-2 transmissibility, as well as interventions aimed at preventing viral transmission.

SARS-CoV-2 pathogenesis in ferrets

Ferrets are also good animal models for exploring the pathogenesis of COVID-19 (Figure 2). A recent study proposed a novel bioinformatics framework to systematically trace animals susceptible to SARS-CoV-2 and predict the binding affinities between mutated/un-mutated ACE2 receptors of susceptible animals. The researchers discovered that the *ACE2* gene in ferrets is phylogenetically identical to that in humans, and their ACE2 protein forms six hydrogen bonds with the SARS-CoV-2 S protein, creating a strong binding force (Sun et al., 2022b) (Figure 2). Shi et al. (2020) found that SARS-CoV-2 can efficiently infect the upper respiratory tract of ferrets for up to 8 days without causing severe disease or death, with all ferrets developing SARS-CoV-2-specific antibodies. The features of SARS-CoV-2 replication and pathogenesis in ferrets makes them an ideal candidate animal model for evaluating the efficacy of antiviral drugs or vaccines against COVID-19 (Shi et al., 2020) (Figure 2). Similarly, Kim et al. (2020) found that ferrets are susceptible to SARS-CoV-2 infection and exhibit elevated body temperatures and acute bronchiolitis (Figure 2), with viral shedding in nasal, saliva, urine, and fecal samples up to 8 days post-infection (dpi). Furthermore, ferrets can effectively transmit the virus to naïve ferrets by direct or indirect contact, as evidenced by the detection of SARS-CoV-2 in all naïve direct contact ferrets 2 days after exposure and in several naïve indirect contact ferrets, suggesting that the virus can be transmitted via the air (Kim et al., 2020). Analysis of age-related disease severity, as observed in COVID-19 patients, also indicates that all ferrets can be infected by SARS-CoV-2 regardless of age, but aged ferrets (≥3 years old) exhibit higher viral loads, longer nasal virus shedding, and more

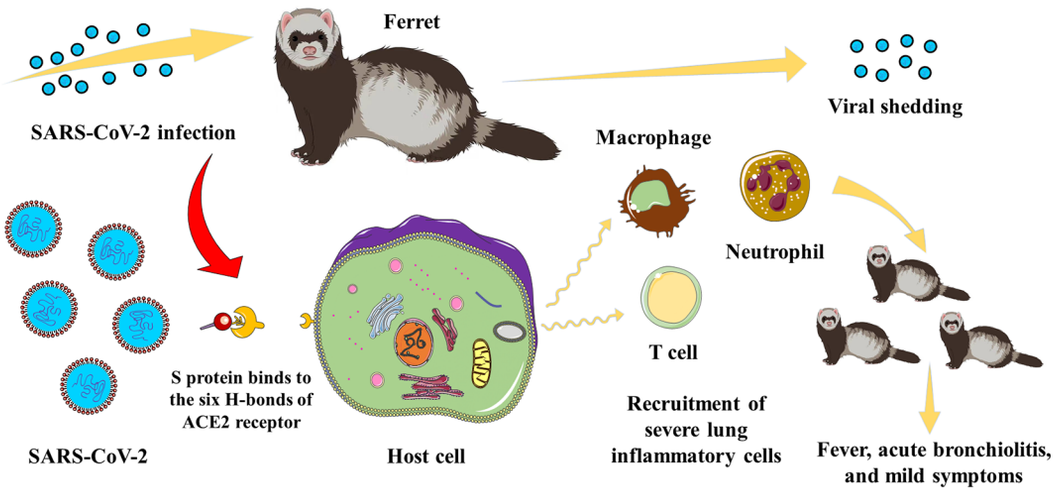

Figure 2 Schematic of pathogenesis in SARS-CoV-2-infected ferret

After SARS-CoV-2 infection in the ferret, the S protein binds to the six H-bonds of the host cell ACE2 receptor. Infected cells are triggered to recruit severe lung inflammatory cells, causing fever, acute bronchiolitis, and other mild symptoms. Aged infected ferrets also show longer nasal viral shedding.

severe lung inflammatory cell infiltration (Figure 2) and clinical symptoms compared to juveniles (≤6 months) and young adults (1–2 years) (Richard et al., 2020). Therefore, age is a critical factor in COVID-19 severity. Although no neurological symptoms or fatalities have been observed in SARS-CoV-2-infected ferrets, various clinical signs and modes of transmission found in COVID-19 patients are also observed in ferrets, indicating that ferrets represent an ideal animal model that should facilitate SARS-CoV-2 research.

Many studies have also suggested that ferrets are suitable animal models for asymptomatic or mild SARS-CoV-2 infection (Au et al., 2022; Everett et al., 2021; Van De Ven et al., 2021). Notably, intranasal SARS-CoV-2 challenge in ferrets does not manifest in overt clinical symptoms but does generate productive infection within the nasal turbinate mucosae, as evidenced by the detection of viral RNA in nasal wash and throat swab samples (Everett et al., 2021). Antibody responses to the S protein and nucleoprotein can be observed from 21 dpi, despite low virus neutralizing activity. Although low levels of viral RNA can also be detected in the fur of some ferrets, infectious virus cannot be re-isolated, highlighting the potential importance of indirect means of transmission, as observed in clinical settings (Everett et al., 2021). Ferrets can also serve as valuable animal models for studying asymptomatic SARS-CoV-2 infection. Au et al. (2022) conducted a natural history/time course study of SARS-CoV-2 infection in ferrets to characterize and assess their suitability as an animal model. In brief, 10 ferrets of each sex were challenged intranasally with 4.64×10^4 $TCID_{50}$ of SARS-CoV-2 isolate Australia/VIC01/2020 and monitored for clinical signs of disease and viral shedding, with tissues collected post-mortem for histopathological and virological assessment at set intervals. SARS-CoV-2 was found to replicate in the upper respiratory tract with consistent viral shedding in nasal wash samples and oral swab samples until 9 dpi. Infectious SARS-CoV-2 was recovered from nasal wash, oral swab, nasal turbinate, pharynx, and olfactory bulb samples within 3–7 dpi. However, only viral RNA was detected in samples collected from the trachea, lung, and parts of the gastrointestinal tract. Viral antigen was observed exclusively in nasal epithelium and associated sloughed cells and in draining lymph nodes upon immunohistochemical staining (Au et al., 2022). Van De Ven et al. (2021) also revealed that intranasally infected ferrets exhibit asymptomatic COVID-19 and possibly aspects of long-COVID. Therefore, ferrets can be experimentally infected with SARS-CoV-2 to model human asymptomatic infection. All infected ferrets develop antibodies against SARS-CoV-2 (Shi et al., 2020) and re-challenge of recovered ferrets shows reduced upper respiratory tract viral shedding and transmission as well as lung pathology (Kim et al., 2021c). Transcriptome analysis of aged ferret lungs revealed strong enrichment in gene sets related to type I interferon, activated T cells, and M1 macrophage responses (Figure 2), suggesting that the severity of COVID-19 in aged population is linked with severe inflammatory response (Kim et al., 2022b). Francis et al. (2021) showed that older males may play a role in viral transmission due to decreased antiviral responses. Martins et al. (2022) also demonstrated that age can affect susceptibility of ferrets to SARS-CoV-2 and confirmed that aged ferrets are more likely to get infected when exposed to lower infectious doses than young ferrets, with enhanced viral replication in the upper respiratory tract as well as viral shedding. These findings suggest that ferrets can be used as a model for studying age-related SARS-CoV-2 infection dynamics and viral replication. Ferret models can also be used to evaluate metabolic profiling of minimally invasive biological samples collected from SARS-CoV-2-infected ferrets during viral shedding and post-shedding periods. Based on multivariate analysis, Beale et al. (2021) identified 29 significant metabolites and three lipids subjected to pathway enrichment and impact analysis. The presence of viral shedding coincided with the challenge dose administered and significant changes in the citric acid cycle, purine metabolism, and pentose phosphate pathways. An elevated immune response in the host was also observed between the two isolates studied. These results support other metabolomic-based findings in clinical observational studies and indicate the utility of metabolomics in ferrets for further COVID-19 research (Beale et al., 2021). Co-infection of ferrets with H1N1 and SARS-CoV-2 extends the clinical duration of COVID-19 and enhances pulmonary damage, but does not reduce viral shedding in throat swabs or viral loads in lungs (Bao et al., 2021). A dose titration study of SARS-CoV-2 in ferrets showed that high (5×10^6 plaque-forming units (pfu)) and medium dose

(5×10^4 pfu) challenge can induce upper respiratory tract RNA shedding associated with lower respiratory tract viral RNA and lung pathology, while re-challenge of recovered ferrets shows reduced upper respiratory tract viral shedding and lung pathology (Ryan et al., 2021). Therefore, the use of such models may aid our understanding of SARS-CoV-2 pathogenesis and naturally acquired immunity.

Ferrets and countermeasure evaluation

The ferret immune system shares many similarities to that of humans. As such, ferrets have been widely used to test vaccines, therapeutics, and antivirals against SARS-CoV-2 (Table 2) (Pandey et al., 2021). A recently developed receptor-binding domain (RBD) protein-based vaccine candidate against SARS-CoV-2 using self-assembling bullfrog ferritin nanoparticles as an antigen delivery system was shown to induce potent neutralizing antibodies against SARS-CoV-2 in ferrets and provide efficient protection from virus challenge (Kim et al., 2021b). Additionally, single vaccination with Ad5-nCoV was found to protect ferrets from wild-type SARS-CoV-2 infection in the upper respiratory tract (Wu et al., 2020). Vaccination with ChAdOx1 nCoV-19, a replication-deficient simian adenoviral vector expressing codon-optimized full-length SARS-CoV-2 S protein, was also found to reduce both viral shedding and lung pathology in ferrets, with a second vaccination increasing antibody titers (Lambe et al., 2021; Marsh et al., 2021). Ferret models have also been used to evaluate the efficacy of protein-based subunit vaccines. Toll-like receptor (TLR)1/2 and TLR3 agonists (L-pampo) can be potent adjuvants for SARS-CoV-2 subunit vaccines in ferrets; notably, SARS-CoV-2 antigens with L-pampo can elicit a neutralizing antibody response and antigen-specific cellular immune response against SARS-CoV-2, resulting in substantially decreased viral load in ferret nasal washes (Jeong et al., 2021; Kim et al., 2021a). Recombinant SARS-CoV-2 S protein formulated with an Advax-SM adjuvant also protects ferrets against COVID-19 infection (Li et al., 2021). COVID-eVax, a DNA plasmid encoding a secreted monomeric form of the SARS-CoV-2 S protein RBD, also confers significant protection to ferrets upon SARS-CoV-2 challenge and can induce a potent T-cell immune response and relatively low neutralizing antibody titers (Compagnone et al., 2022).

The antiviral effects of certain FDA-approved drugs against SARS-CoV-2, including lopinavir-ritonavir, hydroxychloroquine sulfate, emtricitabine-tenofovir, and azathioprine, have also been assessed in ferrets (Park et al., 2020). Of note, all tested antiviral drugs have been shown to marginally reduce the overall clinical score in infected ferrets but have no significant effects on *in vivo* virus titers. The antiviral effects of human placenta hydrolysate (hPH) against SARS-CoV-2 have also been evaluated in ferrets, resulting in minimal body weight loss, attenuated viral replication in nasal washes, turbinates, and lungs, and markedly up-regulated gene expression of type I (IFN-α and IFN-β) and II (IFN-γ) interferons (IFNs) in SARS-CoV-2-infected ferrets (Kim et al., 2021a). Selinexor, an FDA-approved XPO1 inhibitor, was shown to reduce viral load in the lungs and protect against tissue damage in the nasal turbinates and lungs of ferrets (Kashyap et al., 2021). Interestingly, SARS-CoV-2 challenge in ferrets has also been used to assess the effects of probiotics against the virus. In ferrets challenged with SARS-CoV-2, the probiotic consortia OL-1 and OL-2 were found to significantly reduce viral load, modulate immune response, and regulate viral receptor expression compared to placebo (Lehtinen et al., 2022), indicating that the potential benefits of probiotics against SARS-CoV-2 infection should be further explored in human clinical trials.

Table 2 Vaccine and antiviral drug candidates evaluated using ferret models for SARS-CoV-2 prevention and treatment

Category	Name	Features	Effect evaluation	Reference
Vaccines	Self-assembling RBD-nanoparticles	Protein-based vaccine	Antibodies	Kim et al., 2021b
	Ad5-nCoV	Adenoviral-vectored vaccine	Antibodies; T-cell responses	Wu et al., 2020
	ChAdOx1 nCoV-19	Adenoviral-vectored vaccine	Antibodies; T-cell responses	Lambe et al., 2021;Marsh et al., 2021
	SARS-CoV-2 antigens with L-pampo	Protein-based vaccine	Antibodies; T-cell responses	Jeong et al., 2021
	Covax-19™	Protein-based vaccine	Antibodies	Li et al., 2021
	COVID-eVax	DNA vaccine	Antibodies; T-cell responses	Conforti et al., 2022;Compagnone et al., 2022
Antiviral drugs	Lopinavir/Ritonavir	Anti-HIV drugs, protease inhibitor	Clinical symptom (CS) values	Park et al., 2020
	Hydroxychloroquine sulfate	Autophagy inhibitor	Clinical symptom (CS) values	Park et al., 2020
	Emtricitabine/Tenofovir	Nucleotide analog that inhibits RNA-dependent RNA polymerase activity	Clinical symptom (CS) values; virus titers	Park et al., 2020
	Azathioprine	Immunosuppressive drug		Park et al., 2020
	Human placenta hydrolysate (hPH) (Laennec®)		Virus titers	Kim et al., 2021a
	Selinexor	XPO1 inhibitor	Viral load; inflammatory cytokine	Kashyap et al., 2021
	GS-621763	Oral prodrug of remdesivir parent nucleoside	Viral burden	Cox et al., 2021a
	EIDD-2749	Ribonucleoside analog	Viral burden; Viral titers	Sourimant et al., 2022
	CT-P59	Monoclonal antibody	Viral load	Ryu et al., 2021
	Molnupiravir	Orally bioavailable prodrug of ribonucleoside analog EIDD-1931	Viral shedding	Lieber et al., 2022
	OL-1 and OL-2	Probiotic consortia	Viral titers	Lehtinen et al., 2022

Ferrets and SARS-CoV-2 variants

Many SARS-CoV-2 variants, including VOIs and VOCs, have evolved since the initial outbreak of COVID-19, posing an increased risk to global public health (https://www.who.int/activities/tracking-SARS-CoV-2-variants). VOIs and VOCs can affect viral traits, including transmissibility, clinical manifestations, and immune escape, with various VOCs also associated with an increase in global epidemiology and disease severity and a decrease in efficacy of current vaccines and therapeutics. Therefore, it is important to understand the transmission and pathogenesis of these SARS-CoV-2 variants using various animal models, including ferrets.

Using a ferret-based model, Kim et al. (2022a) demonstrated that natural temperature differences between the upper (33 °C) and lower (37 °C) respiratory tract can have profound effects on SARS-CoV-2 replication and transmission. They found that SARS-CoV-2 variants containing the P323L or P323L/G671S mutation in NSP12 RNA-dependent RNA polymerase (RdRp) exhibited enhanced RdRp enzymatic activity at 33 °C compared to 37 °C and high transmissibility in ferrets. They further suggested that the evolutionarily forced NSP12 P323L and P323L/G671S mutations of recent SARS-CoV-2 VOC strains may be associated with increased RdRp complex stability and enzymatic activity, thus promoting the high transmissibility (Kim et al., 2022a). Ryan et al. (2021) found that all ferrets (6/6) challenged intranasally with high (5×10^6 pfu) and medium (5×10^4 pfu) doses of the Victoria/1/202026 SARS-CoV-2 variant exhibited viral RNA shedding in the upper respiratory tract, whereas only one of the six ferrets showed similar signs after low dose (5×10^2 pfu) challenge. Mild multifocal bronchopneumonia in approximately 5%–15% of the lung was also observed on day 3 in the high- and medium-dosed groups. Pulit-Penaloza et al. (2022) performed a comparative analysis of four SARS-CoV-2 strains, including an early pandemic isolate from the United States (WA1) and representatives of the Alpha, Beta, and Delta lineages. Their results showed that the Beta virus failed to replicate in the ferrets, whereas the WA1, Alpha, and Delta viruses replicated efficiently in the upper respiratory tract, causing mild disease with no overt histopathological changes. They also revealed that the WA1 and Delta viruses transmitted in a direct contact setting, whereas the Delta virus was also capable of limited airborne transmission in ferrets.

Ferret models have also been used to test anti-SARS-CoV-2 VOC efficacy of certain drugs. Cox et al. (2021a) assessed the anti-SARS-CoV-2 VOC efficacy of GS-621763, an oral prodrug of the remdesivir parent nucleoside GS-441524, and found that VOC γ did not invade the ferret host more aggressively than WA1/2020 and oral GS-621763 was highly efficacious at reducing viral burden and tissue titers to undetectable levels and at lowering viral RNA copies in nasal lavages and turbinates. The oral antiviral 4'-fluorouridine (EIDD-274) also shows efficacy against SARS-CoV-2 VOCs in ferrets and CT-P59 monoclonal antibodies can reduce viral load of the South African (SA) variant B.1.351 *in vivo* (Ryu et al., 2021; Sourimant et al., 2022). Recently, Lieber et al. (2022) showed that molnupiravir can consistently reduce upper respiratory VOC shedding and prevent viral transmission in ferrets, whereas Omicron-infected dwarf hamsters show significant individual variation in response to treatment, suggesting that approved antivirals should be continuously re-assessed *in vivo* as new VOCs emerge. Furthermore, differences in animal models need to be considered in the development of antivirals and vaccines and different models should be applied to ensure full evaluation.

CONCLUSIONS

The ferret model provides a suitable platform to facilitate the development of SARS-CoV-2 therapeutics and vaccines, and a useful animal model for studying the transmission of SARS-CoV-2. However, although such models can successfully simulate human infection and transmission of SARS-CoV-2, SARS-CoV-2 infected ferrets only show mild clinical symptoms without weight loss or mortality and with relatively low viral titers in the lungs, which may limit their application in the study of severe and critical COVID-19 patients. Consequently, ferrets may be more suitable for studying the pathogenesis of mild SARS-CoV-2 infections. Moreover, as variants continue to evolve worldwide, studies on the transmission, pathogenesis, and countermeasures against emerging SARS-CoV-2 variants using ferret models are warranted.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.C. conceptualized and supervised the project. Y.Z., C.L.W., and Z.Y.G. wrote and edited the manuscript. H.X.Q., W.J.W., and X.Y.L. searched the references. All authors read and approved the final version of the manuscript.

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