Comparative Medicine
Copyright 2023
by the American Association for Laboratory Animal Science

Vol 73, No 3
June 2023
Pages 187–193

Overviews (review articles)

Ferrets as Models for Viral Respiratory Disease

Rachel E Smith, Shambhunath Choudhary, and Julita A Ramirez*

Domestic ferrets (*Mustela putorius furo*) have been used in biomedical research to study influenza viruses since the early 20th century. Ferrets have continued to gain importance for the study of viral respiratory disease due to their disease susceptibility and anatomic similarities to humans. Here we review features of ferret biology and management that should be considered when planning to work with this species, particularly in models of respiratory disease. We specifically discuss biosafety and husbandry, clinical and pathologic assessments, and anesthetic considerations for ferrets with respiratory disease and systemic illness. These considerations are important for animal welfare, fidelity of the model to human disease, and ensuring accuracy and reproducibility of acquired data. Finally, we briefly review the use of ferrets to study respiratory diseases by discussing their respiratory anatomy and 2 frequently studied viral respiratory diseases, influenza and coronavirus disease 2019 (COVID-19).

Abbreviations: ABSL-2/-3, animal biosafety level 2/3; ACE2, angiotensin converting enzyme 2; BSL-3 Ag, biosafety level 3 agriculture; PPE, personal protective equipment; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2; TMPRRS2, transmembrane serine protease 2

DOI: 10.30802/AALAS-CM-22-000064

Introduction

The domestic ferret (*Mustela putorius furo*) was historically kept by humans for hunting and control of rodent and rabbit populations, for fur, and more recently for research and companionship. The curious and active nature, long body, and musky scent of these obligate carnivores make them unique among research species. Indeed, the Latin name for the species loosely translates as "mouse-eating smelly thief."[41] Ferrets became useful models in biomedical research due to their anatomic, physiologic, and metabolic similarities to humans, and also because they are susceptible to many human viral respiratory diseases.[14,28] Ferrets have been used to study several viral respiratory diseases, including both human and avian influenza, coronaviruses, respiratory syncytial virus (RSV), parainfluenza, morbilliviruses such as canine distemper, and emerging viruses such as Nipah (Table 1). Ferrets were first inoculated with human influenza and observed to transmit the disease to other ferrets in 1933,[46] and have been used as respiratory disease models ever since. They are frequently used to study viral transmission and virus-host interactions due to their susceptibility to infection and their size, which allows easier serial sampling (for example, serum, nasal/tracheal washes, etc.) as compared with rodents. Furthermore, ferrets are generally easier to house than dogs or nonhuman primates. Their intermediate size facilitates larger group sizes and thus better statistical assessment of therapeutic effects (Table 1).

Here we will review some of the unique and important aspects of using ferrets to study respiratory disease, including biosafety, handling and husbandry, monitoring and scoring clinical signs of disease, pathology, and anesthetic considerations for animals with respiratory disease. This review is intended to provide information for researchers and facilities new to working with ferrets. The practices we discuss should be tailored to the conditions and goals of specific research projects. We also briefly review the use of ferrets as disease models for influenza and SARS-CoV-2. The frequent use of ferrets for the study of these important viruses highlights their value for research on viral disease pathogenesis and transmission, vaccine development and efficacy, and therapeutic intervention in disease.

Submitted: 07 Jun 2022. Revision requested: 26 Aug 2022. Accepted: 03 Jan 2023.
Pfizer Worldwide Research, Development & Medical, Pearl River, New York 10965, USA
**Corresponding author. Email: julita.ramirez@pfizer.com*

Biosafety and Husbandry Considerations

Ferrets are available from commercial vendors at various levels of conventional or specific pathogen-free (SPF) health status. Infectious disease research often requires ferrets that are seronegative for the organism being studied. However, SPF colonies can be difficult to maintain as disease transmission from humans occurs easily and spreads quickly between ferrets, especially in the case of influenza. Therefore, prevention of disease transmission from humans is of paramount importance when handling and housing ferrets. Although only a few cases document natural transmission of SARS-CoV-2 from human ferret owners to their pets,[15,45] natural transmission of this virus has occurred between humans and the closely related mink in a farm setting.[40] In this instance, evidence indicated humans transmitted the infection to mink followed by transmission among mink and back to humans.

Handling and husbandry of ferrets can be difficult, especially when studying agents at animal biosafety level 2 (ABSL-2) and above. Facilities and enrichment programs designed for small rodents are not adequate. Contemporary circulating human influenza strains (for example H1/H3/B) require ABSL-2 containment, while SARS-CoV-2, noncontemporary wild-type (H2N2), and highly pathogenic influenza viruses such as the 1918 influenza strain require ABSL-3.[12] Facilities built for

Table 1. Viral respiratory diseases studied in ferrets

Pathogen	Primary virus receptors	Selected References
Influenza virus	Sialic acid	4, 46
Respiratory Syncytial Virus (RSV)	Cx3C chemokins receptor 1 (CX3CR1), annexin II, HSPG/GAG	42, 49
Parainfluenza virus	Sialic acid	33
Human Metapneumovirus (hMPV)	Poorly characterized; α5β1 and αv integrins	29
SARS-CoV	ACE2	31
SARS-CoV-2	ACE2	22
Nipah virus	EphrinB2	5

maintaining negative airflow from adjacent rooms into ferret housing room(s) are essential, and the availability of a connected or nearby procedure room will facilitate study work. For ABSL-3 work, 2 approaches for primary containment can be used: sealed containment caging with HEPA-filtered exhaust, or standard caging with modified practices and room-level containment (for example, Animal Biosafety Level 3-Agriculture [ABSL-3Ag] practices).[12] ABSL-3Ag ferret studies often use open air cages in an ABSL-3 facility, with the addition of a bioBUBBLE or similar softwall containment enclosure in the room to satisfy the "box within a box" criteria for ABSL-3Ag.[12] Ventilated negative-pressure sealed primary caging with HEPA-filtered exhaust suitable for rabbits or small nonhuman primates or specifically sized for ferrets can also be used. Caging designed for this level of containment is commercially available.[27,32]. The choice of caging depends on many factors, including the biosafety level, disease agent(s) and infectious strain(s) being studied, the concentration or volume of the inoculum, research design, and handling necessary for ferrets. In addition, administration of the disease agent and other study procedures may not be compatible with isolator caging. Biologic risk should always be assessed in order to evaluate procedures to be used during inoculation or handling of infected animals.[12] These procedures should be thoroughly evaluated in trial runs with naïve ferrets before use of infectious agents.

The personal protective equipment (PPE) used by staff must be considered in order to protect both the ferrets and the personnel from human respiratory pathogens. Ferrets are active, curious, and have sharp teeth and claws that can easily puncture PPE, so measures such as nail trims and socialization must be taken to prevent bite and scratch injuries. Thick leather handling gloves can be worn over PPE to prevent bite or scratch wounds. Socialization of ferrets optimally is done before starting a study by familiarizing them with handlers, the caging environment, PPE worn by the handlers, and handling procedures.[1] Ideally, for studies needing higher biocontainment, newly acquired ferrets should undergo a period of acclimation and socialization outside the high containment facility under similar housing conditions with the same staff. This acclimation period will help familiarize the ferrets with the environment and handling procedures before the study starts.

Litter box training using cat litter in pans can provide a cleaner environment and an easier way for technicians to clean cages, reducing human exposure time and animal stress. Litter box training is easier in younger animals, and husbandry methods used at the vendor should be considered during behavior modification. For example, if the vendor provided litter as enrichment, then the ferrets can be acclimated to using the litter box for elimination by positioning the box in the corner of the cage and securing it to the bars or walls to prevent it from being overturned during play.

Ferrets benefit from environmental enrichment and from social contact with other ferrets and humans, so play opportunities should be provided as often as possible when animals are not actively infected.[1,7,25] All enrichment items used should be safe, durable, and easy to clean and disinfect. For ABSL-3 housing, all items must be either disposable or autoclavable if intended for reuse. Our institution successfully modified rabbit cages for ferrets housed at the ABSL-2 level. Modifications included adding hammocks, hollow balls, chew toys, litter pans, and tunnels connecting horizontal cages to promote socialization. During cage cleaning, ferrets are placed together in a playpen with balls and tunnels for socialization and play; this also increases the ease and efficiency of cage cleaning. However, the necessary containment must always be considered when selecting the ferret housing method, including whether the cage or the room is intended to be the primary method of containment.[12]

Clinical and Pathologic Assessments

Common clinical signs observed in ferrets with respiratory disease include fever, weight loss, lethargy, sneezing, nasal and ocular discharge, difficulty breathing, and abnormal lung sounds such as crackles and wheezes.[3] Clinical parameters that are evaluated often include survival/time to reach endpoint, body temperature, activity, body weight, clinical chemistry, and hematology parameters.[38,43,47] In addition, ferrets with significant systemic disease may experience both fever and hypothermia at different points in the disease course. Each ferret should be weighed and examined clinically at least twice daily during the active phase of disease. Although weight loss is easy to quantify, use of a scoring rubric that includes specific clinical signs enables quantification and ensures uniform evaluation between observers. In addition, observed clinical/behavioral characteristics often constitute a more sensitive sign of morbidity than weight loss.[38] For example, a clinical scoring system based on the sum of 3 parameters (activity level, upper and lower respiratory signs), with each parameter graded on a 0 to 3 scale, has been used to compare disease severity between 2 H1N1 influenza isolates.[36]

We use a weighted scale for scoring clinical signs of ferrets infected intranasally with influenza virus (Table 2). Our system scores the clinical signs of each ferret and sums them to provide an overall score for that animal. Scores are based on the severity of changes after infection, including body temperature, overall activity/behavior/mentation, systemic hydration, and the presence or absence of 8 other clinical signs (Table 2). Higher scores reflect a more severe condition. For example, a ferret with a low-grade fever (less than a 5 °F [2.8 °C] rise in body

Table 2. Sample clinical scoring system for ferrets infected with influenza (N/A, not applicable)

Clinical score	0	1	2	3	4	7
Body temperature change from individual's average baseline temperature	< 5°F change	N/A	N/A	≥ 5°F change	N/A	≥ 7°F change
General appearance • Activity level • Response to stimuli, alertness • Posture (normal posture is arched back; abnormal posture is flattened back)	Normal	N/A	Mild decrease or change	Moderate decrease or change	Severe decrease, unresponsive	N/A
Hydration • Skin turgor • Mucous membranes • Eye position	**Normal** No skin tenting, moist mucus membranes, normal eye positioning	**Mild** Minimal loss of skin turgor, semidry mucus membranes, normal eye positioning	**Moderate** Moderate loss of skin turgor, dry mucus membranes, sunken eyes	**Severe** Major loss of skin turgor, extremely dry mucus membranes, severely sunken eyes	N/A	N/A
Rales (clicking, rattling, or bubbling sound in chest/lungs)	No (not present)	Yes (present)	N/A	N/A	N/A	N/A
Sneezing	No	Yes	N/A	N/A	N/A	N/A
Coughing	No	Yes	N/A	N/A	N/A	N/A
Nasal rattling	No	Yes	N/A	N/A	N/A	N/A
Nasal and/or ocular discharge	No	Yes	N/A	N/A	N/A	N/A
Mouth breathing	No	Yes	N/A	N/A	N/A	N/A
Diarrhea	No	Yes	N/A	N/A	N/A	N/A
Vomiting	No	Yes	N/A	N/A	N/A	N/A

temperature) that is also exhibiting signs of dehydration such as a mild skin tent and mildly decreased activity would receive scores of 0, 1, and 2 for those signs, respectively, and assuming no other clinical signs were displayed, would have an overall score of 3 for that particular observation timepoint. Ferrets that are assessed by husbandry technicians, veterinary technicians, or research staff and receive clinical scores above 5 are assessed by veterinary staff and monitored more closely. These ferrets may receive supportive care or are euthanized if they have a cumulative score of 15 or higher. Nonresponsive ferrets (activity level/response to stimulus = 4) are euthanized.

Although our scoring system does not include body weights, they are collected at least once a day and are used as an additional measure of disease severity. Monitoring body weights daily is important when inoculating ferrets with virus because weight loss through dehydration and/or reduced food consumption is a reliable and quantifiable indicator of severe discomfort or distress.[53] Institutional animal care and use guidelines often require euthanasia of animals that experience weight loss of 20% to 30% or more of the pre-inoculation weight or rapid weight loss (> 20% over a week).[3] Scoring rubrics are frequently used to objectively assess the degree of morbidity and can be used to define experimental endpoints as well as to quantify disease burden.

We provide our scoring system as an example. The scoring system used for specific studies should be customized to the individual study with consideration given to the disease agent, dose and route of infection, animal age, sex, weight, and any comorbidities present. A small pilot study can be performed before conducting actual studies, especially if the infectious agent is new to the institution or if the disease model has been significantly changed.

Pathologic assessments are critical in accurately characterizing, validating, and translating information learned in animal models of respiratory disease. Assessments include collecting and analyzing multiple pathology endpoints, including both anatomic (gross pathology and histopathology) and clinical (clinical chemistry and hematologic) parameters during studies. Gross and histopathologic evaluations of animals that either died spontaneously or were euthanized allow investigators to identify lesions and affected tissue/cell types to better understand viral pathogenesis and develop effective therapies. These anatomic evaluations require knowledge of common background lesions and anticipated lesions that are expected based on clinical findings or are identified in published data. Pathologists use various semiquantitative and quantitative methods to characterize lung lesions and compare treatment groups.[37] Samples for clinical pathology (for example blood, urine, saliva) can be collected multiple times from live animals. Evaluation of these samples allows investigators to monitor disease progression and correlate the ferret data with the clinical pathology of human patients. Considerations for sample collection such as validation of fixation processes for rendering tissue samples noninfectious and preparation for pathology evaluations as well as various scoring systems are reviewed in detail elsewhere.[24,34,37]

Considerations for Anesthesia of Ferrets used to Study Respiratory Disease

Ferrets with respiratory disease and those being inoculated intranasally present a unique challenge for anesthesia. Inhalant and injectable anesthetic agents as well as opiates all cause respiratory depression and should be used sparingly and with caution. Respiratory rate should be carefully monitored from induction to full recovery, and intubation should be carried out when feasible to provide airway control.[21] In addition, at ABSL-3 and higher biosafety levels, work with infected animals must be

conducted in appropriate biosafety cabinets unless ABSL-3-Ag conditions are being employed.[12] If possible, anesthesia equipment should remain outside the biosafety cabinet in order to maximize workspace and minimize disruptions in airflow. All exhaust from anesthetized animals and induction chambers must undergo HEPA filtration and waste gas scavenging. We attach disposable HEPA filters designed for this purpose to waste gas scavenge canisters so that exhaust is filtered immediately before it passes into the canister.

Ferrets are prone to hypoglycemia and thermoregulatory derangements in association with anesthesia.[21] The short gastrointestinal tract of ferrets leads to short transit times, so fasting prior to procedures can be as little as 2 h and should not exceed 4 h.[8] In addition, the high surface area to body mass ratio of ferrets contributes to radiant heat loss. Core body temperature should be monitored every 10 to 15 min until the ferret is fully recovered from anesthesia, as exhibited by the ability to maintain sternal recumbency and move about the cage on its own. We have seen hypothermia develop several hours after the ferret has fully recovered from anesthesia. When first working with any anesthetic protocol, animals should be anesthetized in the morning to facilitate monitoring during and after recovery. Supportive care should be provided as necessary (for example, thermal support, administration of warm fluids). Examples of effective thermal support include circulating warm water blankets (preferably puncture-resistant), thermostat-controlled heated cages, and forced air blankets. If hypothermia occurs after recovery, blood glucose and blood pressure should be checked.

Preoxygenation is recommended for ferrets with pulmonary disease. This can be accomplished by providing a high concentration of inspired oxygen (typically 3-5 minutes on 100% oxygen) immediately before anesthetic administration by using a face mask (if tolerated), an induction chamber, or flow-by oxygen.[21]

Premedication can facilitate handling and reduce the amount of anesthetic needed for induction and maintenance of anesthesia. However, premedication should be used cautiously in ferrets with respiratory disease. The specific drug combinations used to provide anesthesia for inoculation and other procedures vary widely between laboratories and institutions, depending on the age, sex, weight, vendor source, and health status of the animals, the route and dose of inoculation, viral strains used, experimental design/procedures, and many other factors. For example, various studies of influenza in ferrets have used isoflurane alone,[16,52] ketamine/xylazine/atropine,[17] and tiletamine/zolazepam/xylazine.[26] We use isoflurane alone to minimize respiratory depression and facilitate faster recovery. In general, benzodiazepines such as midazolam may be preferable to α-2 adrenergic agonists as the former generally cause milder respiratory and cardiovascular depression.[21] Reversal agents for any sedatives used, such as flumazenil for benzodiazepines or atipamezole for dexmedetomidine, should be easily and quickly available if needed to treat adverse effects/events in patients with cardiopulmonary compromise.[18]

Anatomy and Histology of the Ferret Respiratory Tract

Infectious disease studies in animals require identifying a species that is susceptible to infection with human pathogens under experimental conditions.[2,9] Anatomic/histologic structures and characteristics of the ferret respiratory tract are very similar to those of humans.[2,9] Like humans and most other mammals, the ferret respiratory tract is divided into upper and lower airways. The long trachea of ferrets allows easy targeting of upper or lower respiratory tracts, facilitating study of differential effects of respiratory viruses on these regions.[30] From the nasal cavity to the bronchi, the respiratory tract is lined with pseudostratified columnar ciliated epithelium. The bronchioles are lined with simple columnar to cuboidal epithelium, and the alveoli contain a lining of thin squamous epithelium that allows gas exchange. Detailed anatomy of ferret respiratory tract components is reviewed elsewhere.[20] Ferrets, like humans, have abundant submucosal glands throughout the trachea and bronchi, with the goblet cell being the major secretory cell type. Ferrets also have well-developed respiratory bronchioles.[48] Like humans, the ferret lung can adapt to changes in atmospheric pressures, and both humans and ferrets develop pulmonary vasoconstriction in response to hypoxia.[20] Ozone produces similar epithelial injury in ferrets as in nonhuman primates.[48]

Viral infections involve multiple host–virus interactions that determine viable hosts, tissue tropism, and viral pathogenesis. Host susceptibility depends on the presence of viral receptors on the surface of cells to allow viral entry, on pro- and antiviral factors inside the cell that influence viral translation and replication, and gross and histologic anatomical structures.[2,9,28,50]

All viruses must bind to specific surface receptors on host cells to enter the cell and replicate. Therefore, the presence of specific receptors and efficiency of viral-receptor binding are critical determinants of host susceptibility and disease manifestations. Ferrets are naturally susceptible to many of the same respiratory viral infections as humans because the receptors for these viruses in ferret respiratory tracts are sufficiently similar in structure and distribution to those in humans. For example, sialic acids of cell surface glycoproteins and glycolipids serve as receptors for many viruses including influenza, parainfluenza, mumps, and coronaviruses.[14] A high density of α 2,6-linked sialic acids (the receptor for seasonal low pathogenic H1N1 and H3N2 influenza strains) in the upper respiratory tract of both ferrets and humans results in a mild-to-moderate upper respiratory disease. In contrast, a higher density of α 2,3-linked sialic acids (the specific receptor for highly pathogenic influenza strains) in the lower respiratory tract of both species contributes to widespread infection of the lungs that results in severe pneumonia (inflammation of lungs).[14] Similarly, the structure and distribution of viral receptor angiotensin converting enzyme 2 (ACE2) and cofactor transmembrane protease, serine 2 (TMPRSS2) in the ferret respiratory tract is sufficiently similar to that of humans to make ferrets susceptible to SARS and SARS-CoV-2.[32]

Apart from host factors that allow viral entry, other factors facilitate infection by supporting viral transport, replication, and translation. Antiviral host restriction factors inhibit viral entry or replication inside the cell.[28] Current knowledge regarding these host viral factors is lacking or limited in ferrets. Understanding how variation among these other host determinants influences host-virus interactions will enhance our ability to study different viral respiratory diseases in ferrets.

The similarity of gross anatomic structures and cellular/molecular receptor/pathways in the respiratory tracts of ferrets and humans makes the ferret a prime model for human respiratory disease. These similarities in the respiratory tract underlie their frequent use for the study of human infectious respiratory diseases, including influenza and coronaviruses.[14,20]

Table 3. Characteristics of respiratory disease in ferrets

Pathogen	Virus replication	Clinical signs	Viral transmission	Pathologic characteristics	Viral immune response
Influenza virus	Yes	Fever, lethargy, dehydration, nasal discharge, sneezing, difficulty breathing	Yes; high transmission rates; age-related response similar to that of humans	Focal edema, pulmonary inflammation	Seroconversion; neutralizing antibody titers; T-cell response
SARS-CoV	Yes	Lethargy, conjunctivitis	Yes; transmission by direct contact	Pulmonary inflammation, hepatic lipidosis, wasting	Seroconversion; neutralizing antibody titers; T-cell response
SARS-CoV-2	Yes	Fever, loss of appetite, cough, weight loss, sneezing, nasal discharge, loose stool, wheezing	Yes; high transmission by direct contact and airborne; age-related response similar to that of humans	Pulmonary inflammation	Seroconversion; neutralizing antibody titers; T-cell response

The Use of Ferrets to Study Influenza

Influenza has been of high concern to public health for centuries, whether seasonal, pandemic, or avian in origin. Outbreaks are associated with high disease-related morbidity and significant mortality: in the United States alone, influenza caused an estimated 531,000 to 647,000 hospitalizations and 36,400 to 61,200 deaths during the 2018 to 2019 season, which was only moderately severe.[52] The ability of influenza viruses to infect multiple species, reassort segments of their genome, and quickly sustain mutations contributes to the success of the virus in evading or misdirecting the immune response. Small mammals are indispensable for studying the virulence, pathogenicity, and transmissibility of influenza and the host immune response. These animals provide the foundation of preclinical efficacy studies of vaccines and antiviral drugs. In addition to ferrets, mice, guinea pigs, cotton rats, and hamsters have all been used, although mice have been used most frequently due to their small size, ease of access, and availability of genetically modified strains.[6] Hamsters have been widely used due to their susceptibility to influenza, development of pathogenic effects, and ability to support viral replication. However, hamsters, unlike ferrets, do not transmit type B influenza viruses and do not show clinical signs similar to those of humans.[19] Ferrets have the advantages of similarity to humans in their respiratory anatomy and physiology, virus receptor repertoire, clinical responses, disease transmission, tissue tropism, incubation period, and susceptibility to both human and avian influenza viruses (Table 3).[4] Ferrets also have a well-developed sneeze reflex that allows transmission of influenza to other ferrets.[4] They also develop ocular and nasal discharge and become febrile; mice do not develop these signs. Importantly, ferrets can be infected with circulating strains of influenza without laboratory adaptation, which is usually necessary in mice.[19]

Other advantages of ferrets include a large enough body size to allow serial sampling for serological and virological data, yet small enough to have practical housing/husbandry needs as compared with larger animals and to allow the use of sufficient numbers for statistical assessment of therapeutic efficacy. In addition, ferrets may exhibit comorbidities similar to those of humans. For example, cytomegalovirus (CMV) infects a significant portion of the human population, and latent infection with CMVs can affect the response to influenza vaccines.[35,51]

The Use of Ferrets to Study Severe Acute Respiratory Syndrome (SARS) Coronaviruses

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19), an ongoing global pandemic with hundreds of millions of confirmed cases worldwide.[39] Understanding the pathogenesis of SARS-CoV-2 in an animal model is crucial for development of preventative and therapeutic interventions. ACE2 expression on the cell surface and the compatibility of ACE2 with the receptor binding domain on the viral spike glycoprotein (S) are major determinants in selecting susceptible animals for studying COVID-19. Cell entry of virus also depends on S priming by host cell proteases such as TMPRSS2.[9] In addition to the presence of compatible viral receptor ACE2 and cofactor TMPRSS2,[32] the microanatomy of the ferret respiratory tract is comparable to that of humans, thereby making ferrets a good model for studying COVID-19. Hamsters are also considered good models for studying COVID-19 as they also have an ACE2 suitable for viral S protein binding, and they develop severe clinical disease with viral replication observed in both upper and lower respiratory tracts.[10] While ferrets generally develop mild disease as compared with hamsters and viral replication is usually restricted to upper airways,[11] ferrets have a greater similarity to humans in terms of anatomy. As in humans, ferrets efficiently transmit virus to contact animals.[44] In addition, aged ferrets can develop high viral loads with longer nasal virus shedding, severe pneumonia, and the clinical signs of disease observed in terminally ill COVID-19 patients.[23]

Mice are commonly used to study viral pathogenesis due to their ease of handling, cost-effectiveness, and suitability for use in larger numbers. However, due to low compatibility between the viral S protein and the murine ACE2 receptor, outbred and inbred mouse strains are not considered good models for studying COVID-19.[9] Nonhuman primates (NHPs) are another good model due to the presence of compatible ACE2, but they generally develop mild disease, are expensive to acquire and maintain, and are more difficult to access and house as compared with ferrets. Minks are naturally susceptible to SARS-CoV-2, develop severe disease, and can transmit the virus back to humans.[13,40] However, minks have not been studied widely as they are difficult to acquire and handle in a laboratory setting.

Despite their advantages, ferrets and other widely used COVID-19 animal models do not recapitulate the full spectrum of COVID-19 phenotypes observed in humans including lethality, extrapulmonary manifestations (for example, vascular complications, cardiac disease, neurologic disease), and long COVID (long-term problems related to SARS-CoV-2 infection).[9]

Conclusion

Ferrets are often used as a model for human viral respiratory disease because of their similarities with respect to pathology, clinical signs, and transmission. However, working with ferrets poses unique challenges related to their biology, size, and

behavior. We discuss the ferret model with regard to husbandry, biosafety, clinical and anesthetic perspectives. Systems for scoring clinical disease in animals are often used to provide a more objective assessment of disease severity and endpoint criteria. However, although many methods have been published for scoring commonly used species such as rodents, few scoring systems for ferrets are available in the literature. We have presented the system we use as an example of a rubric for scoring clinical signs of disease, specifically in ferrets with respiratory disease. This example may be useful as a template for researchers new to using ferrets. We also review highlights of the ferret model for 2 commonly studied viral respiratory diseases, influenza and COVID-19, and review characteristics of respiratory disease in ferrets to aid researchers in selecting the best model for their studies.

Acknowledgments

We thank Terese (Beth) Bennett, Anand Giddabasappa, and Brian O'Shea for manuscript review and Deanne Illenberger, Kim Froeschl, Kate Breyer, and Ryan Misonznick for valuable discussions.

References

1. **Ball RS.** 2006. Issues to consider for preparing ferrets as research subjects in the laboratory. ILAR J **47**:348–357. https://doi.org/10.1093/ilar.47.4.348.
2. **Baxter VK, Griffin DE.** 2016. Chapter 10: Animal models: No model is perfect, but many are useful, p 125–138. In: Katze MG, Korth MJ, Law GL, Nathanson N, eds. Viral pathogenesis (third edition). Boston (MA): Academic Press.
3. **Belser JA, Eckert AM, Tumpey TM, Maines TR.** 2016. Complexities in ferret influenza virus pathogenesis and transmission models. Microbiol Mol Biol Rev **80**:733–744. https://doi.org/10.1128/MMBR.00022-16.
4. **Belser JA, Katz JM, Tumpey TM.** 2011. The ferret as a model organism to study influenza A virus infection. Dis Model Mech **4**:575–579. https://doi.org/10.1242/dmm.007823.
5. **Bossart KN, Zhu Z, Middelton D, Klippel J, Crameri G, Bingham J, McEachern JA, Green D, Hancock TJ, Chan YP, Hickey AC, Dimitrov DS, Wang LF, Broder CC.** 2009. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Pathog **5**:e1000642. https://doi.org/10.1371/journal.ppat.1000642.
6. **Bouvier NM, Lowen AC.** 2010. Animal models for influenza virus pathogenesis and transmission. Viruses **2**:1530–1563. https://doi.org/10.3390/v20801530.
7. **Burn CC, Raffle J, Bizley JK.** 2020. Does 'playtime' reduce stimulus-seeking and other boredom-like behaviour in laboratory ferrets? Anim Welf **29**:19–29. https://doi.org/10.7120/09627286.29.1.019.
8. **Cantwell SL.** 2001. Ferret, rabbit, and rodent anesthesia. Vet Clin North Am Exot Anim Pract **4**:169–191. https://doi.org/10.1016/S1094-9194(17)30056-7.
9. **Choudhary S, Kanevsky I, Tomlinson L.** 2022. Animal models for studying covid-19, prevention, and therapy: Pathology and disease phenotypes. Vet Pathol **59**:516–527. https://doi.org/10.1177/03009858221092015.
10. **Choudhary S, Kanevsky I, Yildiz S, Sellers RS, Swanson KA, Franks T, Rathnasinghe R, Munoz-Moreno R, Jangra S, Gonzalez O.** 2022. Modeling SARS-CoV-2: Comparative pathology in rhesus macaque and Golden Syrian hamster models. Toxicol Pathol **50**:280–293. https://doi.org/10.1177/01926233211072767.
11. **Ciurkiewicz M, Armando F, Schreiner T, de Buhr N, Pilchová V, Krupp-Buzimikic V, Gabriel G, von Köckritz-Blickwede M, Baumgärtner W, Schulz C, Gerhauser I.** 2022. Ferrets are valuable models for SARS-CoV-2 research. Vet Pathol **59**:661–672. https://doi.org/10.1177/03009858211071012.
12. **Centers for Disease Control and Prevention.** 2020. Biosafety in microbiological and biomedical laboratories (BMBL), 6th edition. St Louis (MO): US Government Printing Office. Available at: Biosafety in Microbiological and Biomedical Laboratories—6th Edition (cdc.gov).
13. **Eckstrand CD, Baldwin TJ, Rood KA, Clayton MJ, Lott JK, Wolking RM, Bradway DS, Baszler T.** 2021. An outbreak of SARS-CoV-2 with high mortality in mink (*Neovison vison*) on multiple Utah farms. PloS Pathog **17**:e1009952. https://doi.org/10.1371/journal.ppat.1009952.
14. **Enkirch T, von Messling V.** 2015. Ferret models of viral pathogenesis. Virology **479-480**:259–270. https://doi.org/10.1016/j.virol.2015.03.017.
15. **Giner J, Villanueva-Saz S, Tobajas AP, Pérez MD, González A, Verde M, Yzuel A, García-García A, Taleb V, Lira-Navarrete E, Hurtado-Guerrero R, Pardo J, Santiago L, Paño JR, Ruíz H, Lacasta D, Fernández A.** 2021. SARS-CoV-2 seroprevalence in household domestic ferrets (*Mustela putorius furo*). Animals (Basel) **11**:667. https://doi.org/10.3390/ani11030667.
16. **Govorkova EA, Rehg JE, Krauss S, Yen H-L, Guan Y, Peiris M, Nguyen TD, Hanh TH, Puthavathana P, Long HT, Buranathai C, Lim W, Webster RG, Hoffmann E.** 2005. Lethality to ferrets of H5N1 influenza viruses isolated from humans and poultry in 2004. J Virol **79**:2191–2198. https://doi.org/10.1128/JVI.79.4.2191-2198.2005.
17. **Gustin KM, Maines TR, Belser JA, van Hoeven N, Lu X, Dong L, Isakova-Sivak I, Chen L-M, Voeten JTM, Heldens JG.** 2011. Comparative immunogenicity and cross-clade protective efficacy of mammalian cell-grown inactivated and live attenuated H5N1 reassortant vaccines in ferrets. J Infect Dis **204**:1491–1499. https://doi.org/10.1093/infdis/jir596.
18. **Hawkins MG, Pascoe PJ.** Chapter 37: Anesthesia, analgesia, and sedation of small mammals, p 536–558. In: Quesenberry KE, Orcutt CJ, Mans C, Carpenter JW, eds. Ferrets, rabbits, and rodents (4th edition). Philadelphia (PA): W.B. Saunders.
19. **Iwatsuki-Horimoto K, Nakajima N, Ichiko Y, Sakai-Tagawa Y, Noda T, Hasegawa H, Kawaoka Y.** 2018. Syrian hamster as an animal model for the study of human influenza virus infection. J Virol **92**:e01693-17. https://doi.org/10.1128/JVI.01693-17.
20. **Johnson-Delaney CA, Orosz SE.** 2011. Ferret respiratory system: Clinical anatomy, physiology, and disease. Vet Clin North Am Exot Anim Pract **14**:357–367. https://doi.org/10.1016/j.cvex.2011.03.001.
21. **Kapaldo N, Eshar D.** 2022. Ferret sedation and anesthesia. Vet Clin North Am Exot Anim Pract **25**:273–296. https://doi.org/10.1016/j.cvex.2021.08.006.
22. **Kim YI, Kim SG, Kim SM, Kim EH, Park SJ, Yu KM, Chang JH, Kim EJ, Lee S, Casel MAB, Um J, Song MS, Jeong HE, Lai VD, Kim Y, Chin BS, Park JS, Chung KH, Foo SS, Poo H, Mo IP, Lee OJ, Webby RJ, Jung JU, Choi YK.** 2020. Infection and rapid transmission of SARS-CoV-2 in ferrets. Cell Host Microbe **27**:704–709. https://doi.org/10.1016/j.chom.2020.03.023.
23. **Kim YI, Yu KM, Koh JY, Kim EH, Kim SM, Kim EJ, Casel MAB, Rollon R, Jang SG, Song MS, Park SJ, Jeong HW, Kim EG, Lee OJ, Kim YD, Choi Y, Lee SA, Choi YJ, Park SH, Jung JU, Choi YK.** 2022. Age-dependent pathogenic characteristics of SARS-CoV-2 infection in ferrets. Nat Commun **13**:21. https://doi.org/10.1038/s41467-021-27717-3.
24. **Knoblaugh SE, Hohl TM, La Perle KM.** 2018. Pathology principles and practices for analysis of animal models. ILAR J **59**:40–50. https://doi.org/10.1093/ilar/ilz001.
25. **Larrat S, Summa N.** 2021. Ferret behavior medicine. Vet Clin North Am Exot Anim Pract **24**:37–51. https://doi.org/10.1016/j.cvex.2020.09.001.
26. **Lee D-H, Kim J-I, Lee J-W, Chung W-H, Park J-K, Lee Y-N, Han JS, Kim H-Y, Lee S-W, Song C-S.** 2014. Quantitative measurement of influenza virus replication using consecutive bronchoalveolar lavage in the lower respiratory tract of a ferret model. J Vet Sci **15**:439–442. https://doi.org/10.4142/jvs.2014.15.3.439.

27. **Lednicky JA, Hamilton SB, Tuttle RS, Sosna WA, Daniels DE, Swayne DE.** 2010. Ferrets develop fatal influenza after inhaling small particle aerosols of highly pathogenic avian influenza virus A/Vietnam/1203/2004 (H5N1). Virol J **7**:1–15. https://doi.org/10.1186/1743-422X-7-231.
28. **Long JS, Mistry B, Haslam SM, Barclay WS.** 2019. Host and viral determinants of influenza A virus species specificity. Nat Rev Microbiol **17**:67–81. https://doi.org/10.1038/s41579-018-0115-z.
29. **MacPhail M, Chickli JH, Tang RS, Kaur J, Robinson C, Fouchier RAM, Osterhaus ADME, Spaete RR, Haller AA.** 2004. Identification of small-animal and primate models for evaluation of vaccine candidates for human metapneumovirus (hMPV) and implications for hMPV vaccine design. J Gen Virol **85**:1655–1663. https://doi.org/10.1099/vir.0.79805-0.
30. **Maher JA, DeStefano J.** 2004. The ferret: An animal model to study influenza virus. Lab Anim (NY) **33**:50–53. https://doi.org/10.1038/laban1004-50.
31. **Martina BE, Haagmans BL, Kuiken T, Fouchier RA, Rimmelzwaan GF, Van Amerongen G, Peiris JS, Lim W, Osterhaus AD.** 2003. SARS virus infection of cats and ferrets. Nature **425**:915. https://doi.org/10.1038/425915a.
32. **Martins M, Fernandes MHV, Joshi LR, Diel DG.** 2022. Age-related susceptibility of ferrets to SARS-CoV-2 infection. J Virol **96**:e01455-12. https://doi.org/10.1128/jvi.01455-21.
33. **Mascoli CC, Gower TA, Capilupo FA, Metzgar DP.** 1976. Further studies on the neonatal ferret model of infection and immunity to and attenuation of human parainfluenza viruses. Dev Biol Stand **33**:384–390.
34. **McClure DE.** 1999. Clinical pathology and sample collection in the laboratory rodent. Vet Clin North Am Exot Anim Pract **2**:565–590. https://doi.org/10.1016/S1094-9194(17)30111-1.
35. **Merani S, Pawelec G, Kuchel GA, McElhaney JE.** 2017. Impact of aging and cytomegalovirus on immunological response to influenza vaccination and infection. Front Immunol **8**:784. https://doi.org/10.3389/fimmu.2017.00784.
36. **Meunier I, Embury-Hyatt C, Stebner S, Gray M, Bastien N, Li Y, Plummer F, Kobinger GP, von Messling V.** 2012. Virulence differences of closely related pandemic 2009 H1N1 isolates correlate with increased inflammatory responses in ferrets. Virology **422**:125–131. https://doi.org/10.1016/j.virol.2011.10.018.
37. **Meyerholz DK, Beck AP.** 2020. Histopathologic evaluation and scoring of viral lung infection, p 205–220. In: Vijay R, ed. MERS Coronavirus. Methods Mol Biol **2099**. New York (NY): Springer US. https://doi.org/10.1007/978-1-0716-0211-9_16.
38. **Oh DY, Barr IG, Hurt AC.** 2015. A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity. PloS One **10**:e0118780. https://doi.org/10.1371/journal.pone.0118780.
39. **World Health Organition.** 2022. [Internet] WHO Coronavirus (COVID-19) Dashboard. Geneva (Switzerland): World Health Organization. [Cited 01 August 2022]. Available at: https://covid19.who.int/.
40. **Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, van der Spek A, Tolsma P, Rietveld A, Brouwer M, Bouwmeester-Vincken N, Harders F, Hakze-van der Honing R, Wegdam-Blans MCA, Bouwstra RJ, Geurts van Kessel C, van der Eijk AA, Velkers FC, Smit LAM, Stegeman A, van der Poel WHM, Koopmans MPG.** 2021. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science **371**:172–177. https://doi.org/10.1126/science.abe5901.
41. **Powers LV, Perpiñán D.** 2020. Chapter 1: Basic anatomy, physiology, and husbandry of ferrets, p 1–12. In: Quesenberry KE, Orcutt CJ, Mans C, Carpenter JW, eds. Ferrets, rabbits, and rodents (fourth edition). Philadelphia (PA): W.B. Saunders.
42. **Prince GA, Porter DD.** 1976. The pathogenesis of respiratory syncytial virus infection in infant ferrets. Am J Pathol **82**:339–352.
43. **Reuman PD, Keely S, Schiff GM.** 1989. Assessment of signs of influenza illness in the ferret model. J Virol Methods **24**:27–34. https://doi.org/10.1016/0166-0934(89)90004-9.
44. **Richard M, Kok A, de Meulder D, Bestebroer TM, Lamers MM, Okba N, Fentener van Vlissingen M, Rockx B, Haagmans BL, Koopmans MP.** 2020. SARS-CoV-2 is transmitted via contact and via the air between ferrets. Nat Commun **11**:3496. https://doi.org/10.1038/s41467-020-17367-2.
45. **Sawatzki K, Hill NJ, Puryear WB, Foss AD, Stone JJ, Runstadler JA.** 2021. Host barriers to SARS-CoV-2 demonstrated by ferrets in a high-exposure domestic setting. Proc Natl Acad Sci USA **118**:e2025601118. https://doi.org/10.1073/pnas.2025601118.
46. **Smith W, Andrewes CH, Laidlaw PP.** 1933. A virus obtained from influenza patients. Lancet **222**:66–68. https://doi.org/10.1016/S0140-6736(00)78541-2.
47. **Stark GV, Long JP, Ortiz DI, Gainey M, Carper BA, Feng J, Miller SM, Bigger JE, Vela EM.** 2013. Clinical profiles associated with influenza disease in the ferret model. PloS One **8**:e58337. https://doi.org/10.1371/journal.pone.0058337.
48. **Sterner-Kock A, Kock M, Braun R, Hyde DM.** 2000. Ozone-induced epithelial injury in the ferret is similar to nonhuman primates. Am J Respir Crit Care Med **162**:1152–1156. https://doi.org/10.1164/ajrccm.162.3.9812153.
49. **Stittelaar KJ, de Waal L, van Amerongen G, Veldhuis Kroeze EJ, Fraaij PL, van Baalan CA, van Kampen JJ, van der Vries E, Osterhaus AD, de Swart RL.** 2016. Ferrets as a novel animal model for studying human respiratory syncytial virus infections in immunocompetent and immunocompromised hosts. Viruses **8**:168. https://doi.org/10.3390/v8060168.
50. **Swearengen JR, Colby LA, Zitzow L, Asfaw YG, Reynolds R, Alderman S, Norton JN, Villano JS, Vleck SE, Felt SA.** 2018. Contemporary safety topics in animal research. ILAR J **59**
51. **Van den Berg SPH, Lanfermeijer J, Jacobi RHJ, Hendriks M, Vos M, van Schuijlenburg R, Nanlohy NM, Borghans JAM, van Beek J, van Baarle D, de Wit J.** 2021. Latent CMV infection is associated with lower influenza virus-specific memory T-cell frequencies, but not with an impaired T-cell response to acute influenza virus infection. Front Immunol **12**:663664. https://doi.org/10.3389/fimmu.2021.663664.
52. **Xu X, Blanton L, Elal AIA, Alabi N, Barnes J, Biggerstaff M, Brammer L, Budd AP, Burns E, Cummings CN, Garg S, Kondor R, Gubareva L, Kniss K, Nyanseor S, O'Halloran A, Rolfes M, Sessions W, Dugan VG, Fry AM, Wentworth DE, Stevens J, Jernigan D.** 2019. Update: Influenza activity in the United States during the 2018–19 season and composition of the 2019–20 influenza vaccine. MMWR Morb Mortal Wkly Rep **68**:544–551. https://doi.org/10.15585/mmwr.mm6824a3.
52. **Zaraket H, Baranovich T, Kaplan BS, Carter R, Song M-S, Paulson JC, Rehg JE, Bahl J, Crumpton JC, Seiler J.** 2015. Mammalian adaptation of influenza A (H7N9) virus is limited by a narrow genetic bottleneck. Nat Commun **6**:6553. https://doi.org/10.1038/ncomms7553.
53. **Zitzow LA, Rowe T, Morken T, Shieh WJ, Zaki S, Katz JM.** 2002. Pathogenesis of avian influenza A (H5N1) viruses in ferrets. J Virol **76**:4420–4429. https://doi.org/10.1128/JVI.76.9.4420-4429.2002.