ILAR Advanced Articles

**Landi et al. March 2021. Bioethical, Reproducibility, and Translational Challenges of Animal Models. ILAR Journal, ilaa027,**[**https://doi.org/10.1093/ilar/ilaa027**](https://doi.org/10.1093/ilar/ilaa027)

Domain 5: Regulatory Responsibilities, K3 protocol review, K8 responsible conduct of research

SUMMARY: In the typical protocol review process, there is no standardized point where an animal model is reviewed for reproducibility and translatability. The role of the IACUC is to review animal protocols for study justification and the effect of the study on animal welfare. However, this paper argues that perhaps the protocols should also be reviewed for reproducibility and translatability as well. This would increase the likelihood of the protocol benefitting human medicine and decreasing the amount of unnecessary harm placed on animal models. There are three target areas that have been identified to fully define the translatability of an animal model: face validity, construct validity, and predictive validity. Face validity is the similarity in the model and human phenotype of the disease. Construct validity is when the phenotype's etiology is the same in the animal model and humans. Predictive validity is whether the model accurately predicts features of the human disease that are not presently understood. These three areas should be reviewed in all animal models to ensure that they are translatable to human medicine.

NIH-funded research protocols do undergo peer review of the scientific and technical merit of an application by NIH Scientific Review Groups, but the practice is highly variable. This paper argues that this process should be standardized and also implemented for animal model protocols that are not NIH-funded, since they do not undergo a scientific review process to ensure translatability and reproducibility. The DEPART and PREPARE initiatives are similar to the ARRIVE guidelines, but the two former focus on guidelines for planning animal studies. The ARRIVE guidelines are a checklist of what is important to include in a publication on an animal study. The DEPART and PREPARE guidelines could be used by scientific review groups within institutions if such groups were implemented. The paper argues that our present method of protocol review at IACUCs may inadvertently lower reproducibility and translatability by targeting discussion to the 3Rs and harm-benefit analysis alone. Although it is not the role of the IACUC to perform in-depth scientific review, IACUCs must acknowledge that not all protocols receive such a review if they are not NIH-funded.

QUESTIONS

1. What does ARRIVE stand for in the ARRIVE guidelines?

a. Animal Research Review for in vivo Experimentation

b. Animal Research Review in Virology Experimentation

c. Animals in Research: Reporting in vivo Experiments

d. Animal Reports: Research in Virology Experiments

2. True or False: The IACUC is not responsible for scientific review.

3. True or False: There are no standards or guidelines for scientific review for the animal research component.

ANSWERS

1. c

2. True

3. True

# **Mangus et al. March 2021. Research-Relevant Background Lesions and Conditions in Common Avian and Aquatic Species. ILAR Journal, ilab008,**[**https://doi.org/10.1093/ilar/ilab008**](https://doi.org/10.1093/ilar/ilab008)

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

One-Line Summary: This is an overview of the research-relevant anatomic features, non-infectious conditions, and infectious diseases that impact research colonies of birds and aquatic animals, including fish and Xenopus species.

SUMMARY: The aim of this paper was to provide a broad-ranging but accessible reference describing the unique anatomic and physiologic features and disease conditions affecting commonly used birds, fish, and Xenopus species. The information provided in the article is concise, thorough, and invaluable.  The authors used a short introduction on each type of animal and tables to present the large quantity of information in a succinct manner.

For birds, the authors listed anatomic and physiologic features of Galliformes, Psittaciformes, and Passeriformes.  In this table they included special anatomical or physiologic features that help differentiate the different orders of birds. Tables were also used to list the anatomic and physiologic features of commonly used fish species and Xenopus species.  The authors included tabular comparisons of the two Xenopus species used in research and information on the commonly used fish in research.

When addressing diseases in birds and Xenopus species the authors split the tables into two types: 1. Metabolic and Spontaneous conditions and 2: Common infectious diseases affecting the animals.  For birds these tables were done separately to reflect conditions seen in different types of birds, such as: Chickens, Turkeys and Quail; Parrots and Budgerigars; and Finches.  For fish, the tables covering diseases included: Spontaneous Conditions of Fish Used in Research, Common Infectious Diseases of Captive Research Fish and Neoplastic Conditions of Captive Research Fish.

QUESTIONS

1.  List the advantages of using birds in research.

2.  List the advantages of using fish in research.

3.  List the major disadvantages of using fish in research.

4.  Why are beak trimming and toe trimming done in poultry flocks?

5.   True/ False: Marek’s disease is caused by a poxvirus.

ANSWERS

1.  Advantages of using birds in research – short generation time, small size, low cost, and ease of animal handling and care.

2.  Advantages of using fishes in research - small size, rapid generation time, fecundity, a wide variety of species, and the ability to expose animals to toxicants via dermal, respiratory, and gastrointestinal routes simultaneously.

3.  Disadvantages of using fish in research - highly labor intensive, and the technical support of long-term experiments is challenging.

4.  Beak trimming and toe trimming are done to prevent cannibalism and cutaneous trauma in poultry flocks.

5.  FALSE. It is caused by a herpesvirus.

*This article is a comprehensive and very concise review of the anatomy and disease affecting birds, fish and Xenopus sp.  For those studying for board exams it is worth saving the article and taking the time to read through and study the information presented in this article. In my opinion it can be used as a quick reference guide or study tool.*

# **Veenhuis and Zeiss. April 2021. Animal Models of COVID-19 II. Comparative Immunology. ILAR Journal, ilab010,**[**https://doi.org/10.1093/ilar/ilab010**](https://doi.org/10.1093/ilar/ilab010)

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions; K3. Immunobiology

Domain 3: Research; K3. animal models

SUMMARY: This review is the second part of a 2-part series.  In the first part, the virology and pathogenesis of SARS-CoV-2 was introduced.  In this review, prominent immunological characteristics of COVID-19 in humans and how animal models have been used thus far to study COVID-19 are reviewed.  The authors address immunological questions regarding SARS-CoV-2 infection and COVID-19 disease as well as how animal models can be used to develop vaccines and therapeutics.  (See Table 1 for a very nice summary of the immunological features of COVID-19 animal models).  Although a clear understanding of the innate immune system’s role in development of COVID-19 disease have not fully been elucidated, studies with other coronaviruses indicate that the viruses can evade the immune system by altering inflammatory mediator signaling. (See Table 2 for a detailed summary of innate immune mediators known to be involved in COVID-19 infection).  Dysregulation of interferon signaling and interferon stimulated genes is covered because some patients have a cytokine storm that inhibits normal physiologic function and can cause tissue damage. Elegant mouse studies indicate that IFN-signaling is exploited to enhance infection and disease. Unfortunately, no animal model is available that simulates the cytokine storm induced by COVID-19 in people.  In humans, pro-inflammatory macrophages and monocytes are recruited to the lung and play a rule in pulmonary damage.  Animal models including primates and hACE2 transgenic mice will be important in teasing out the pathogenesis of pneumonia in humans, the roles of myeloid cells in the development of disease, and the potential identification of therapeutic immune targets.  Animal models of SARS-CoV-2 neuropathogenesis and thrombosis are still under development, but there are significant neurologic consequences (such as the loss of taste/smell) and thrombosis causes significant morbidity and mortality.  To improve vaccines and vaccine responses, a better understanding of B- and T-cell responses to coronaviruses is warranted. Studies with rodents and monkeys are proving useful to increase understanding.  Mice, guinea pigs, rats, and rabbits have been used in COVID-19 studies to test he immunogenicity of vaccine candidates prior to them being tested in large anima models. There is still much to learn about SARS-CoV-2 immunopathology and using effective animal models will surely contribute to our increased understanding.

QUESTIONS

1.  Why is angiotensin-converting enzyme 2 so important in SARS-CoV-2 pathogenesis?

a.  ACE2 is an immune mediator in the heart and lungs

b.   ACE2 deficiency results in worsening COVID-19 pathology

c.  The ACE2 receptor is the site of cellular entry for SARS-CoV-2

d.   ACE2 is an enzyme that chews up viral particles and is important for viral clearance

2.   True/False: The optimal animal model to study neurologic consequences of SARS-CoV-2 infection is the ferret.

ANSWERS

1.  d

2.   False: SARS-CoV-2 has not been detected in the brains of ferrets infected with the virus. Only mice expressing human SARS-CoV-2 or mice infected with mouse-adapted SARS-CoV-2 have been useful as neuroimmunologic models.

# **Zeiss et al. April 2021. Animal Models of COVID-19. I. Comparative Virology and Disease Pathogenesis. ILAR Journal, ilab007,**[**https://doi.org/10.1093/ilar/ilab007**](https://doi.org/10.1093/ilar/ilab007)

Domain 3: Research

SUMMARY:The article describes the biology of coronaviruses, its taxonomy, host range, genomic structure and replication and the SARS-CoV-2 Receptor tropism across species. It lists various animal models that have evolved to study the disease pathogenesis of COVID-19, test therapeutics and support vaccine development.

There are four genera of Coronaviridae: Alpha, Beta, Delta and Gammacoronavirus. The recently relevant human betacoronaviruses have emerged in 2002 (Severe Acute Respiratory Syndrome, SARS-CoV-1), 2012 (Middle East Respiratory Syndrome Coronavirus, MERS-CoV) and 2019 (SARS-CoV-2 = COVID 19) and have caused 778, 8858 and 2.7 million deaths respectively. It is believed that the virus jumped from an animal host into humans and animal populations can serve as a reservoir in which the virus is constantly evolving. SARS-CoV-1 is believed to have jumped from Himalayan palm civets or racoon dogs to humans, MERS-CoV is transmitted from dromedary camels and SARS-CoV-2 is believed to be a bat coronavirus that may use Malayan pangolins as an intermediate host prior to entry in humans. The loss of natural habitats and urbanization make other coronaviral pandemics more likely as humans increasingly come in contact with wild animals that harbor coronaviruses. The paper lists various coronaviruses, their natural host species and the disease in a table. A few well known of these are: Feline infectious peritonitis virus of cats, Shipping fever of cows, Mouse hepatitis virus, Sialodacryoadenitis virus of rats (to name a few).

Coronaviruses have a large non-segmented, positive sense single-stranded RNA genome. Coronaviruses encode for the following 4 structural proteins: spike/surface (S), envelope/small membrane (E), membrane (M), and nucleocapsid (N) proteins. Some Betacoronaviruses also encode a hemagglutinin-esterase (HE) protein. The S glycoprotein forms the characteristic spikes protruding from the envelope of the virus. It binds to cell surface receptors to mediate viral entry into the cell. The ACE2 receptor of the host cell is used by SARS-CoV-2 to bind and enter the cells.

The core aspects of SARS-CoV-2 infection in humans are modelled truthfully in spontaneous animal models, these are animals that are susceptible to the infection and develop more or less severe clinical signs. Animals that spontaneously become infected with SARS-CoV-2 are: Rhesus macaque, cynomolgus macaque, African Green monkeys, common marmoset, mink, Syrian hamster, ferret, deer mouse, and to a lesser degree cat. These animals show the acute nature and predominantly respiratory source of viral shedding, acute transient and nonfatal disease with predominantly pulmonary symptoms and similar short-term immune responses. These animals do not model the more severe COVID infections in humans (including systemic inflammation and hematologic abnormalities) very well.  The paper then summarizes the disease pathogenesis clinical presentation of SARS-CoV-2 infection in each of the various spontaneous animal models.

SARS-CoV-2 infection has also been modelled in mice. Wild type mice cannot easily be infected with SARS-CoV-2 because of insufficient interaction between viral S protein and murine Ace2. Therefore different approaches have been employed to increase susceptibility to SARS-CoV-2 infection in laboratory mice: genetic alteration of the virus so that it is able to bind mouse Ace2, expression of human ACE2 under a variety of promoters and transfection of mice with human ACE2 cDNA using viral vectors. A table is provided that lists the various mouse models for SARS-CoV-2 infections and the disease pathogenesis and clinical presentation is then described in more detail for each mouse model.

The paper concludes that animal models are indispensable to the understanding of pulmonary and immune phenotype central to preclinical testing of vaccines and therapies for SARS coronaviruses.

QUESTIONS

1.  The recently emerged human coronaviruses SARS-CoV-1, MERS-CoV and SARS-CoV-2 belong to which genera of coronaviruses?

a.   Alphacoronavirus

b.  Betacoronavirus

c.  Deltacoronavirus

d. Gammacoronavirus

2.  Which two of the following animals are susceptible to SARS-CoV-2 (viral replication) and are therefore a good animal model to study the disease?

a.   Rhesus macaque

b.  Mouse

c. Syrian hamster

d.  Dog

3.       Which receptor does the SARS-CoV-2 virus use to bind to the cell surface?

a.  NMDA-Receptor

b.   Glu-Receptor

c.  ACE2-Receptor

d. IL-2 Receptor

4.   Why will it not be possible to eradicate Coronaviruses and their potential threat as a human pathogen?

5.   Which animals can be infected with SARS-CoV-2 (viral replication) but do not show overt clinical signs?

a.  Common marmoset

b.   African Green monkey

c.   Deer mouse

d.  Cat

ANSWERS

1. b

2.  a and c

3.  c

4.   Because Coronaviruses can infect a rich diversity of mammalian and avian hosts and these animal populations act as reservoirs for repeated human infections.

5.   a, b, c and d are correct

# **Kinter et al. June 2021. A Brief History of Use of Animals in Biomedical Research and Perspectives on Non-Animal Alternatives. *ILAR Journal*,  ilab020,**[**https://doi.org/10.1093/ilar/ilab020**](https://doi.org/10.1093/ilar/ilab020)

Domain 3: Research

SUMMARY: Results from the observation of the environment, plants, animals and their medicinal use are evident in historical evidence from as far as 17.000 years ago. The first known European to perform “experiments” was Aristotle (Greece, 4th century BCE), and others followed. Kinter and DeGeorge concluded that humans were the species most frequently used in ancient scientific inquiries, due to practicalities that made animal use difficult. The period around the First Industrial Revolution (circa 1760–1840) resulted in improvement of the conditions of human life, increase of interest for scientific discoveries in the field of physiology and the first social efforts to recognize and promote the more humane treatment of animals, including the founding of the British Society for the Prevention of Cruelty to Animals in Britain in 1824. The Second Industrial Revolution (circa 1860–1914) brought more elegant instrumentation such as the ECG, and developments in pharmacology. The first laboratory animal breeders and suppliers were founded, including Jackson Laboratories (1929), Harlan Laboratories (1931), Marshall BioResources (1939), and Charles River Laboratories (1947). The end of the 20th century signified an explosion of biomedical research using various animal models, including many species and transgenic animals, and the development of the theory of the 3Rs.

Numbers of animal use per species are recorded after 1973 in the US, and after 1945 in the UK. Incomplete reporting of early years, and differences in reporting requirements (e.g. specific species for USDA reporting) makes animal use difficult to compare. Animal use seems to be decreasing since the 80’s, and lately focuses on genetically modified animals.

Examples on non-animal alternatives:

* + Non–animal-sourced synthetic human insulin for diabetes management. First experiments from insulin were conducted with dogs. Insulin was then extracted from production animals (bovine, porcine sources).
  + Replacement of the rabbit pyrogen test (RPT) by the limulus amoebocyte lysate (LAL) from horseshoe crabs for endotoxin testing. The crabs were initially sacrificed but as the method evolved they are released.

QUESTIONS

1. The limulus amoebocyte lysate (LAL) test replaced:

a. Draize test

b. Rabbit pyrogen test

c. Guinea pig maximization test

d. LD50 tests

2. The first attested animal experiments were performed by:

a. Socrates

b. Plato

c. Aristotle

d. Galen

ANSWERS

1. b

2. c

**Layton et al. June 2021. Type I Hypersensitivity in Ferrets Following Exposure to SARS-CoV-2 Inoculum: Lessons Learned. *ILAR Journal*, ilab019,**[**https://doi.org/10.1093/ilar/ilab019**](https://doi.org/10.1093/ilar/ilab019)

Task 3: Diagnose disease or condition as appropriate

Secondary Species: Ferret (*Mustela putorius furo*)

SUMMARY: This report details a root-cause investigation following suspected hypersensitivity reactions in two groups of ferrets undergoing COVID-19 vaccine candidate trials. 6 animals out of 56 total were affected, with cases seen across vaccine candidates, routes of administration, and controls. Affected animals presented with signs of anaphylaxis (respiratory distress, epistaxis, hemoptysis) approximately 1 hour following intranasal exposure to SARS-CoV-2 inoculum. Of the 6 affected, 3 were euthanized while the others recovered and continued to the study endpoint. Histopathology of all affected animals, plus others within the cohort without clinical signs, was indicative of peracute to acute hypersensitivity reaction (anaphylaxis) and eosinophilic airway disease. An Ishikawa (“fishbone”) technique was used to catalog and categorize factors that may have contributed to the observed hypersensitivity reactions. Ultimately, it was determined that off-label protective vaccination with a canine distemper vaccine containing fetal bovine serum (FBS) acted as a priming agent; ferrets who had received both an initial and booster dose displayed overt anaphylaxis, while animals who received only a single dose had no clinical signs but did have histological evidence of allergic response. In addition to outlining a strategy for root-cause investigation, this report also encourages veterinary staff to consider the necessity of certain vaccines, and to opt for serum-free products when possible.

QUESTIONS

1. Name the established rodent model for allergic asthma (eosinophilic granulomatous pneumonia).
2. Match the following examples to the appropriate hypersensitivity reaction:

|  |  |
| --- | --- |
| Type I | Pemphigus vulgaris |
| Type II | PPD skin test |
| Type III | Peanut anaphylaxis |
| Type IV | Arthus reaction |

ANSWERS

1. Brown Norway rat

|  |  |
| --- | --- |
| Type I | Peanut anaphylaxis |
| Type II | Pemphigus vulgaris |
| Type III | Arthus reaction |
| Type IV | PPD skin test |



Histopathology in 1 of the ferrets that developed peracute respiratory distress after receiving the SARS-CoV-2 inoculum intranasally. (A) Severe edema in bronchial submucosa (arrowheads). Black rectangular box indicates the region

shown in panel B. (B) Moderate infiltrate of eosinophils and mononuclear cells in the bronchial mucosa. There are also eosinophils marginating in adjacent blood vessels (arrowhead). (C) Regionally extensive area of epithelial ulceration (arrows), submucosal edema, and hemorrhage (arrowhead) in the trachea. Black rectangular box indicates the region shown in panel D. (D) In the ulcerated

region, there is a light infiltrate of eosinophils in superficial lamina propria and some lining the denuded basement membrane of the tracheal mucosa (arrows). There are also numerous extravasated erythrocytes (hemorrhage) and a

moderate amount of fibrin admixed with edema in the lamina propria. All slides are stained with H&E stain. Magnification: (A) at 5×; (B) at 40×; (C) at 10×; and (D) at 40×.



Ishikawa (fishbone) diagram for root cause analysis of hypersensitivity reaction in ferrets. A comprehensive review of all possible causal factors contributing to the observed clinical signs was visualized in an Ishikawa (fishbone) diagram. The diagram lists potential causal factors in useful categories to aid a comprehensive

investigation. All factors were reviewed, and those highlighted were further examined after a systematic process of elimination.

**Navarro et al. June 2021. Mouse Anesthesia: The Art and Science. *ILAR Journal*, ilab016,**[**https://doi.org/10.1093/ilar/ilab016**](https://doi.org/10.1093/ilar/ilab016)

Domain 4

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Anesthesia is a science and also an art. In mice is especially important due its particular biology  and the implication of mice in  research projects.  The authors  review the science of mouse anesthesia  together with the art  to apply this knowledge in the mice involved in their research projects. The authors do a review  of definitions, drugs and dosages, anesthetic planes, monitoring , analgesia, factors that affect anesthesia and  complications. They also include their experience in both injectable and inhalant anesthesia in mice, and concluded that experience is as important as knowledge when using anesthesia. It is recommended to look the figure 1, that shows the pain pathway and the sites where anesthetic and analgesic drug act.

QUESTIONS

1. What are the four main components of anesthesia?

2. How sedation is different from tranquilization?

3. The indicators of unconsciousness in anesthetized animals are..

4. What are the four major components of pain pathway?

5. What are the four stages of anesthesia?

6. What is MAC (minimum alveolar concentration?

7. What is the isoflurane’s MAC for most species?

1. 1.2-1.8 %
2. 1.4-2%
3. 1.6-2.2%

d. 1.8-2.4%

8. T/F: Surgical plane of anesthesia is the one in which there are no response to a noxious stimulus.

9. T/F: The amount of anesthetic required to inhibit the spinal reflexes is no dependent of noxious stimulus.

10. How much anesthesia is needed for loss of consciousness?

a. 20% MAC

b. 40% MAC

c. 50% MAC

d. 70% MAC

11. Which of the next analgesics act in three of the four components of the pain pathway?

a. Buprenorphine

b. Butorphanol

c. Lidocaine

d. All of above

12. What influences mice response to anesthetics?

a. Strain

b. Age

c. Genetic manipulation

d. All of above

13. Why is necessary to be cautious when applying anesthetic protocols described in the literature in similar experiments?

14. What are the benefits to use inhalant anesthetics?

15. What are the disadvantages of inhalant anesthetics in mice?

16. Which of the following injectable anesthetics also provides analgesia?

a. Ketamine

b. Opiates

c. Local anesthetics

d. All of above

17. What is the advantage when using analgesia prior inhalant anesthesia in surgery?

18. In which way inhalant anesthetics affect respiratory function?

19. Why is better to use oxygen instead of room air in inhalant anesthesia?

20. Inhalant anesthetics induce hypotension by direct effect on three points that are…

21. What is the isoflurane MAC value for mice?

1. 1.1-1.6 %
2. 1.3-1.8 %
3. 1.5-2 %

d. 1.7-2.2%

22. What is the isoflurane range to anesthetize mice for surgery?

a. 1-2 %

b. 1.5-2.5 %

c. 2-3 %

d. 2.5-3.5 %

23. What is multimodal anesthesia?

24. T/F: Ketamine decreased MAC values for sevoflurane.

25. T/F: Combination of midazolam and butorphanol significantly decreases MAC values.

26. What do Ketamine/xylazine/acepromazine bring when used combination?

27. Where act and what produce xylazine?

28. What is the half-life of ketamine in mice if administered by IP injection?

a. 10 minutes

b. 13 minutes

c. 16 minutes

d. 19 minutes

29. What effects do mice experience when receiving a ketamine/xylazine/acepromazine combination?

30. T/F: Ketamine/xylazine can induce a surgical plane when combined with buprenorphine or carprofen.

31. T/F: Lidocaine added to ketamine/xylazine combination allow to achieve a surgical anesthesia plane, but lidocaine dose needed is higher than lidocaine DL50.

32. T/F: Redosing anesthesia in mice when they are close to emerge from surgical plane results in 50% of mortality.

33. T/F: When mice achieve a positive pedal withdrawal reflex they can be redosed with 50% of initial ketamine dose.

34. T/F: One advantage to use xylazine in anesthetic cocktails is that it can be reversed at the end of procedure.

35. What is true related to Alfaxalone?

1. Minimal cardiovascular effects
2. Females are more sensitive than males
3. Induces myoclonic activity in recovery

d. All of above

36. T/F: Although neither medetomidine, midazolam and butorphanol are hypnotic agents, hypnotic effect is achieved when administered as combination.

37. Why 2,2,2, tribromoethanol is not recommended to use as anesthetic agent?

38. T/F: Atipamezole reverses xylazine better than yohimbine.

39. What is the reversal agent of fentanyl) and for midazolam? And for medetomidine?

40. The three potential risks to use reversal agents are

41. What are the five categories of ASA PS preoperative evaluation?

42. What are the parameters for physical evaluation in mice?

43. In addition to physical condition, anesthesia risk is related to other six factors, that are…

44. Why do mice not need to be fasted prior anesthesia?

45. T/F: Physical restrain and handling can increase stress in mice and as consequence more anesthesia dose will be needed.

46. How can be evaluated in mice the respiratory function? The body circulation?  Hydration? Body condition?

47. T/F: The majority of anesthetic related deaths occurs during maintenance and post operative period.

48. Supportive care mitigates \_\_\_\_\_\_\_\_\_, \_\_\_\_\_\_\_\_\_\_ and \_\_\_\_\_\_\_    \_\_\_\_\_\_\_\_\_.

49. Why mice are vulnerable to fluid loss and how it can be minimized?

50. What is true about mice hypothermia?

a. Can prevent it with surgical draper

b. Is due to mice high surface area

c. Surgical scrub can be associated with it

d. All of above

51. Why is essential to do an anesthetic record?

52. T/F: The use of translucent surgical drape improves continuous control of skin coloration.

53. What is true related with respiratory rate of mice?

a. Awake mice have 120-200 breaths per minute

b. Inhalant anesthetics lows respiratory rate to 40-100

c. Ketamine based protocol increases respiratory rate to 230 per minute

d. All of above

54. T/F: Paw withdrawal is used to assess mouse anesthetic depth and do it in front paws is more valuable indicator.

55. T/F: Paw withdrawal has to be continuous monitored during surgery anesthesia because anesthetic plane continuously evolves.

56  What are the negative consequences of hypothermia?

57. T/F: Infrared thermometers in mice provide temperature 2ºC lower than rectal temperature.

58. What is a pulse oximeter?

59. Where can be placed the probes of a pulse oximeter?

60. What measurement shows a pulse oximeter if the mouse is breathing air room? and if breathing oxygen?

61. T/F: It is not needed to control respiratory patterns when using a pulse oximeter.

62. What is the heart rate of an anesthetized mouse?

a. 300-840 bpm

b. 250-300 bpm

c. 350-450 bpm

d. 400-540 bpm

63. T/F: Although a mouse shows a normal ECG, it can have circulatory complications.

64. Why is low blood pressure measured in mice?

65. What capnography measurements are difficult to interpret in mice?

66. T/F: The most sensitive parameter to detect mouse’s changes in anesthetic plane is respiratory characteristics .

67. What are the mortality risk factors in mice anesthetic recovery period?

68. T/F: To prevent overheating no more than one half of the recovery cage needs to be warmed.

69. Are allodynia and nociception correlated or no? Why?

70. The most intense pain peaks post operatively in mice occurs between

a. 2-24 h

b. 4-24 h

c. 6-24 h

d. 8-24 h

71. T/F: The most common opioids used in mice target the delta receptor.

72. T/F: NAIDS anti-inflammatory effects are due to inhibition of cyclo-oxygenase enzymes, COX-1 and COX-2.

73. What is windup pain?

74. What is important mice strain when selecting an anesthetic protocol?

75. T/F: In mice MAC of volatile inhalant anesthesia increases with age.

76. T/F: It is recommended normothermia support and the shortest duration when performing mice procedures that include anesthesia.

77. What is the most anesthetic method reported in neonates?

78. Why are not recommended injectable anesthetics in neonates?

79. T/F: The MAC of isoflurane  in 10 days old mice is higher than adult mice.

80. What causes CNS complications in mice anesthesia?

81. What causes respiratory complications in mice anesthesia?

82. How to correct a fast respiratory rate pattern in anesthetized mice?

83. T/F: In blood loss, fluid replacement has to be 3 times the volume of blood loss.

84. What causes excessive salivation in anesthetized mice?

85. T/F: Recovery after  anesthesia in mice takes 30-35 minutes after anesthesia discontinuation.

ANSWERS

1. Unconsciousness, amnesia, immobility/muscle relaxation, analgesia

2. Sedation implies central depression while tranquilization aware of the surroundings is maintained

3. Loss of movement, loss of right reflex

4. Transduction, transmission, modulation, perception

5. Loss of memory, loss of consciousness, loss of motor response to noxious stimulus

6. Is the inhalant anesthetic concentration whereby 50% of a specie will not respond to a noxious stimuli

7. b

8. True

9. False

10. c

11. d

12. d

13. Because usually next information related with anesthetic protocol is not included: protocol success, redosing procedures, confirmation of anesthesia depth, report of side effects, report of research outcome

14. They have an extremely response curve, due to its activation effect on  gamma aminobutyric acid and glycine receptors and inhibitors of NMDA receptor. Also, their blood solubility allows rapid induction and rapid recovery when changing de alveolar concentration

15. Needed of close animal monitoring, need of special equipment (vaporizer), risk of waste anesthetic gas in the environment and lack of analgesia

16. d

17. It decreases the total amount of inhalant anesthesia needed

18. A decrease in respiratory rate, tidal volume and blood oxygen concentration by inhibiting central and peripheral chemoreceptors for oxygen, and inducing an airway closure originating atelectasis

19. To compensate hypoxia induced by anesthetics

20. Smooth muscle vasculature , stroke volume. Autonomic nervous system

21. b

22. b

23. The use of multiple anesthetic drugs with complementary modes of action to minimize the dose of each drug used.

24. False

25. True

26. Ketamine analgesia and preservation of heart rate and blood pressure, xylazine sedation and analgesia, acepromazine tranquilization

27. Produces sedation in brain and analgesia in spinal cord

28. b

29. Ketamine suppresses respiration, xylazine induces bradycardia, acepromazine induces hypotension

30. False

31. True

32. True

33. True

34. True

35. d

36. True

37. Because there is not a grade formulation available, and it is needed to be reconstituted from a chemical grade product and stored.  This induces high drugs and quality variability.

38. True

39. Fentanyl is naloxone, midazolam is flumazenil, medetomidine is atipamezole

40. To experience pain, force the recovery from the non-reversed agent,  can be metabolized faster than anesthetic

41. ASA1 (normal healthy), ASA2 (mild systemic disease), ASA3 (severe systemic disease), ASA4 (systemic disease that can threat to life) ASA5 (moribund patient not expected to survive without surgery)

42. Overall appearance, respiratory function, mucous membrane or skin coloration, hydration, body condition scoring

43. Anesthetic agents, surgery performed, procedure length, monitoring and supportive care, equipment available, surgeon skill and training

44. Because they cannot vomit and prolonged fasting can lead to hypoglycemia

45. True

46. Respiratory function by observing nares and respiratory pattern, rate and depth. Blood circulation by observing color of mucous membrane, ears, paw pads. Tail. Hydration by observing eyes, piloerection, doing skin turgor test. Body condition by palpation of sacroiliac bones.

47. True

48. Hypovolemia, hypothermia, respiratory depression

49. Due to its small size and high metabolic rate. It can be minimized by irrigation of surgical site, control of blood loss and replacement with warmed balanced fluid

50. d

51. Because anesthesia problems occur gradually rather than abruptly

52. True

53. b

54. False

55. True

56. Cardiac arrhythmias, hypercoagulability, pain, increase susceptibility to infection, prolonged recovery time, decreased MAC

57. True

58. An equipment to measure the percentage of oxygenated hemoglobin in blood and the hearth rate

59. Paws, thigh, tail, neck

60. Room air 95-98%, oxygen 100%

61. False

62. c

63. True

64. Due to its high pulse rate and decreased arterial pulse

65. Due to its rapid respiration pattern and small tidal volume

66. True

67. dehydration, influence of residual anesthetic, hypothermia, hypoglycemia, sickness, age, respiratory complications

68. True

69. No, nociception is the detection of a noxious stimuli by CNS and allodynia is pain due to a normally non-noxious stimuli

70. b

71. False

72. True

73. A post-surgery hyperalgesia related with surgery incision. It can be minimized infiltration the tissues around the incision with local anesthetic

74. Because there are differences in anesthetic and analgesic sensitivity related with the background and with  genetic changes

75. False

76. True

77. Hypothermia

78. Because the answer is unpredictable, dosage is difficult and risk of mortality is high

79. True

80. Incorrect animal weight, improper route of administration, under or over dosage

81. Wrong anesthetic plane, overdose, anesthetic side effects, underlying health issues, equipment malfunction

82. Pause surgical manipulation, increase inhalant anesthesia ( 0,25% steps every 3-5 minutes), administer injectable anesthetics or buprenorphine, use balanced/multimodal analgesia

83. True

84. Use of ketamine, prolonged anesthesia

85. True

# **Brill et al. August 2021. The Symbiotic Relationship Between Scientific Quality and Animal Research Ethics. *ILAR Journal*, ilab023,**[**https://doi.org/10.1093/ilar/ilab023**](https://doi.org/10.1093/ilar/ilab023)

Domain 5: Regulatory responsibilities

SUMMARY: This article explores various ways that animal research ethics and scientific quality are closely linked. There is no standardized way to measure quality of science, but throughout history, discussions of ethics have frequently emphasized scientific quality to some degree. The ethical justification of animal research is rooted in utilitarianism, which argues that if the scientific yield of a study is higher than the expected harm to an animal involved in the study, the animal use is justified. Some type of harm-benefit analysis (HBA) is often incorporated into animal research planning, and is required by law in Europe. HBAs are not straightforward, though, as they attempt to weigh different levels of harm against different levels of benefit, and they are difficult to conduct for basic science research. While medical advancement is the typical benefit thought of for an HBA, benefits in economics, education, conservation, and veterinary medicine also positively affect society, and should also be taken into account. Rigorous study design has an increased likelihood of producing benefits, which applies to any kind of study, including basic science. Reducing potential harm to animals has been shown to improve scientific quality, further supporting the HBA. Species-specific needs both during an animal research study and prior to the study are 2 things that can alter study results and reproducibility, with improvement in both if those needs are addressed effectively.

The authors propose that a concrete Harm-Yield Analysis (HYA) process be developed and used for animal research studies. The first key element of the HYA is establishing minimum and maximum thresholds for variables for pain, benefit, and scientific quality. The second element is that it may be beneficial for specific fields to develop HYA formulations that suit their specialty. The last key element of the HYA is for it to be conducted in an integral manner, with previous results being used to inform and improve future HYAs. In order to get scientists to adopt these HYAs, training on the relationship between the responsible conduct of research and scientific quality could be built into various facets of the standard curriculum for graduate students, and refresher training could be provided to current animal researchers at regular intervals.

QUESTIONS

1. These 2 authors introduced the 3 Rs, but also included an entire section on the importance of sound study design in that same document.

2. What guidelines, introduced in 2010 by NC3Rs, are aimed at improving reproducibility and reliability by urging the inclusion of enough details in publications so they effectively add to the knowledge base?

ANSWERS

1. Russell and Burch

2. ARRIVE guidelines (Animal Research: Reporting of in vivo experiments)

# **Shriver and Hutchison. August 2021. An Introduction to Ethical Questions Around Animal Research. *ILAR Journal*,  ilab026,**[**https://doi.org/10.1093/ilar/ilab026**](https://doi.org/10.1093/ilar/ilab026)

Domain 6: Education

SUMMARY:The Principle investigator is an expert on the topic under investigation and the veterinarian is the expert for the health and wellness of the animal.  What then can ethicists add to the conversation? This special short issue of ILAR addresses this question through a review of various ethics centered articles and books that offers insight.

Ethics and ethical debate is essentially an expression of one's opinion on ‘how one ought to behave or live’.  At the very least, one should agree that any ethical perspective should make assumptions clear, consider alternative approaches, and employ airtight reasoning to justify moving away from initial assumptions to practical implications.  In general, it is important to demonstrate how clear thinking about values and the overall implications of those values impacts *what* type of research is conducted and *how*.  Some authors express the importance of including ethical foundations as an essential component of IACUC’s and other animal research discussion.  Recent literature on animal research ethics centers around the comparison of harm, costs, and benefits analysis.  Arguments for research suggest that the potential benefits, whether to all mankind or animals (depending on the nature of the research) would benefit far more than any potential harm endured by animals.  However, opponents to this rationale argue that the benefits of any research are not sufficient to justify the harm.  Additionally, there may be flaws in *evaluating* the potential benefits and this must be re-thought and re-considered.  Another major emerging thought surrounding harm-benefit analysis is informed consent from research subjects.  There is some discussion in literature about what informed consent would look like and what that would actually mean or represent for research animals and overall animal “rights”.

QUESTIONS

1. According to the review article, which of the following is one of the emerging perspectives on animal research?

a.   Benefit outweighs the harm and cost analysis

b.   Harm outweighs the benefits and cost analysis

c.   Harm, cost and benefit analysis

2. What appears to be the consensus among the literature regarding ethics and the role this philosophy plays in animal research?

a.  Ethical consideration should strongly be represented

b.   Ethical consideration should not be represented

c.    Ethical consideration should only be considered when working with NHP’s

ANSWERS

1. c

2. a

# **Kuiper et al. January 2022.** [**“But Mouse, You Are Not Alone”: On Some Severe Acute Respiratory Syndrome Coronavirus 2 Variants Infecting Mice**](https://academic.oup.com/ilarjournal/advance-article/doi/10.1093/ilar/ilab031/6503941)**. ILAR Journal, ilab031,**[**https://doi.org/10.1093/ilar/ilab031**](https://doi.org/10.1093/ilar/ilab031)

Domain 3: Research; T3. Design and conduct research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: This article describes use of molecular biology data and bioinformatics to understand the changes in SARS-CoV-2 during the current global pandemic. Using sequencing data for SARS-CoV-2 variants, and the software program Alphafold, and comparing to published epidemiologic, *in vitro* and *in vivo* SARS-CoV-2 research, the authors make a case for multidisciplinary approach to understanding evolution of infectious agents over time within populations. Current studies of the COVID-19 pandemic and SARS-CoV-2 changes can help to refine and model predictions for future epidemics/pandemics with regard to characterizing infectious agent changes and how those might be vulnerable to immunization and treatment protocols. It also provides a systematic methodology to analyze the potential for other animal reservoirs and their likely locations. Finally, the authors state that assessing the risk of viruses adapting to new hosts requires careful interpretation of all available data from *in silico*, *in vivo*, *in vitro*, and *in situ* sources; understanding host adaptation at a molecular level via modeling helps reconcile seemingly conflicting, experimental, and clinical observations while a pandemic is still in progress.

KEY POINTS

* + Mouse adaptation of the severe acute respiratory syndrome 2 virus requires an aromatic substitution in position 501 or position 498 (but not both) of the spike protein’s receptor binding domain
  + Definitions
  + Angiotensin-Converting Enzyme 2 (ACE2) Receptor: The receptor for the SARS-CoV-2 viral entry. A potential target for anti-viral therapeutics. Physiological functions of ACE2 in the cardiovascular system and the lungs include the activation of ACE2/MAS/G protein coupled receptor, contributing to reducing acute injury and inhibiting pulmonary fibrogenesis and protecting the cardiovascular system.
  + Receptor Binding Domain (RBD): A key part of a virus located on its ‘spike’ domain that allows it to dock to cell receptors to gain entry into those cells and lead to infection. These are also the primary targets in the prevention and treatment of viral infections.
  + Variant Of Concern (VOC): A category used for variants of the virus where mutations in their spike protein receptor binding domain (RBD) substantially increase binding affinity (e.g., N501Y) in RBD-hACE2 complex (genetic data), while also being linked to rapid spread in human populations (epidemiological data)
  + *In Silico*: In biology and other experimental sciences, an *in silico* experiment is one performed on computer or via computer simulation.
  + Alphafoldr Protein Folding Software: Provides open access to >992,316 protein structure predictions for the human proteome and other key proteins of interest
  + Alpha, Beta, Gamma, and Omicron variants of concern can infect mice, whereas Delta and “Delta Plus” lack a similar biomolecular basis to do so.
  + Currently 11 countries (Brazil, Chile, Djibouti, Haiti, Malawi, Mozambique, Reunion, Suriname, Trinidad and Tobago, Uruguay, and Venezuela) have programs for targeted local field surveillance of mice, based on the possibilities that they may have come in contact with humans who had the virus with adaptive mutation(s).
  + This work provides a systematic methodology to analyze the potential for other animal reservoirs and their likely locations.
  + The virus was adapted through techniques such as
* Sequential passaging in mouse lung tissues and modifying the receptor binding domain (RBD).
* Strategies for infecting mice with the original form of the virus or the G614 including transgenic mice expressing human ACE2 (hACE2)
* Sensitizing the mouse respiratory tract through transduction with adenovirus or adeno-associated virus expressing hACE2.
  + Surveillance for and recognition of virus variants of concern (VOC) originating from Brazil, South Africa, and the United Kingdom (which contain the common mutation N501Y in the RBD) were shown to infect mice.
  + Protein prediction software AlphaFold was used to recalculate RBD structures, and AlphaFold predictions were found to be in excellent agreement with the authors’ initial homology models
  + With time, the quality of AlphaFold predictions of protein complexes will improve thanks to additional experimental observations, and thus it is expected to play a more central role in structural interpretation during pandemics such as COVID-19 and future “disease-X.”
  + Public database called Global Initiative on Sharing All Influenza Data (GISAID) used to compare sequences and changes within the pandemic-associated virus variants through time, and relate those to related epidemiologic data.
  + Early in silico predictions based on comparative structural analysis of ACE2 suggested that mouse has a very low probability of being infected. Although correct about mouse, those analyses also made inconsistent and erroneous predictions that ferrets would not be susceptible, pigs would be susceptible, etc., thus exposing the need for experimental inputs into the model.
  + Practical thoughts for interspecies infection and virus changes: mice can be kept as pets or come into contact with other pets like cats, which are known to be susceptible. Also, mouse plague (extreme mouse overpopulation, seen at times in Australia) can occur in areas of COVID-19 outbreaks or endemicity.

QUESTIONS

1. Which of the following is the point of entry for SARS-CoV-2 into cells?

a. CCR5 - chemokine receptor 5

b. ACE2 receptor - angiotensin-converting enzyme 2 receptor

c. Nectin-4

d. CXCR4

2. Which of the following refers to experiments performed on computer or via computer simulation?

a. *In vitro*

b. *In vivo*

c. *In situ*

d. *In silico*

3. Which of the following is not a technique that has been described to adapt a virus found in one species to a new species (mice in this case)?

a. Sequential passaging in mouse lung tissues and modifying the receptor binding domain (RBD).

b. Creation of mice genetically engineered to express the viral-infection receptor from the host species to the new mouse line

c. Injection of mice with virus culture media containing very high virus concentrations

d. Modifying the mouse respiratory tract cell population through transduction with adenovirus or adeno-associated virus expressing the original host species virus receptor

ANSWERS

1. b. ACE2 receptor - angiotensin-converting enzyme 2 receptor

2. d. I*n silico*

3. c. Injection of mice with virus culture media containing very high virus concentrations

# **Cooper et al. January 2022. Research-Relevant Conditions and Pathology of Laboratory Mice, Rats, Gerbils, Guinea Pigs, Hamsters, Naked Mole Rats, and Rabbits. *ILAR Journal*, ilab022,**[**https://doi.org/10.1093/ilar/ilab022**](https://doi.org/10.1093/ilar/ilab022)

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Domain 3: Research

Primary Species: Mouse (*Mus musculus*), Rat (*Rattus norvegicus*), Rabbit (*Oryctolagus cuniculus*)

Secondary Species: Gerbil (*Meriones unguiculatus*), Guinea Pig (*Cavia porcellus*), Syrian Hamster (*Mesocricetus auratus*)

Tertiary Species: Other Rodents

SUMMARY:This review aims to provide a comprehensive resource to identify species/strain specific normal and expected findings (background lesions or incidental findings) to precisely define experimental research related outcomes for laboratory Mice, Rats, Gerbils, Guinea Pigs, Hamsters, Naked Mole rats, and Rabbits. Authors have elegantly summarized the information in tabulated form in a total of 44 tables for 7 species. Additionally, almost 900 citations have been referenced in this review which itself provides an extensive bibliography to be utilized for appropriate studies.

The tabulated information for each species has been described based on:

* Taxonomy, Genetics, Nomenclature, and Common Strains/Stocks
* Anatomic and Physiologic Features
* Non-Infectious Non-Neoplastic Conditions
* Neoplastic Conditions
* Infectious diseases due to Viruses, Bacteria and Fungi, Protists, Metazoan Parasites

In biomedical research mice are the most commonly used animals and both mice and rats make up 95% of all laboratory animals. Having summarized invaluable and extensive biological, phenotypical, physiological and morphological information on rodents and rabbits under one resource will serve as a guidance lexicon for laboratory animal veterinarian (including student residents), pathologist and scientist. In my personal opinion, an effort to summarize already succinct information will be a futile attempt. It is highly advisable to the lab animal community at large to keep this resource handy and refer to this article as per their specific research need.