Animal Models for Influenza Research

Even though numerous studies have been performed in the over 100 years since the 1918 influenza pandemic, knowledge of the host factors influencing influenza disease severity remains elusive. Shortcomings include understanding the transmission mechanisms, natural history and precise pathogenesis of influenza disease, and host immune responses. In addition, knowledge gaps exist regarding the relationship between clinical presentation, transmission, and protection levels. Given that universal influenza vaccines are still unavailable, there remains prodigious potential for influenza to reassort and cause severe human epidemics and pandemics. Therefore, it is essential to continuously assess host-virus interactions, transmission mechanisms, and the host immune response to different influenza viruses in various animal models. The selection of appropriate animal models for specific research questions is prerequisite for accurate understanding of influenza virus properties prior to clinical trials for novel universal influenza vaccines. In this review, the advantages and disadvantages of different animal models used for influenza research, including mice, ferrets, guinea pigs, swine, felines, canines, and non-human primates, will be discussed.

1. Mouse Model

Mice are the experimental tool of choice for the study of infectious diseases for several reasons: (1) easily manipulated genome, (2) rapid reproductive rate and ease of handling, (3) ease of husbandry, (4) low cost, including animals and housing, and (5) decreased variability.

In influenza research, mice are the most widely used animal model, and among inbred mice, C57BL/6 and BALB/c are the most commonly used. Influenza-infected mice develop clinical symptoms, such as anorexia, malaise, and cytokine storm, depending on mouse strain, virus strain, and challenge dose [1]. As in humans, reduced blood oxygen saturation increased lactate dehydrogenase, and total protein levels can be used to measure pneumonia in mice. Bodyweight loss and survival rate of mice are also good markers of influenza disease severity. However, studies have shown that the pathogenesis of the influenza virus in mice does differ to some extent from that in humans. Unlike humans, mice infected with the influenza virus do not show fever, instead exhibits hypothermia [2]. Some clinical signs, such as cyanosis, dyspnea, and hemoptysis, are also not as easily observed in mice [3]. Studies demonstrated that the use of DBA/2 mice enhanced influenza virus pathogenesis, resulting in significant body weight loss, higher mortality, enhanced cytokine production, and more severe lung pathology compared to C57BL/6 mice [4][5]. Other mouse models, such as Tmprss2$^{-/-}$ mice, have also been used to study the critical role of host protease TMPRSS2 in influenza viral pathogenesis [6][7][8][9].

Compared to other animal models, there appears to be limited utility for mouse models in the study of influenza virus transmission [10]. Although Schumal et al. successfully developed a transmission mouse model in the 1960s, it was found that only H2N2 subtypes (meaning no other subtypes, including H1N1, H3N2, or H5N2) were transmissible in their system [11][12][13][14][15]. Thus, the transmission of influenza in mice is only possible under certain conditions, or with specific mouse strains and mouse-adapted virus isolates. For example, Edenboroughand et al. demonstrated that direct contact is an important factor for virus transmission in mice [16]. However, Ortigoza and colleagues recently showed that an infant C57BL/6 mouse model might be useful for studying influenza virus transmission [17], and DRAGA mice have also been recently used as a transmissible model for influenza A virus infections [18]. Thus, further studies should be conducted to better understand influenza transmission in a mouse model.

Although the pathogenesis of the influenza virus is not completely recapitulated in mice, the convenience of monitoring clinical signs, including bodyweight loss, has resulted in their use for the investigation of influenza vaccines and antiviral drug efficacy in many preclinical studies. Furthermore, the availability of commercial reagents specific for mice facilitates the investigation of the immune response against influenza virus infection. For instance, the availability of cytokine and chemokine detection kits helps scientists to observe the influenza-induced cytokine storm, which is also seen in humans,

and to investigate Th1, Th2, and Th17 cytokine responses after immunization. The use of mouse-specific antibodies, together with flow cytometric analysis, enzyme-linked immunosorbent assay (ELISA), or other relevant techniques, allows measurement of immune cell responses and antigen-specific antibody responses after vaccination. In addition, researchers have used antibodies to deplete immune cells, such as CD4, CD8, or NK cells, to study the contribution of these cells to the protection against influenza infection in vaccinated mice [19][20]. Preclinical studies using knockout or deficient mouse models have also elucidated the roles of immune cells, including B cells, T cells, NK cells, and monocytes [21][22][23][24][25][26][27][28][29][30], as well as the IFN signaling pathway [31][32]. Transgenic mice, such as B6-Mx1$^{-/-}$ carrying mutant $Mx1$ alleles, B6-Mx1$^{r/r}$ carrying a functional A2G $Mx1$ allele, and SPRET/Ei, which carry another $Mx1$ wild-type allele, have been applied to study the importance of the $MX1$ gene in influenza virus resistance [33][34]. DRAGA mice that lack the murine immune system and express a functional human immune system were recently used to generate cross-reactive, anti-human influenza monoclonal antibodies [35]. The employment of humanized mice allows studies of influenza vaccine and antiviral drug efficacy and safety [36][37][38][39][40]. For example, HLA-A2 mice expressing a hybrid HLA-A2 transgene comprising the human α1/α2 domains and β2-microglobulin fused with a murine α3 domain were used to investigate the effects of influenza polypeptide vaccines [37]. Nonobese, diabetic, severe combined immunodeficiency $β_2$ microglobulin$^{-/-}$ (NOD/SCID $β2m^{-/-}$) mice (engrafted with human CD34$^+$ hematopoietic progenitors and further reconstituted with T cells), can be used to test influenza vaccines that cannot be tested in human volunteers [38]. The NOD/SCID/Jak3$^{-/-}$ (NOJ) mouse strain, which has defective T and B cells and impaired NK cell development, was transplanted with human peripheral blood mononuclear cells (huPBMC) to recapitulate the low immunogenicity of H7N9 influenza vaccines [39]. Rag2$^{-/-}$γc$^{-/-}$ mice reconstituted with huPBMC were used to investigate the effect of antiviral aminobisphosphonate pamidronate [36]. NOD/Shi-scid $IL2ry^{null}$ mice, retaining human CD14$^+$ cells, plasmacytoid dendritic cells, CD4$^+$ and CD8$^+$ T cells, and B cells in the lungs were also demonstrated to be suitable for evaluating the safety of influenza vaccines [40]. Kenney et al. applied IFITM3$^{-/-}$ mice, in which IFITM3 genes were deleted by CRISPR/Cas9-based deletion strategy, to evaluate influenza-induced cardiac pathogenesis [41].

In spite of the significant advantages, there are concerns over the use of mouse models for biomedical research in general. These include the differences between murine and human immune systems, such as frequencies of lymphocytes and neutrophils in peripheral blood, and differences in toll-like receptors, defensins, and IgG classes [42]. With the exception of high pathogenetic strains, such as pandemic H1N1 2009 [43], H5N1 [44], H7N7 [45], and H7N9 [46], mice are not naturally infected with influenza viruses. Therefore, viruses must be adapted through multiple lung passages to allow for infection and replication. Moreover, because influenza viruses do not transmit well among mice, more suitable animal models must be used to study this aspect [1][47]. In addition, unlike humans, mice exhibit influenza A virus replication in the lower respiratory tract; thus, IAV virulence is not preserved between these species [48].

2. Ferret Model

Ferrets have been used as an animal model for influenza research since 1933 when Smith et al. successfully isolated the human influenza A virus via inoculation into ferrets [49]. Recently, the successful genome sequencing of ferrets [50] has facilitated the use of this mammalian model in infectious disease research. Thus, ferrets have been used in a wide array of influenza research, including assessment of virus pathogenicity, transmissibility, viral tropism, host immune responses, and evaluation of novel vaccines and antiviral treatments [51].

Ferrets and humans share similar lung pathology and cellular receptor distribution [52], making ferrets attractive models for studies of the pathogenicity, transmissibility, and tropism of influenza viruses [53]. In addition, ferrets are naturally and highly susceptible to many different influenza virus strains, including influenza A and B viruses [54]. Previous studies showed that ferrets also exhibit predominant infection of influenza virus in the upper respiratory tract, as seen in humans. In addition, highly virulent influenza viruses can also infect the lower respiratory tract of ferrets [53]. Furthermore, influenza-infected ferrets exhibit similar clinical symptoms as seen in humans, such as fever, nasal secretions, coughing, gastrointestinal complications, serum abnormalities, neurological complications, weight loss and/or anorexia, lymphopenia, hypercytokinemia, and lethargy [10][53][54][55][56].

Human-to-human transmission of influenza viruses remains poorly understood due to knowledge gaps in the relationship between clinical symptoms, virus shedding, and transmission [57]. Unlike mice, influenza-infected ferrets are capable of transmitting the virus through both direct and indirect contact (aerosol, respiratory droplet, or airborne transmission) and thus, can be used as models to study influenza virus transmission [10]. The assessment of transmission is performed by detecting the virus in nasal secretions and investigating seroconversion [58]. Zhou and colleagues used this model to demonstrate that the size of airborne particles dictated the transmission through the air of 2009 pandemic H1N1, recombinant human seasonal H3N2, and triple-reassortant swine H1N2 influenza viruses in ferrets [59]. A previous study used ferrets to assess virus transmission by direct contact of the highly pathogenic avian H5N2 and H5N6 viruses, which

cause lethal diseases in humans. The study showed that the transmission between co-housed ferrets was limited to H5N6, but not the H5N2 virus [60]. Herfst et al. reported that the H5N6 virus causes severe disease, but does not transmit between ferrets via the airborne route [61]. In addition, R195K mutation in the PA-X protein was found to increase the virulence and transmission of pandemic H1N1 influenza A viruses via direct contact and aerosol transmission in the ferret model [62]. The impact of HA stability on the transmission of swine H1N1 gamma influenza virus (airborne transmission) [63] and avian H10N7 influenza virus (aerosol or respiratory droplet transmission) [64] was also demonstrated using ferret models. It should be noted that although ferrets are currently used as models for influenza transmission studies, there is a debate about the potential influenza pandemics associated with studies on the transmission of highly pathogenic influenza viruses using ferrets [65][66][67][68].

Ferrets are also an indispensable animal model for evaluating the host immune response to influenza as well as antiviral drug and vaccine efficacies. Early studies using the ferret model showed that cellular immune responses, especially cytotoxicity T cells, play an important role in recovery from influenza virus infection [69][70]. Furthermore, several influenza vaccine platforms have been studied using ferret models [71][72][73][74][75][76][77][78]. A study by Holzer et al. described differential protective efficacy of the Signal Minus FLU vaccine candidate in ferrets and pigs [79]. In addition, researchers demonstrated that vaccination of ferrets with chimeric hemagglutinin-based live attenuated vaccine provided durable protection against influenza A viruses [80]. Cross-protection of different influenza vaccines [77][78][81] and the role of adjuvants in influenza vaccines [72][74][82][77] have also been studied using ferret models. Additionally, many previous studies used ferrets to assess the effects of antiviral drugs against influenza virus infection [83][84][85][86][87]. Beale et al. employed ferrets to demonstrate that metabolomics could be used as indicators for the early diagnosis of influenza infection and assess drug therapies' effectiveness [87]. Treatment with human plasma-derived IgG product (FLU-IGIV) reduced viral loads in the lungs of pandemic H1N1-infected ferrets [86]. Lee et al. showed that Baloxavir, a recently licensed antiviral drug for influenza, could reduce onward transmission of pandemic H1N1 virus in infected ferrets [83]. However, it should be noted that Jones et al. reported that there is a risk of transmission of Baloxavir drug-resistant viruses from treated ferrets [84].

A limitation of the ferret model is the lack of reagents, including ferret-specific monoclonal antibodies, for analysis of immune responses to influenza virus infection and vaccination. Other disadvantages of ferrets as an experimental model are their size, high cost, and husbandry requirements, which make this model inaccessible to some researchers. Thus, due to the limited number of ferrets used in experiments, there is a possibility of misinterpretation of the results, which requires researchers trained well in doing experiments using this model. Of note, there is the possibility of danger in handling highly pathogenic influenza viruses in this model due to the risk of transmission. Experiments should be carefully performed with appropriate equipment in high-level biological safety laboratories.

3. Guinea Pig Model

Since 1963, guinea pigs (*Cavia porcellus*) have been occasionally used to study the pathogenesis of the influenza virus [88][89][90]. Of note, the lung anatomy and physiology of the guinea pig resemble that of humans [91]. In addition, the commercial availability, small size, ease of handling and housing, and relatively low cost strengthen the use of this model for the study of influenza viruses.

Guinea pigs are naturally susceptible to various influenza virus strains without prior adaptation. Following infection of guinea pigs, the influenza virus mainly replicates in the upper respiratory tract; however, signs of disease are not regularly observed [10]. Further, influenza virus infection does not cause mortality in this model [92].

Together with ferrets, guinea pigs have been widely used to evaluate the transmission potential of influenza viruses. Lina et al. employed guinea pigs to show that adaptive amino acid substitutions account for the increased transmissibility of H9N2 avian influenza virus [93]. Mutations in the HA and PB1 genes were also demonstrated to increase the pathogenicity and transmissibility of influenza viruses in the guinea pig model [94][95]. Previous studies using guinea pigs have shown that IAVs are transmissible via aerosolized fomites, microscopic particulates released from virus-contaminated surfaces (such as fur, skin, or bedding) [96], or respiratory droplets [97]. More recently, Asadi et al. described some unknown fraction of transmission events between guinea pigs in which aerosolized fomites were thought to be more important than respiratory droplets [98]. Wild bird-origin H5N6 avian influenza virus was also reported to be transmissible in guinea pigs [99].

Numerous influenza vaccine studies have been conducted in guinea pigs. McMahon et al. reported the role of neuraminidase-specific mucosal immunity in preventing influenza B virus transmission in guinea pigs [100]. In addition, vaccination with a live attenuated influenza vaccine prevented highly pathogenic H7N9 virus replication and transmission in guinea pigs [101]. Moreover, a consensus influenza D virus hemagglutinin-esterase fusion DNA vaccine completely

protected guinea pigs from challenge with influenza D virus [102]. In addition, the effects of plant-produced influenza human monoclonal antibody on preventing influenza infection and transmission in guinea pigs [103] suggest an alternative approach for the development of immunotherapeutics for influenza treatment.

It should be noted that the study of influenza viruses using guinea pigs has limitations. Guinea pigs show very few clinical symptoms following influenza infection, making it difficult to study viral pathogenesis. In addition, the lack of reagents specific to this model is also a drawback.

4. Hamster Model

The Syrian hamster (*Mesocricetus auratus*) is another of the animal models used for influenza virus pathogenesis, transmission, and vaccine efficacy studies, due to its ease in handling, reproduction capability, and relatively inexpensive maintenance cost [47]. This model was established due to its susceptibility to human influenza (H5N1, H9, and H3N2) and also to various avian influenza viruses without prior adaptation [47][104]. Replication and pathogenicity of several H9 isolates were investigated in this animal model [104]. Iwatsuki-Horimoto and colleagues [105] reported that Syrian hamsters were susceptible to H3N2 influenza viruses. Moreover, airborne transmission of pandemic H1N1 and seasonal H3N2 influenza virus isolates were also reported using Syrian hamsters. Heath et al. utilized hamsters and hamster tracheal organ cultures for the virulence study of recombinant influenza viruses [106]. The dissemination of ingested H5N1 influenza viruses was also explored using the hamster model [107]. Results revealed that H5N1 can systemically spread through the hematogenous route, causing interstitial lung lesions.

Hamsters are also a crucial animal model for investigating influenza vaccine efficacy. Similar to humans, hamsters have a body temperature of 37 °C; thus, this animal model is suitable to study cold-adapted influenza virus vaccine efficacy [47]. Using hamsters as an experimental animal model, Potter et al. showed that vaccination with inactivated H3N2 influenza vaccine provided protection against influenza virus challenge [108]. The enhanced antibody responses to inactivated influenza virus vaccine were also demonstrated in this model [109].

It should be noted that hamsters have not been extensively used in influenza studies given the disadvantage of the absence of clinical signs even with high viral titers in the respiratory tract. In addition, the limited availability of reagents for immunological assays poses a disadvantage to attempts to further elucidate immunological responses in this animal model.

5. Chicken Model

The chicken (*Gallus gallus domesticus*) is also an ideal animal model for influenza research due to the high abundance of α2, 3 receptors present in the lower respiratory tract. This species has the advantage of low cost, high availability, with medium availability of species-specific reagents. It can also be genetically modified [110]. The use of the chicken model has provided insights into the understanding of influenza virus pathogenesis. A study reported that chickens infected with influenza virus typically produce signs of coughing, nasal discharge, respiratory distress, diarrhea, lethargy, and death [110]. Using chicken models, researchers have found that the high pathogenicity in H5N1 influenza viruses was caused by a wider tissue tropism of the viruses. Ranaware et al. utilized chickens to study host genetic components and their responses to influenza virus infection [111].

Chickens have also been used to investigate the efficacy of influenza vaccines. A study by Steel et al. reported that vaccination with a live attenuated vaccine containing non-structural protein 1 completely protected chickens from challenges of homologous high pathogenic avian influenza H5N1 virus (A/Viet Nam/1203/04) and showed a high level of protection against heterologous H5N1 virus (A/egret/Egypt/01/06) [112]. Van der Goot and colleagues investigated the effects of vaccine candidates on H7N7 influenza virus transmission using chicken models [113]. The use of T-cell-suppressed chickens demonstrated the role of T cell responses in influenza virus clearance after challenge with lowly pathogenic H9N2 virus [114].

Although the chicken model has been used for a wide array of influenza virus studies, this animal model is not susceptible to human influenza virus strains. Thus, chickens are not the experimental tool of choice for studying human influenza viruses.

6. Swine Model

Pigs (Sus scrofa) are a natural host of influenza A viruses. The abundance of α2, 6-linked sialic acid in the upper respiratory tract and α2,3-linked sialic acid in the lower respiratory tract of pigs makes them ideal hosts and "mixing vessels" for both avian and human influenza virus infections and reassortment events [92]. It was previously reported that swine and humans share marked similarities in genome sequences, anatomy, and physiology [115]. Therefore, the pig is an animal model of choice for studying influenza pathogenesis and transmission and for evaluating influenza vaccines.

A study by Barnard reported that pigs develop signs of diseases, including fever, coughing, loss of appetite, and labored breathing, after influenza virus infection [3]. Moreover, swine are susceptible to highly pathogenic avian H5N1 [116], and influenza B viruses infect domestic pigs and cause influenza-like symptoms and lung lesions [117]. The pathogenesis of coinfection between H1N1 influenza virus and swine Streptococcus suis serotype 2 was investigated in a swine model [118]. Due to the possession of receptors for both avian and human influenza viruses, pigs are potential intermediate hosts for the adaptation of avian influenza viruses to humans [119]. In fact, using a swine model, Rajao et al. recently detected two novel reassortant, human-like H3N2 and H3N1 influenza viruses [120].

Numerous studies have shown that swine are suitable for evaluating influenza vaccine efficacy. A previous study reported similarities in the innate and adaptive immune responses of humans and swine, including elevation of inflammatory cytokines (IL-6, IFN-γ, IFN-α, TNF-α, and IL-10) after IAV infection [115]. HA-specific neutralizing antibodies were demonstrated to correlate with protection against influenza infections in swine, which is also seen in humans [115]. As in humans, an important role of $CD4^+$ and/or $CD8^+$ T cell responses for influenza virus clearance was also observed in infected pigs [115]. Recently, McNee et al. established the swine model for evaluating influenza monoclonal antibody treatment. Treatment using monoclonal antibodies significantly reduced viral load and pathology in this model [121]. Studies to understand and evaluate vaccine-associated enhanced respiratory disease (VAERD), which results in the intensification of clinical signs and the development of more severe lung lesions, have also been performed using the swine model [92].

Although pigs are suitable animal models for the study of influenza, the high cost, difficulties in animal handling, housing, and waste management make them less favorable [110]. Recently, the microminipig animal model was established to overcome the limitations posed by the large size of regular pigs in influenza virus studies. There was no significant difference in genomic sequence, receptor distribution, and susceptibility to influenza A viruses between microminipigs and regular-sized pigs, indicating that this model could be a good alternative model for influenza studies [122].

7. Feline and Canine Models

Canine and feline species were not considered natural hosts of influenza virus until an H3N8 equine influenza virus was isolated from dogs in 1999. This was followed by the identification of an H3N2 avian influenza virus in domestic dog populations in Asia and the United States in 2004, and an outbreak of H7N2 among cats in the United States in 2016 [123].

The susceptibility of dogs to avian influenza without prior adaptation was attributed to the abundance of α2, 3-linked sialic acid receptors in their lower respiratory tract [124]. Further, H5N1 infection of dogs is possible due to their close contact with wild and domestic birds as well as humans. Following experimental infection, clinical manifestation in dogs was characterized by the development of conjunctivitis and fever within 2 days, which resolved with no other adverse events by day 7. While dogs have been found to be susceptible to experimental infection, thus far, they have not been found to be capable of transmitting the virus to other mammals [125]. However, continued surveillance for canine influenza infections is imperative due to the high possibility that the virus can adapt to dogs and could transmit to humans while retaining its virulence. Given the increasing number of companion animals (dogs and cats) worldwide, their close contact with humans is a potential risk for zoonotic transmission of infectious viral diseases like influenza. In this sense, dogs may be considered more useful as a sentinel for human disease than as a model of human influenza [126].

In 2004, a study of highly pathogenic avian influenza H5N1 using a feline animal model showed severe and fatal disease manifestation as well as transmission of the virus via direct contact between infected and naive animals [127]. The virulence of H5N1 in cats was assessed through intratracheal infection and ingestion of virus-infected chicks. Clinical signs such as fever, conjunctivitis, lethargy, and labored breathing were observed in infected animals. After inoculation, the virus systematically spread and was isolated from the respiratory tract, digestive tract, liver, kidney, heart, brain, and lymph nodes. Moreover, the virus was found to be shed in the respiratory tract and also in the stool [127]. Although cats were reported to have susceptibility to IAV infection and there has been some work toward utilizing a feline influenza model for evaluating pathogenesis of H5N1 infections, few (if any) studies have been published which describe the suitability of felines for efficacy studies of influenza vaccines and antivirals [125][128].

8. Non-Human Primate Model

The close genetic relationship of non-human primates (NHPs) to humans, coupled with anatomic, physiologic, and immune feature similarities, makes this animal model invaluable for studying seasonal and highly pathogenic influenza viruses [129]. Several NHP species have been used for influenza studies, including rhesus (*Macaca mulatta*), cynomolgus (*Macaca fascicularis*), and pig-tailed (*Macaca nemestrina*) macaques, African green monkeys (*Chlorocebus aethiops*), New World NHPs, and common marmosets (*Callithrix jacchus*) [130]. While rhesus macaques have been selected for pathogenic studies and the evaluation of therapeutic and prophylactic strategies [131], the pig-tailed macaque is recommended for transcriptional studies of influenza virus [132]. Cynomolgus macaques have recently been used for various influenza studies [133][134][135].

NHPs were found to be susceptible to many avian and human influenza A isolates without prior adaptation. Clinical symptoms can range from asymptomatic to mild infections, which include conjunctivitis, listlessness, anorexia, and nasal discharge, all consistent with human clinical manifestations [129][131][136]. A primate model was also used to study the pathogenesis of H5N1 influenza virus infection [137]. Recently, Fukuyama et al. used aged cynomolgus macaques to study the severity of H7N9 influenza infection and the mechanism underlying the increased susceptibility of the elderly [138]. Nonetheless, NHPs are rarely used as models for influenza virus transmission. In 2013, Moncla et al. first demonstrated that human influenza virus isolates could infect and transmit between common marmosets, suggesting that this model could be used for studying influenza virus transmission [139].

NHPs are an overlooked animal model for the evaluation of the immunogenicity and efficacy of novel influenza vaccine candidates and treatments. Cross-reactive T cells have been shown to be involved in the rapid viral clearance and heterologous protection of NHPs against the pandemic H1N1 influenza virus [140][141]. Suzuki and colleagues employed cynomolgus macaques to investigate the efficacy of a cap-dependent endonuclease inhibitor and neuraminidase inhibitors on protection against high pathogenic avian H7N9 influenza virus [133]. A study by Darricarrère et al. investigated the ability of an HA stem-based influenza vaccine to induce broadly cross-reactive neutralizing antibodies against H1 and H3 using an NHP model [142]. The potential effects of quadrivalent influenza nanoparticle vaccines were also investigated in NHPs [143]. Furthermore, Koutsakos et al. recently developed flow cytometry-based assays to detect influenza-specific B and T cells in cynomolgus macaques. The use of these assays could be helpful for studying immune responses to influenza viruses in this model [135].

To date, NHPs have been used in many laboratories in order to study influenza virus pathogenesis and evaluate influenza vaccine and antiviral drug efficacies. However, ethical considerations, prohibitive costs, complicated husbandry requirements, and the need for extremely experienced personnel make this model less accessible for influenza research.

References

1. Margine, I.; Krammer, F. Animal models for influenza viruses: Implications for universal vaccine development. Pathogens 2014, 3, 845–874.

2. Yang, Y.T.; Evans, C.A. Hypothermia in mice due to influenza virus infection. Proc. Soc. Exp. Biol. Med. 1961, 108, 776–780.

3. Barnard, D.L. Animal models for the study of influenza pathogenesis and therapy. Antivir. Res. 2009, 82, A110–A122.

4. Srivastava, B.; Błażejewska, P.; Heßmann, M.; Bruder, D.; Geffers, R.; Mauel, S.; Gruber, A.D.; Schughart, K. Host genetic background strongly influences the response to influenza a virus infections. PLoS ONE 2009, 4, e4857.

5. Pica, N.; Iyer, A.; Ramos, I.; Bouvier, N.M.; Fernandez-Sesma, A.; García-Sastre, A.; Lowen, A.C.; Palese, P.; Steel, J. The DBA. 2 mouse is susceptible to disease following infection with a broad, but limited, range of influenza A and B viruses. J. Virol. 2011, 85, 12825–12829.

6. Lambertz, R.L.O.; Gerhauser, I.; Nehlmeier, I.; Gärtner, S.; Winkler, M.; Leist, S.R.; Kollmus, H.; Pöhlmann, S.; Schughart, K. H2 influenza A virus is not pathogenic in Tmprss2 knock-out mice. Virol. J. 2020, 17, 1–7.

7. Sakai, K.; Sekizuka, T.; Ami, Y.; Nakajima, N.; Kitazawa, M.; Sato, Y.; Nakajima, K.; Anraku, M.; Kubota, T.; Komase, K. A mutant H3N2 influenza virus uses an alternative activation mechanism in TMPRSS2 knockout mice by loss of an oligosaccharide in the hemagglutinin stalk region. J. Virol. 2015, 89, 5154–5158.

8. Lambertz, R.L.; Gerhauser, I.; Nehlmeier, I.; Leist, S.R.; Kollmus, H.; Pöhlmann, S.; Schughart, K. Tmprss2 knock-out mice are resistant to H10 influenza A virus pathogenesis. J. Gen. Virol. 2019, 100, 1073–1078.

9. Hatesuer, B.; Bertram, S.; Mehnert, N.; Bahgat, M.M.; Nelson, P.S.; Pöhlman, S.; Schughart, K. Tmprss2 is essential for influenza H1N1 virus pathogenesis in mice. PLoS Pathog. 2013, 9, e1003774.

10. Thangavel, R.R.; Bouvier, N.M. Animal models for influenza virus pathogenesis, transmission, and immunology. J. Immunol. Methods 2014, 410, 60–79.

11. Lowen, A.C.; Mubareka, S.; Tumpey, T.M.; García-Sastre, A.; Palese, P. The guinea pig as a transmission model for human influenza viruses. Proc. Natl. Acad. Sci. USA 2006, 103, 9988–9992.

12. Schulman, J.L. Experimental transmission of influenza virus infection in mice. IV. Relationship of transmissibility of different strains of virus and recovery of airborne virus in the environment of infector mice. J. Exp. Med. 1967, 125, 479–488.

13. Schulman, J.L. Experimental transmission of influenza virus infection in mice. 3. Differing effects of immunity induced by infection and by inactivated influenza virus vaccine on transmission of infection. J. Exp. Med. 1967, 125, 467–478.

14. Schulman, J.L.; Kilbourne, E.D. Experimental Transmission of Influenza Virus Infection in Mice. II. Some Factors Affecting the Incidence of Transmitted Infection. J. Exp. Med. 1963, 118, 267–275.

15. Schulman, J.L.; Kilbourne, E.D. Experimental Transmission of Influenza Virus Infection in Mice. I. The Period of Transmissibility. J. Exp. Med. 1963, 118, 257–266.

16. Edenborough, K.M.; Gilbertson, B.P.; Brown, L.E. A mouse model for the study of contact-dependent transmission of influenza A virus and the factors that govern transmissibility. J. Virol. 2012, 86, 12544–12551.

17. Ortigoza, M.B.; Blaser, S.B.; Zafar, M.A.; Hammond, A.J.; Weiser, J.N. An infant mouse model of influenza virus transmission demonstrates the role of virus-specific shedding, humoral immunity, and sialidase expression by colonizing Streptococcus pneumoniae. MBio 2018, 9.

18. Mendoza, M.; Gunasekera, D.; Pratt, K.P.; Qiu, Q.; Casares, S.; Brumeanu, T.-D. The humanized DRAGA mouse (HLA-A2. HLA-DR4. RAG1 KO. IL-2R gc KO. NOD) establishes inducible and transmissible models for influenza type A infections. Hum. Vaccines Immunother. 2020, 16, 2222–2237.

19. Yang, J.; Shim, S.-M.; Nguyen, T.Q.; Kim, E.-H.; Kim, K.; Lim, Y.T.; Sung, M.-H.; Webby, R.; Poo, H. Poly-γ-glutamic acid/chitosan nanogel greatly enhances the efficacy and heterosubtypic cross-reactivity of H1N1 pandemic influenza vaccine. Sci. Rep. 2017, 7, 44839.

20. Kwak, C.; Nguyen, Q.T.; Kim, J.; Kim, T.-H.; Poo, H. Influenza chimeric protein (3M2e-3HA2-NP) adjuvanted with PGA/alum conferred cross-protection against heterologous influenza A viruses. J. Microbiol. Biotechnol. 2021, 31, 304–316.

21. Jayasekera, J.P.; Moseman, E.A.; Carroll, M.C. Natural antibody and complement mediate neutralization of influenza virus in the absence of prior immunity. J. Virol. 2007, 81, 3487–3494.

22. Wu, H.; Haist, V.; Baumgärtner, W.; Schughart, K. Sustained viral load and late death in Rag2-/-mice after influenza A virus infection. Virol. J. 2010, 7, 1–6.

23. Palladino, G.; Mozdzanowska, K.; Washko, G.; Gerhard, W. Virus-neutralizing antibodies of immunoglobulin G (IgG) but not of IgM or IgA isotypes can cure influenza virus pneumonia in SCID mice. J. Virol. 1995, 69, 2075–2081.

24. Lin, K.L.; Suzuki, Y.; Nakano, H.; Ramsburg, E.; Gunn, M.D. CCR2+ monocyte-derived dendritic cells and exudate macrophages produce influenza-induced pulmonary immune pathology and mortality. J. Immunol. 2008, 180, 2562–2572.

25. Graham, M.B.; Braciale, T.J. Resistance to and recovery from lethal influenza virus infection in B lymphocyte–deficient mice. J. Exp. Med. 1997, 186, 2063–2068.

26. Nguyen, H.H.; van Ginkel, F.W.; Vu, H.L.; McGhee, J.R.; Mestecky, J. Heterosubtypic immunity to influenza A virus infection requires B cells but not CD8+ cytotoxic T lymphocytes. J. Infect. Dis. 2001, 183, 368–376.

27. Lee, B.O.; Rangel-Moreno, J.; Moyron-Quiroz, J.E.; Hartson, L.; Makris, M.; Sprague, F.; Lund, F.E.; Randall, T.D. CD4 T cell-independent antibody response promotes resolution of primary influenza infection and helps to prevent reinfection. J. Immunol. 2005, 175, 5827–5838.

28. Mozdzanowska, K.; Maiese, K.; Gerhard, W. Th cell-deficient mice control influenza virus infection more effectively than Th-and B cell-deficient mice: Evidence for a Th-independent contribution by B cells to virus clearance. J. Immunol. 2000, 164, 2635–2643.

29. Eichelberger, M.; Allan, W.; Zijlstra, M.; Jaenisch, R.; Doherty, P. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted CD8+ T cells. J. Exp. Med. 1991, 174, 875–880.

30. Allan, W.; Tabi, Z.; Cleary, A.; Doherty, P.C. Cellular events in the lymph node and lung of mice with influenza. Consequences of depleting CD4+ T cells. J. Immunol. 1990, 144, 3980–3986.

31. Arimori, Y.; Nakamura, R.; Yamada, H.; Shibata, K.; Maeda, N.; Kase, T.; Yoshikai, Y. Type I interferon limits influenza virus-induced acute lung injury by regulation of excessive inflammation in mice. Antivir. Res. 2013, 99, 230–237.

32. Davidson, S.; Crotta, S.; McCabe, T.M.; Wack, A. Pathogenic potential of interferon αβ in acute influenza infection. Nat. Commun. 2014, 5, 1–15.

33. Vanlaere, I.; Vanderrijst, A.; Guénet, J.-L.; De Filette, M.; Libert, C. Mx1 causes resistance against influenza A viruses in the Mus spretus-derived inbred mouse strain SPRET/Ei. Cytokine 2008, 42, 62–70.

34. Shin, D.-L.; Hatesuer, B.; Bergmann, S.; Nedelko, T.; Schughart, K. Protection from severe influenza virus infections in mice carrying the Mx1 influenza virus resistance gene strongly depends on genetic background. J. Virol. 2015, 89, 9998–10009.

35. Mendoza, M.; Ballesteros, A.; Qiu, Q.; Pow Sang, L.; Shashikumar, S.; Casares, S.; Brumeanu, T.-D. Generation and testing anti-influenza human monoclonal antibodies in a new humanized mouse model (DRAGA: HLA-A2. HLA-DR4. Rag1 KO. IL-2Ryc KO. NOD). Hum. Vaccines Immunother. 2018, 14, 345–360.

36. Tu, W.; Zheng, J.; Liu, Y.; Sia, S.F.; Liu, M.; Qin, G.; Ng, I.H.; Xiang, Z.; Lam, K.-T.; Peiris, J.M. The aminobisphosphonate pamidronate controls influenza pathogenesis by expanding a γδ T cell population in humanized mice. J. Exp. Med. 2011, 208, 1511–1522.

37. Miles, J.J.; Tan, M.P.; Dolton, G.; Edwards, E.S.; Galloway, S.A.; Laugel, B.; Clement, M.; Makinde, J.; Ladell, K.; Matthews, K.K. Peptide mimic for influenza vaccination using nonnatural combinatorial chemistry. J. Clin. Investig. 2018, 128, 1569–1580.

38. Yu, C.I.; Gallegos, M.; Marches, F.; Zurawski, G.; Ramilo, O.; García-Sastre, A.; Banchereau, J.; Palucka, A.K. Broad influenza-specific CD8+ T-cell responses in humanized mice vaccinated with influenza virus vaccines. Blood J. Am. Soc. Hematol. 2008, 112, 3671–3678.

39. Wada, Y.; Nithichanon, A.; Nobusawa, E.; Moise, L.; Martin, W.D.; Yamamoto, N.; Terahara, K.; Hagiwara, H.; Odagiri, T.; Tashiro, M. A humanized mouse model identifies key amino acids for low immunogenicity of H7N9 vaccines. Sci. Rep. 2017, 7, 1283.

40. Sasaki, E.; Momose, H.; Hiradate, Y.; Furuhata, K.; Mizukami, T.; Hamaguchi, I. Development of a preclinical humanized mouse model to evaluate acute toxicity of an influenza vaccine. Oncotarget 2018, 9, 25751.

41. Kenney, A.D.; McMichael, T.M.; Imas, A.; Chesarino, N.M.; Zhang, L.; Dorn, L.E.; Wu, Q.; Alfaour, O.; Amari, F.; Chen, M. IFITM3 protects the heart during influenza virus infection. Proc. Natl. Acad. Sci. USA 2019, 116, 18607–18612.

42. Mestas, J.; Hughes, C.C. Of mice and not men: Differences between mouse and human immunology. J. Immunol. 2004, 172, 2731–2738.

43. Maines, T.R.; Jayaraman, A.; Belser, J.A.; Wadford, D.A.; Pappas, C.; Zeng, H.; Gustin, K.M.; Pearce, M.B.; Viswanathan, K.; Shriver, Z.H. Transmission and pathogenesis of swine-origin 2009 A (H1N1) influenza viruses in ferrets and mice. Science 2009, 325, 484–487.

44. Lu, X.; Tumpey, T.M.; Morken, T.; Zaki, S.R.; Cox, N.J.; Katz, J.M. A mouse model for the evaluation of pathogenesis and immunity to influenza A (H5N1) viruses isolated from humans. J. Virol. 1999, 73, 5903–5911.

45. Munster, V.J.; de Wit, E.; van Riel, D.; Beyer, W.E.; Rimmelzwaan, G.F.; Osterhaus, A.D.; Kuiken, T.; Fouchier, R.A. The molecular basis of the pathogenicity of the Dutch highly pathogenic human influenza A H7N7 viruses. J. Infect. Dis. 2007, 196, 258–265.

46. Xu, L.; Bao, L.; Deng, W.; Zhu, H.; Chen, T.; Lv, Q.; Li, F.; Yuan, J.; Xiang, Z.; Gao, K. The mouse and ferret models for studying the novel avian-origin human influenza A (H7N9) virus. Virol. J. 2013, 10, 1–8.

47. Bouvier, N.M.; Lowen, A.C. Animal models for influenza virus pathogenesis and transmission. Viruses 2010, 2, 1530–1563.

48. Rodriguez, L.; Nogales, A.; Martínez-Sobrido, L. Influenza A virus studies in a mouse model of infection. J. Vis. Exp. 2017, 127, e55898.

49. Smith, W.; Andrewes, C.H.; Laidlaw, P.P. A virus obtained from influenza patients. Lancet 1933, 222, 66–68.

50. Peng, X.; Alföldi, J.; Gori, K.; Eisfeld, A.J.; Tyler, S.R.; Tisoncik-Go, J.; Brawand, D.; Law, G.L.; Skunca, N.; Hatta, M. The draft genome sequence of the ferret (Mustela putorius furo) facilitates study of human respiratory disease. Nat. Biotechnol. 2014, 32, 1250–1255.

51. Belser, J.A.; Pulit-Penaloza, J.A.; Maines, T.R. Ferreting out influenza virus pathogenicity and transmissibility: Past and future risk assessments in the ferret model. Cold Spring Harb. Perspect. Med. 2020, 10, a038323.

52. Jayaraman, A.; Chandrasekaran, A.; Viswanathan, K.; Raman, R.; Fox, J.G.; Sasisekharan, R. Decoding the distribution of glycan receptors for human-adapted influenza A viruses in ferret respiratory tract. PLoS ONE 2012, 7, e27517.

53. Belser, J.A.; Eckert, A.M.; Tumpey, T.M.; Maines, T.R. Complexities in ferret influenza virus pathogenesis and transmission models. Microbiol. Mol. Biol. Rev. 2016, 80, 733–744.

54. Maher, J.A.; DeStefano, J. The ferret: An animal model to study influenza virus. Lab. Anim. 2004, 33, 50–53.

55. Belser, J.A.; Barclay, W.; Barr, I.; Fouchier, R.A.; Matsuyama, R.; Nishiura, H.; Peiris, M.; Russell, C.J.; Subbarao, K.; Zhu, H. Ferrets as models for influenza virus transmission studies and pandemic risk assessments. Emerg. Infect. Dis. 2018, 24, 965.

56. Enkirch, T.; Von Messling, V. Ferret models of viral pathogenesis. Virology 2015, 479, 259–270.

57. Paules, C.I.; Marston, H.D.; Eisinger, R.W.; Baltimore, D.; Fauci, A.S. The pathway to a universal influenza vaccine. Immunity 2017, 47, 599–603.

58. Belser, J.A.; Katz, J.M.; Tumpey, T.M. The ferret as a model organism to study influenza A virus infection. Dis. Models Mech. 2011, 4, 575–579.

59. Zhou, J.; Wei, J.; Choy, K.-T.; Sia, S.F.; Rowlands, D.K.; Yu, D.; Wu, C.-Y.; Lindsley, W.G.; Cowling, B.J.; McDevitt, J. Defining the sizes of airborne particles that mediate influenza transmission in ferrets. Proc. Natl. Acad. Sci. USA 2018, 115, E2386–E2392.

60. Pulit-Penaloza, J.A.; Brock, N.; Pappas, C.; Sun, X.; Belser, J.A.; Zeng, H.; Tumpey, T.M.; Maines, T.R. Characterization of highly pathogenic avian influenza H5Nx viruses in the ferret model. Sci. Rep. 2020, 10, 12700.

61. Herfst, S.; Mok, C.K.; van den Brand, J.M.; van der Vliet, S.; Rosu, M.E.; Spronken, M.I.; Yang, Z.; de Meulder, D.; Lexmond, P.; Bestebroer, T.M. Human clade 2.3. 4.4 A/H5N6 influenza virus lacks mammalian adaptation markers and does not transmit via the airborne route between ferrets. Msphere 2018, 6, 3.

62. Sun, Y.; Hu, Z.; Zhang, X.; Chen, M.; Wang, Z.; Xu, G.; Bi, Y.; Tong, Q.; Wang, M.; Sun, H. An R195K mutation in the PA-X protein increases the virulence and transmission of influenza A virus in mammalian hosts. J. Virol. 2020, 94, 11.

63. Hu, M.; Yang, G.; DeBeauchamp, J.; Crumpton, J.C.; Kim, H.; Li, L.; Wan, X.-F.; Kercher, L.; Bowman, A.S.; Webster, R.G. HA stabilization promotes replication and transmission of swine H1N1 gamma influenza viruses in ferrets. Elife 2020, 9, e56236.

64. Herfst, S.; Zhang, J.; Richard, M.; McBride, R.; Lexmond, P.; Bestebroer, T.M.; Spronken, M.I.; de Meulder, D.; van den Brand, J.M.; Rosu, M.E. Hemagglutinin traits determine transmission of avian A/H10N7 influenza virus between mammals. Cell Host Microbe 2020, 28, 602–613.e607.

65. Lipsitch, M.; Inglesby, T.V. Moratorium on research intended to create novel potential pandemic pathogens. Am. Soc. Microbiol. 2014.

66. Fouchier, R.A. Studies on influenza virus transmission between ferrets: The public health risks revisited. MBio 2015, 6.

67. Lipsitch, M.; Inglesby, T.V. Reply to "studies on influenza virus transmission between ferrets: The public health risks revisited". MBio 2015, 6.

68. Lipsitch, M. Why do exceptionally dangerous gain-of-function experiments in influenza? Influenza Virus 2018, 589–608.

69. Kauffman, C.; Schiff, G.; Phair, J. Influenza in ferrets and guinea pigs: Effect on cell-mediated immunity. Infect. Immun. 1978, 19, 547–552.

70. McLAREN, C.; Butchko, G.M. Regional T-and B-cell responses in influenza-infected ferrets. Infect. Immun. 1978, 22, 189–194.

71. Reneer, Z.B.; Skarlupka, A.L.; Jamieson, P.J.; Ross, T.M. Broadly Reactive H2 Hemagglutinin Vaccines Elicit Cross-Reactive Antibodies in Ferrets Preimmune to Seasonal Influenza A Viruses. Msphere 2021, 6, e00052-21.

72. Stadlbauer, D.; de Waal, L.; Beaulieu, E.; Strohmeier, S.; Kroeze, E.J.V.; Boutet, P.; Osterhaus, A.D.; Krammer, F.; Innis, B.L.; Nachbagauer, R. AS03-adjuvanted H7N9 inactivated split virion vaccines induce cross-reactive and protective responses in ferrets. NPJ Vaccines 2021, 6, 40.

73. Vidaña, B.; Brookes, S.M.; Everett, H.E.; Garcon, F.; Nuñez, A.; Engelhardt, O.; Major, D.; Hoschler, K.; Brown, I.H.; Zambon, M. Inactivated pandemic 2009 H1N1 influenza A virus human vaccines have different efficacy after homologous challenge in the ferret model. Influenza Other Respir. Viruses 2021, 15, 142–153.

74. De Jonge, J.; van Dijken, H.; de Heij, F.; Spijkers, S.; Mouthaan, J.; de Jong, R.; Roholl, P.; Adami, E.A.; Akamatsu, M.A.; Ho, P.L. H7N9 influenza split vaccine with SWE oil-in-water adjuvant greatly enhances cross-reactive humoral immunity and protection against severe pneumonia in ferrets. NPJ Vaccines 2020, 5, 38.

75. Yeolekar, L.R.; Guilfoyle, K.; Ganguly, M.; Tyagi, P.; Stittelaar, K.J.; van Amerongen, G.; Dhere, R.M.; BerlandaScorza, F.; Mahmood, K. Immunogenicity and efficacy comparison of MDCK cell-based and egg-based live attenuated influenza vaccines of H5 and H7 subtypes in ferrets. Vaccine 2020, 38, 6280–6290.

76. Hatta, Y.; Boltz, D.; Sarawar, S.; Kawaoka, Y.; Neumann, G.; Bilsel, P. Novel influenza vaccine M2SR protects against drifted H1N1 and H3N2 influenza virus challenge in ferrets with pre-existing immunity. Vaccine 2018, 36, 5097–5103.

77. Christensen, D.; Christensen, J.P.; Korsholm, K.S.; Isling, L.K.; Erneholm, K.; Thomsen, A.R.; Andersen, P. Seasonal influenza split vaccines confer partial cross-protection against heterologous influenza virus in ferrets when combined with the CAF01 adjuvant. Front. Immunol. 2018, 8, 1928.

78. Govorkova, E.A.; Webby, R.J.; Humberd, J.; Seiler, J.P.; Webster, R.G. Immunization with reverse-genetics–produced H5N1 influenza vaccine protects ferrets against homologous and heterologous challenge. J. Infect. Dis. 2006, 194, 159–167.

79. Holzer, B.; Morgan, S.B.; Matsuoka, Y.; Edmans, M.; Salguero, F.J.; Everett, H.; Brookes, S.M.; Porter, E.; MacLoughlin, R.; Charleston, B. Comparison of heterosubtypic protection in ferrets and pigs induced by a single-cycle influenza vaccine. J. Immunol. 2018, 200, 4068–4077.

80. Liu, W.-C.; Nachbagauer, R.; Stadlbauer, D.; Strohmeier, S.; Solórzano, A.; Berlanda-Scorza, F.; Innis, B.L.; García-Sastre, A.; Palese, P.; Krammer, F. Chimeric Hemagglutinin-Based Live-Attenuated Vaccines Confer Durable Protective Immunity against Influenza A Viruses in a Preclinical Ferret Model. Vaccines 2021, 9, 40.

81. Baras, B.; Stittelaar, K.J.; Simon, J.H.; Thoolen, R.J.; Mossman, S.P.; Pistoor, F.H.; Van Amerongen, G.; Wettendorff, M.A.; Hanon, E.; Osterhaus, A.D. Cross-protection against lethal H5N1 challenge in ferrets with an adjuvanted pandemic influenza vaccine. PLoS ONE 2008, 3, e1401.

82. Wang, S.H.; Chen, J.; Smith, D.; Cao, Z.; Acosta, H.; Fan, Y.; Ciotti, S.; Fattom, A.; Baker Jr, J. A novel combination of intramuscular vaccine adjuvants, nanoemulsion and CpG produces an effective immune response against influenza A virus. Vaccine 2020, 38, 3537–3544.

83. Lee, L.Y.Y.; Zhou, J.; Frise, R.; Goldhill, D.H.; Koszalka, P.; Mifsud, E.J.; Baba, K.; Noda, T.; Ando, Y.; Sato, K. Baloxavir treatment of ferrets infected with influenza A (H1N1) pdm09 virus reduces onward transmission. PLoS Pathog. 2020, 16, e1008395.

84. Jones, J.C.; Pascua, P.N.Q.; Fabrizio, T.P.; Marathe, B.M.; Seiler, P.; Barman, S.; Webby, R.J.; Webster, R.G.; Govorkova, E.A. Influenza A and B viruses with reduced baloxavir susceptibility display attenuated in vitro fitness but retain ferret transmissibility. Proc. Natl. Acad. Sci. USA 2020, 117, 8593–8601.

85. Toots, M.; Yoon, J.-J.; Hart, M.; Natchus, M.G.; Painter, G.R.; Plemper, R.K. Quantitative efficacy paradigms of the influenza clinical drug candidate EIDD-2801 in the ferret model. Transl. Res. 2020, 218, 16–28.

86. Qiu, H.; Andersen, H.; Tarbet, E.B.; Muhammad, F.S.; Carnelley, T.; Pronyk, R.; Barker, D.; Kodihalli, S. Efficacy of anti-influenza immunoglobulin (FLU-IGIV) in ferrets and mice infected with 2009 pandemic influenza virus. Antivir. Res. 2020, 180, 104753.

87. Beale, D.J.; Oh, D.Y.; Karpe, A.V.; Tai, C.; Dunn, M.S.; Tilmanis, D.; Palombo, E.A.; Hurt, A.C. Untargeted metabolomics analysis of the upper respiratory tract of ferrets following influenza A virus infection and oseltamivir treatment. Metabolomics 2019, 15, 33.

88. Janssen, R.J.; Chappell, W.A.; Gerone, P.J. Synergistic Activity between PR8 Influenza Virus and Staphylococcus Aureus in the Guinea Pig; Army Biological Labs Frederick Md: Fort Detrick, MD, USA, 1963.

89. Fehlmann, H.; Gasser, M.; Jegge, S. Comparison of cellular and humoral immunity to influenza virus in the guinea pig. Pathol. Et Microbiol. 1974, 40, 231–233.

90. Phair, J.P.; Kauffman, C.A.; Jennings, R.; Potter, C.W. Influenza virus infection of the guinea pig: Immune response and resistance. Med. Microbiol. Immunol. 1979, 165, 241–254.

91. Canning, B.J.; Chou, Y. Using guinea pigs in studies relevant to asthma and COPD. Pulm. Pharmacol. Ther. 2008, 21, 702–720.

92. D'Alessio, F.; Koopman, G.; Houard, S.; Remarque, E.J.; Stockhofe, N.; Engelhardt, O.G. Workshop report: Experimental animal models for universal influenza vaccines. Vaccine 2018, 36, 6895–6901.

93. Lina, L.; Saijuan, C.; Chengyu, W.; Yuefeng, L.; Shishan, D.; Ligong, C.; Kangkang, G.; Zhendong, G.; Jiakai, L.; Jianhui, Z. Adaptive amino acid substitutions enable transmission of an H9N2 avian influenza virus in guinea pigs. Sci. Rep. 2019, 9, 19734.

94. Dreier, C.; Resa-Infante, P.; Thiele, S.; Stanelle-Bertram, S.; Walendy-Gnirß, K.; Speiseder, T.; Preuss, A.; Müller, Z.; Klenk, H.-D.; Stech, J. Mutations in the H7 HA and PB1 genes of avian influenza a viruses increase viral pathogenicity and contact transmission in guinea pigs. Emerg. Microbes Infect. 2019, 8, 1324–1336.

95. Wang, Z.; Yang, H.; Chen, Y.; Tao, S.; Liu, L.; Kong, H.; Ma, S.; Meng, F.; Suzuki, Y.; Qiao, C. A single-amino-acid substitution at position 225 in hemagglutinin alters the transmissibility of Eurasian avian-like H1N1 swine influenza virus in guinea pigs. J. Virol. 2017, 91.

96. Asadi, S.; ben Hnia, N.G.; Barre, R.S.; Wexler, A.S.; Ristenpart, W.D.; Bouvier, N.M. Influenza A virus is transmissible via aerosolized fomites. Nat. Commun. 2020, 11, 4062.

97. Zhao, Z.; Liu, L.; Guo, Z.; Zhang, C.; Wang, Z.; Wen, G.; Zhang, W.; Shang, Y.; Zhang, T.; Jiao, Z. A Novel Reassortant Avian H7N6 Influenza Virus Is Transmissible in Guinea Pigs via Respiratory Droplets. Front. Microbiol. 2019, 10, 18.

98. Asadi, S.; Tupas, M.J.; Barre, R.S.; Wexler, A.S.; Bouvier, N.M.; Ristenpart, W.D. Non-respiratory Particles Emitted by Guinea Pigs in Airborne Disease Transmission Experiments. Sci. Rep. 2021. under review.

99. Xiang, B.; Chen, L.; Xie, P.; Lin, Q.; Liao, M.; Ren, T. Wild bird-origin H5N6 avian influenza virus is transmissible in guin ea pigs. J. Infect. 2020, 80, e20–e22.

100. McMahon, M.; Kirkpatrick, E.; Stadlbauer, D.; Strohmeier, S.; Bouvier, N.M.; Krammer, F. Mucosal immunity against ne uraminidase prevents influenza B virus transmission in Guinea Pigs. MBio 2019, 10.

101. Yang, W.; Yin, X.; Guan, L.; Li, M.; Ma, S.; Shi, J.; Deng, G.; Suzuki, Y.; Chen, H. A live attenuated vaccine prevents rep lication and transmission of H7N9 highly pathogenic influenza viruses in mammals. Emerg. Microbes Infect. 2018, 7, 1– 10.

102. Wan, Y.; Kang, G.; Sreenivasan, C.; Daharsh, L.; Zhang, J.; Fan, W.; Wang, D.; Moriyama, H.; Li, F.; Li, Q. A DNA vacci ne expressing consensus hemagglutinin-esterase fusion protein protected guinea pigs from infection by two lineages of influenza D virus. J. Virol. 2018, 92.

103. Park, J.-G.; Ye, C.; Piepenbrink, M.S.; Nogales, A.; Wang, H.; Shuen, M.; Meyers, A.J.; Martinez-Sobrido, L.; Kobie, J. J. A Broad and potent H1-specific human monoclonal antibody produced in plants prevents Influenza virus infection an d transmission in Guinea Pigs. Viruses 2020, 12, 167.

104. Saito, T.; Lim, W.; Suzuki, T.; Suzuki, Y.; Kida, H.; Nishimura, S.-I.; Tashiro, M. Characterization of a human H9N2 influe nza virus isolated in Hong Kong. Vaccine 2001, 20, 125–133.

105. Iwatsuki-Horimoto, K.; Nakajima, N.; Ichiko, Y.; Sakai-Tagawa, Y.; Noda, T.; Hasegawa, H.; Kawaoka, Y. Syrian hamster as an animal model for the study of human influenza virus infection. J. Virol. 2018, 92.

106. Heath, A.; Addison, C.; Ali, M.; Teale, D.; Potter, C.J. In vivo and in vitro hamster models in the assessment of virulence of recombinant influenza viruses. Antivir. Res. 1983, 3, 241–252.

107. Shinya, K.; Makino, A.; Tanaka, H.; Hatta, M.; Watanabe, T.; Le, M.Q.; Imai, H.; Kawaoka, Y.J. Systemic dissemination of H5N1 influenza A viruses in ferrets and hamsters after direct intragastric inoculation. J. Virol. 2011, 85, 4673–4678.

108. Potter, C.; Jennings, R.J. The hamster as a model system for the study of influenza vaccines. Postgrad. Med. 1976, 52, 345–351.

109. Jennings, R.; Potter, C.W.; McLaren, C. Effect of preinfection and preimmunization on the serum antibody response to subsequent immunization with heterotypic influenza vaccines. J. Immunol. 1974, 113, 1834–1843.

110. Hemmink, J.D.; Whittaker, C.J.; Shelton, H.A. Animal models in influenza research. In Influenza Virus; Springer: Berlin/ Heidelberg, Germany, 2018; pp. 401–430.

111. Ranaware, P.B.; Mishra, A.; Vijayakumar, P.; Gandhale, P.N.; Kumar, H.; Kulkarni, D.D.; Raut, A. Genome wide host ge ne expression analysis in chicken lungs infected with avian influenza viruses. PLoS ONE 2016, 11, e0153671.

112. Steel, J.; Lowen, A.C.; Pena, L.; Angel, M.; Solórzano, A.; Albrecht, R.; Perez, D.R.; García-Sastre, A.; Palese, P. Live a ttenuated influenza viruses containing NS1 truncations as vaccine candidates against H5N1 highly pathogenic avian inf luenza. J. Virol. 2009, 83, 1742–1753.

113. Van der Goot, J.; Koch, G.d.; De Jong, M.; Van Boven, M.J. Quantification of the effect of vaccination on transmission o f avian influenza (H7N7) in chickens. Proc. Natl. Acad. Sci. USA 2005, 102, 18141–18146.

114. Kwon, J.-S.; Lee, H.-J.; Lee, D.-H.; Lee, Y.-J.; Mo, I.-P.; Nahm, S.-S.; Kim, M.-J.; Lee, J.-B.; Park, S.-Y.; Choi, I.-S. Imm une responses and pathogenesis in immunocompromised chickens in response to infection with the H9N2 low pathoge nic avian influenza virus. Virus Res. 2008, 133, 187–194.

115. Rajao, D.S.; Vincent, A.L. Swine as a model for influenza A virus infection and immunity. Ilar J. 2015, 56, 44–52.

116. Meseko, C.; Globig, A.; Ijomanta, J.; Joannis, T.; Nwosuh, C.; Shamaki, D.; Harder, T.; Hoffman, D.; Pohlmann, A.; Bee r, M. Evidence of exposure of domestic pigs to Highly Pathogenic Avian Influenza H5N1 in Nigeria. Sci. Rep. 2018, 8, 5 900.

117. Ran, Z.; Shen, H.; Lang, Y.; Kolb, E.A.; Turan, N.; Zhu, L.; Ma, J.; Bawa, B.; Liu, Q.; Liu, H. Domestic pigs are suscepti ble to infection with influenza B viruses. J. Virol. 2015, 89, 4818–4826.

118. Lin, X.; Huang, C.; Shi, J.; Wang, R.; Sun, X.; Liu, X.; Zhao, L.; Jin, M. Investigation of pathogenesis of H1N1 influenza virus and swine Streptococcus suis serotype 2 co-infection in pigs by microarray analysis. PLoS ONE 2015, 10, e0124 086.

119. Bourret, V. Avian influenza viruses in pigs: An overview. Vet. J. 2018, 239, 7–14.

120. Rajão, D.S.; Gauger, P.C.; Anderson, T.K.; Lewis, N.S.; Abente, E.J.; Killian, M.L.; Perez, D.R.; Sutton, T.C.; Zhang, J.; Vincent, A.L. Novel reassortant human-like H3N2 and H3N1 influenza A viruses detected in pigs are virulent and antigenically distinct from swine viruses endemic to the United States. J. Virol. 2015, 89, 11213–11222.

121. McNee, A.; Smith, T.R.; Holzer, B.; Clark, B.; Bessell, E.; Guibinga, G.; Brown, H.; Schultheis, K.; Fisher, P.; Ramos, S. Establishment of a pig influenza challenge model for evaluation of monoclonal antibody delivery platforms. J. Immunol. 2020, 205, 648–660.

122. Iwatsuki-Horimoto, K.; Nakajima, N.; Shibata, M.; Takahashi, K.; Sato, Y.; Kiso, M.; Yamayoshi, S.; Ito, M.; Enya, S.; Otake, M. The microminipig as an animal model for influenza a virus infection. J. Virol. 2017, 91, 91.

123. Wasik, B.R.; Voorhees, I.E.; Parrish, C.R. Canine and feline influenza. Cold Spring Harb. Perspect. Med. 2021, 11, a038562.

124. Song, D.; Kang, B.; Lee, C.; Jung, K.; Ha, G.; Kang, D.; Park, S.; Park, B.; Oh, J. Transmission of avian influenza virus (H3N2) to dogs. Emerg. Infect. Dis. 2008, 14, 741.

125. Giese, M.; Harder, T.C.; Teifke, J.P.; Klopfleisch, R.; Breithaupt, A.; Mettenleiter, T.C.; Vahlenkamp, T.W. Experimental infection and natural contact exposure of dogs with avian influenza virus (H5N1). Emerg. Infect. Dis. 2008, 14, 308.

126. Halliday, J.E.; Meredith, A.L.; Knobel, D.L.; Shaw, D.J.; Bronsvoort, B.M.d.C.; Cleaveland, S. A framework for evaluating animals as sentinels for infectious disease surveillance. J. R. Soc. Interface 2007, 4, 973–984.

127. Kuiken, T.; Rimmelzwaan, G.; van Riel, D.; van Amerongen, G.; Baars, M.; Fouchier, R.; Osterhaus, A. Avian H5N1 influenza in cats. Science 2004, 306, 241.

128. Fouchier, R.A.; Schneeberger, P.M.; Rozendaal, F.W.; Broekman, J.M.; Kemink, S.A.; Munster, V.; Kuiken, T.; Rimmelzwaan, G.F.; Schutten, M.; Van Doornum, G.J. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc. Natl. Acad. Sci. USA 2004, 101, 1356–1361.

129. Baskin, C. The role and contributions of systems biology to the non-human primate model of influenza pathogenesis and vaccinology. Syst. Biol. 2012, 363, 69–85.

130. Bodewes, R.; Morick, D.; de Mutsert, G.; Osinga, N.; Bestebroer, T.; van der Vliet, S.; Smits, S.L.; Kuiken, T.; Rimmelzwaan, G.F.; Fouchier, R.A. Recurring influenza B virus infections in seals. Emerg. Infect. Dis. 2013, 19, 511.

131. Baas, T.; Baskin, C.; Diamond, D.L.; Garcia-Sastre, A.; Bielefeldt-Ohmann, H.; Tumpey, T.; Thomas, M.; Carter, V.; Teal, T.; Van Hoeven, N. Integrated molecular signature of disease: Analysis of influenza virus-infected macaques through functional genomics and proteomics. J. Virol. 2006, 80, 10813–10828.

132. Baskin, C.R.; García-Sastre, A.; Tumpey, T.M.; Bielefeldt-Ohmann, H.; Carter, V.S.; Nistal-Villán, E.; Katze, M.G. Integration of clinical data, pathology, and cDNA microarrays in influenza virus-infected pigtailed macaques (Macaca nemestrina). J. Virol. 2004, 78, 10420–10432.

133. Suzuki, S.; Nguyen, C.T.; Ogata-Nakahara, A.; Shibata, A.; Osaka, H.; Ishigaki, H.; Okamatsu, M.; Sakoda, Y.; Kida, H.; Ogasawara, K. Efficacy of a cap-dependent endonuclease inhibitor and neuraminidase inhibitors against H7N9 highly pathogenic avian influenza virus causing severe viral pneumonia in cynomolgus macaques. Antimicrob. Agents Chemother. 2021, 65, 65.

134. Mooij, P.; Stammes, M.A.; Mortier, D.; Fagrouch, Z.; van Driel, N.; Verschoor, E.J.; Kondova, I.; Bogers, W.M.; Koopman, G. Aerosolized Exposure to H5N1 Influenza Virus Causes Less Severe Disease Than Infection via Combined Intrabronchial, Oral, and Nasal Inoculation in Cynomolgus Macaques. Viruses 2021, 13, 345.

135. Koutsakos, M.; Sekiya, T.; Chua, B.Y.; Nguyen, T.H.O.; Wheatley, A.K.; Juno, J.A.; Ohno, M.; Nomura, N.; Ohara, Y.; Nishimura, T. Immune profiling of influenza-specific B-and T-cell responses in macaques using flow cytometry-based assays. Immunol. Cell Biol. 2021, 99, 97–106.

136. Iwatsuki-Horimoto, K.; Nakajima, N.; Kiso, M.; Takahashi, K.; Ito, M.; Inoue, T.; Horiuchi, M.; Okahara, N.; Sasaki, E.; Hasegawa, H. The marmoset as an animal model of influenza: Infection with A (H1N1) pdm09 and highly pathogenic A (H5N1) viruses via the conventional or tracheal spray route. Front. Microbiol. 2018, 9, 844.

137. Rimmelzwaan, G.; Kuiken, T.; Van Amerongen, G.; Bestebroer, T.; Fouchier, R.A.M.; Osterhaus, A.D.M. A primate model to study the pathogenesis of influenza A (H5N1) virus infection. Avian Dis. 2003, 47, 931–933.

138. Fukuyama, S.; Iwatsuki-Horimoto, K.; Kiso, M.; Nakajima, N.; Gregg, R.W.; Katsura, H.; Tomita, Y.; Maemura, T.; da Silva Lopes, T.J.; Watanabe, T. Pathogenesis of Influenza A (H7N9) Virus in Aged Nonhuman Primates. J. Infect. Dis. 2020, 222, 1155–1164.

139. Moncla, L.H.; Ross, T.M.; Dinis, J.M.; Weinfurter, J.T.; Mortimer, T.D.; Schultz-Darken, N.; Brunner, K.; Capuano III, S.V.; Boettcher, C.; Post, J. A novel nonhuman primate model for influenza transmission. PLoS ONE 2013, 8, e78750.

140. Weinfurter, J.T.; Brunner, K.; Capuano III, S.V.; Li, C.; Broman, K.W.; Kawaoka, Y.; Friedrich, T.C. Cross-reactive T cells are involved in rapid clearance of 2009 pandemic H1N1 influenza virus in nonhuman primates. PLoS Pathog. 2011, 7, e1002381.

141. Koday, M.T.; Leonard, J.A.; Munson, P.; Forero, A.; Koday, M.; Bratt, D.L.; Fuller, J.T.; Murnane, R.; Qin, S.; Reinhart, T. A. Multigenic DNA vaccine induces protective cross-reactive T cell responses against heterologous influenza virus in n onhuman primates. PLoS ONE 2017, 12, e0189780.

142. Darricarrère, N.; Qiu, Y.; Kanekiyo, M.; Creanga, A.; Gillespie, R.A.; Moin, S.M.; Saleh, J.; Sancho, J.; Chou, T.-H.; Zho u, Y. Broad neutralization of H1 and H3 viruses by adjuvanted influenza HA stem vaccines in nonhuman primates. Sci. Transl. Med. 2021, 13, eabe5449.

143. Boyoglu-Barnum, S.; Ellis, D.; Gillespie, R.A.; Hutchinson, G.B.; Park, Y.-J.; Moin, S.M.; Acton, O.J.; Ravichandran, R.; Murphy, M.; Pettie, D. Quadrivalent influenza nanoparticle vaccines induce broad protection. Nature 2021, 592, 623–62 8.