Animal Models to Study Influenza Virus Pathogenesis and Control

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Abstract: Influenza A virus causes a highly contagious respiratory disease with potentially fatal outcomes in both humans and animals. Animal models for studying the pathogenesis of the influenza virus are of considerable importance, both for practical treatments of the disease and for the development of vaccines to prevent it. Ideal animal models that accurately reflect the disease, respond to antiviral therapy, and induce a protective immune response to influenza infection or vaccination are important for advances in research. In the veterinary field, natural hosts can be utilized, although the application of vaccine and antiviral therapy in animals should be considered carefully because of the possible latency of viral infection and acceleration of viral mutations. In a laboratory setting, ferrets have been used extensively in influenza research because the pathogenesis of the influenza virus in ferrets is very similar to that observed in humans. Contact ferret models have also been used to evaluate transmissibility of the influenza virus in humans in order to determine the pandemic potential. Laboratory mice are also experimentally infected with the influenza virus, although mice are not naturally infected and usually do not cause lethal disease without adaptation of the virus. Recently, cotton rat as a small animal model has proved useful because, as adaptation to human influenza strains is not required for the virus to replicate in the lower respiratory tract, subsequent disease develops. Non-human primates such as rhesus and cynomolgus macaques can be experimentally infected with the influenza virus and can become ill. Although the use of this model is limited, influenza models in non-human primates may be more predictive of the responses in humans due to their close evolutionary relationship. In this review, we will discuss the characteristics of these species as a potential influenza model. We will also highlight data obtained from animal models that are expected to contribute to the development of vaccines and treatments to improve the lives of both humans and animals from infection in the future.

Keywords: Influenza, animal model, ferret, mouse, cotton rat, non-human primate.

1. ANIMAL MODELS FOR INFLUENZA

1.1. Influenza Viruses

Influenza viruses belong to the Orthomyxoviridae family and cause highly contagious respiratory diseases with potentially fatal outcomes in humans and animals. With the realization that animal influenza viruses can be transmitted to humans, influenza viruses are now considered to be one of the zoonotic diseases, and are therefore a major global health concern. The economic burden of influenza is prominent, especially when it becomes pandemic in humans. The combined costs of seasonal epidemics in humans each year as well as the occasional outbreaks that occur in animals, such as avian and horse influenzas, are also significant and potentially serious.

There are three known types of influenza viruses—influenza A, B, and C—based on antigenic differences between viral nucleoproteins (NPs) and matrix 1 (M1) proteins. Influenza A viruses are further designated into 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes. Influenza A viruses have been isolated from a variety of animals, including humans, pigs, horses, sea mammals, and birds [1]. Phylogenetic studies of type A isolates reveal that viral genes form species-specific lineages, and that aquatic birds are the source of all influenza A viruses in other animal species. Influenza A viruses do not usually produce disease in wild aquatic birds, except for some H5N1 highly pathogenic strains [2], thus indicating that they have achieved an optimal level of adaptation in their natural reservoir. All HA and NA subtypes of type A viruses are maintained in aquatic bird populations, particularly in ducks, shorebirds, and gulls.

In addition to viruses already established in mammals, those that have been transmitted to both mammals and poultry from wild waterfowl have also caused outbreaks in recent years. Experimentally, however, viruses from one species of animals do not grow efficiently in other species. For example, human influenza viruses do not replicate in ducks or horses, indicating host range restriction [3].

1.2. Roles of Animal Models

Animal models for influenza are essential for understanding the pathogenicity of the viruses and for developing vaccines and therapeutic treatments. Animal models that replicate the major features of illness in humans or in target animals provide selective, sensitive and reproducible results.

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In the field of veterinary research, natural hosts can be directly used for experimental infection as long as the study is properly designed. Concepts of vaccination and therapeutic treatment for animals sometimes differ to those for humans: food animals may not be vaccinated, because vaccination can induce viral infection in the animals without any clinical signs, thus allowing the viruses to spread, which may accelerate viral mutation and result in the generation of new viruses. The principles of controlling contagious diseases in food animals are traditionally to restrict animal movement, to quarantine, and to remove the affected animals [4]. Although several vaccines for poultry have been developed and licensed [5], and vaccinations against avian influenzas (AI) caused by viruses of the H5 and H7 subtypes have recently been used on several occasions to control and in some cases eradicate the disease [6], the use of vaccines and therapies for food animals should be considered carefully.

Despite the diversity of mammalian species that are infected by influenza viruses present in nature, only a few species are amenable to study in the laboratory. For example, ferrets are considered the best model for influenza because of their high susceptibility to the viruses. However, the mouse is often chosen as a primary model because of its low cost, ready availability, and ease of handling. In addition, the cotton rat is an attractive rodent species because of its susceptibility to many pathogens. Rodent models such as mice and cotton rats are also advantageous in terms of their small size and fast reproduction rate. Nevertheless, non-human primates more closely resemble humans. It is important to consider that commonly used laboratory animal species may not be fully permissive to infection with non-adapted wild-type isolates of influenza viruses, and can vary in their susceptibility to infection by specific virus strains and subtypes [7].

Evaluation parameters commonly include body temperature elevation, virus recovery from respiratory tissues and nasal washes, and pneumonia-associated mortality [8]. A serum hemagglutination-inhibiting (HAI) antibody titer of 1:32 or 1:40, or even greater, is associated with protection from seasonal influenza, and this is used as a measure to predict the protective efficacy of seasonal inactivated vaccines. However, the correlates of protection for live-attenuated vaccines are less clear-cut. These vaccines elicit systemic and mucosal immune responses as well as mucosal antibodies in the respiratory tract and so are believed to play a major role in the protection afforded by these vaccines [7]. Most studies that evaluate immune responses to influenza vaccines have been conducted in mice and ferrets [7]. Measurement of antibody responses in animal models is very straightforward since HAI and neutralizing antibody assays do not require species-specific reagents.

In this report, we will first review the natural infection of influenza A viruses in animals. Observations from natural cases are important for understanding the nature of viruses in a variety of animal species. Then, we will discuss the main features of animal models that are used for studying influenza viruses, with a specific focus on ferrets, mice, cotton rats, and non-human primates.

2. NATURAL INFECTIONS WITH INFLUENZA A VIRUSES IN ANIMALS

2.1. Avian Influenza in Poultry

Avian influenza viruses can be sorted on the basis of their virulence in poultry: highly pathogenic avian influenza (HPAI) viruses cause systemic lethal infection and kill birds quickly, while low pathogenic avian influenza (LPAI) viruses rarely generate outbreaks of severe disease in the field so that their associated morbidity and mortality rates are much lower than that of HPAI viruses [9]. Avian influenza viruses have two geographically separate sub-lineages (Eurasian and American), which reflect the separation of viruses due to the distinct migratory patterns of the host birds in these two distinct regions of the world. Both HPAI and LPAI viruses are found within these two sub-lineages. Without exception, all of the HPAI viruses belong to the H5 or H7 subtype, for reasons that remain elusive. There does not appear to be any association of specific NA subtypes with HPAI viruses.

In chickens infected with HPAI viruses, swelling of the microvascular endothelium, systemic congestion, multifocal hemorrhages, perivascular mononuclear cell infiltration, and thrombosis are typically common [10]. These viruses replicate efficiently in the vascular endothelium and in perivascular parenchymatous cells, a property important for viral dissemination and systemic infection. Viral antigens are found in necrotic cardiac myocytes as well as in cells of other organs that display necrotic and inflammatory changes. Thus, involvement of the cardiovascular system plays an important role in the pathogenesis of avian influenza [11]. High concentrations of HPAI viruses, which replicate systemically in poultry, are also shed in feces; however, these viruses are more readily transmitted among birds in densely populated flocks via the nasal and oral passages through contact with virally contaminated materials. In contrast, LPAI viruses replicate mainly in intestinal and respiratory organs and are shed in the feces of infected birds. Thus, transmission of viruses via the fecal-contaminated water-oral route is a major mechanism of virus dissemination among birds.

The HA glycoprotein mediates virus binding to host-cell receptors and, through membrane fusion, promotes the release of viral RNA complexed with polymerase and nucleoprotein NP (ribonucleoprotein, RNP). Post-translational cleavage of a precursor HA molecule (HA0) into HA1 and HA2 subunits by host proteases, with the generation of a fusogenic domain at the amino terminus of HA2, mediates fusion of the viral envelope with the endosomal membrane. This proteolytic activation is essential for viral infectivity and dissemination. The HAs of LPAI viruses possess a single Arg at the cleavage site and are usually cleaved in only a limited number of organs, resulting in mild or asymptomatic infection. Proteases capable of cleaving the HAs of LPAI viruses are often called “trypsin-like” enzymes, although the proteases responsible for HA cleavage *in vivo* are yet to be identified. By contrast, the HAs of HPAI viruses possess a series of basic amino acids at the cleavage site, which are cleaved by ubiquitous proteases, such as furin, and are present in a broad range of host cells that support lethal

systemic infection in poultry [12, 13]. Therefore, HA cleavability is considered the main determinant of tissue tropism of avian influenza viruses, whereby differences in tissue distribution of the proteases and HA susceptibility to these enzymes can determine the outcome of virus infection [14].

2.2. Influenza Infections in Mammals, Excluding Humans

During the Spanish influenza of 1918-1919, pigs presented with influenza-like symptoms such as nasal discharge, coughing, fever, and conjunctivitis [15]; the first swine influenza virus (H1N1) was isolated in 1930 [16]. Since then, swine H1N1 influenza virus, referred to as the "classic swine virus", has become enzootic in pigs in North America, Europe, and Asia, causing mild respiratory disease in these animals. In 1979, an avian H1N1 virus was introduced into pigs in Europe as "avian-like swine virus", thereby establishing a stable virus lineage [17]. Following this, reassortant viruses possessing human-like H3N2 and avian-like internal genes emerged and are now common in Europe. Human-like H3N2 viruses, the "human-like swine viruses", were first isolated from pigs in Taiwan in 1970, as well as the human-like H1N1 viruses that transmit easily to pigs. Recently, "reassortant swine viruses" containing H1N2 swine/human and H3N2 swine/avian/human reassortants have spread among pig populations in North America [18]. Pigs have been postulated to be the intermediate host for the generation of pandemic viruses because their respiratory epithelia contain both avian- and human-type receptors, which provide a "mixing vessel" for gene reassortment between avian and human viruses.

Two different subtypes of influenza A viruses have been identified in horses: H7N7 (first isolated in 1956) and H3N8 subtypes (first isolated in 1963). Equine influenza is typically associated with fever, a dry hacking cough, loss of appetite, muscular soreness, and tracheobronchitis. There has been no confirmed outbreak of the H7N7 virus in horses since 1979 [19].

Transmission of influenza A viruses to seals has also occurred, albeit occasionally, and this is mainly from aquatic birds. Seals have been infected with viruses of avian origin that cause respiratory diseases with primary viral pneumonia [20]. H13 viruses have also been isolated from the lungs and hilar nodes of a stranded pilot whale [21].

In 2004, equine H3N8 viruses were transmitted from horses to racing greyhounds in Florida, causing fatal diseases in some dogs [22]. Serological studies indicate that the virus spread to the general dog population. Interspecies transmission of equine H3N8 virus to dogs by close contact was also confirmed with experimentally infected horses [23]. In 2007, avian H3N2 viruses were transmitted to domestic dogs in Korea [24].

Highly pathogenic H5N1 avian viruses have been isolated from several mammals such as domestic cats, zoo tigers and leopards, civet cats, dogs, a stone marten, raccoon dogs, and pikas, causing fatal outcomes with systematic infections in most of the animals [25-27]. Interestingly, the susceptibility of pigs to H5N1 viruses is low, and causes only asymptomatic infections [28].

3. FERRET MODELS

The first human influenza virus was isolated from ferrets in 1933 [29]. Since then, ferrets have been used extensively for studying various aspects of human influenza virus infection and its course of action. In the opinion of many researchers, the ferret remains the ideal small animal model for influenza virus research [7, 30-32].

Pathogenesis of the seasonal influenza virus in ferrets is very similar to that observed in humans. Non-adapted isolates replicate efficiently in the respiratory tract of this animal. Signs of illness include fever, sneezing, rhinorrhea, and weight loss. Infection in these animals only rarely progresses to pneumonia [33]. Researchers have shown that even though nasal turbinates are the primary site of viral replication, highly virulent strains of influenza A are also capable of infecting the lower respiratory tract. The pathological changes seen in both ferrets and humans are most prominent in the upper airways. The influenza virus attaches to "human-type" receptors on the surface of respiratory epithelia in ferrets.

The ferret is believed to be a good model system for the study of HPAI viruses. Since the direct transmission of HPAI H5N1 viruses from birds to humans was observed in Hong Kong in 1997, the avian H5N1 viruses isolated from humans were evaluated on their ability to replicate and cause disease in outbred ferrets. The 1997 wild-type human H5N1 viruses from Hong Kong were highly virulent in the outbred ferret model, unlike the differential pathogenicity documented in inbred BALB/c mice [34]. The 2004 wild-type human H5N1 viruses from Vietnam and Thailand were fatal to intranasally inoculated ferrets. High fever, weight loss, anorexia, extreme lethargy, and diarrhea were observed [35].

To assess the possibility of alimentary transmission of H5N1, infection with and pathogenesis of H5N1 viruses were studied in ferrets by inoculating them intragastrically with virus in liquid, or by administering them infected raw chicken meat or minced meat into the stomach by gavage with a tube. While no evidence of infection was observed in the ferrets after intragastric inoculation, consumption of infected meat by the ferrets resulted in respiratory system infection only, or in both severe respiratory and systemic infection with predominant involvement of the liver, pancreas, and the large and small intestine. On the other hand, direct intragastric exposure to infected meat resulted in lethal systemic disease mainly affecting the intestine, liver, and pancreas but not involving the lungs. It was therefore concluded that exposure of the digestive system to H5N1 influenza viruses could initiate infection either through the tonsils, with spread to respiratory tissues, or through intestinal infection, with spread to the liver and pancreas [36].

To investigate whether transmissible H5 subtype human-avian reassortant viruses could be generated *in vivo*, ferrets were co-infected with 2004 human isolates of avian H5N1, and 2003 human H3N2 viruses. The relatively high incidence of reassortant viruses were identified from the tissues of the upper airway in the ferret, and it was concluded that continued exposure of humans and animals to H5N1 viruses, alongside seasonal influenza viruses, increases the risk of

generating H5 subtype reassortant viruses that may be shed from upper airway secretions [37].

Plasmid-derived human 1918 pandemic H1N1 influenza virus was found to replicate to high titers in the respiratory tract of ferrets [38]. Severe disease was observed, including lethargy, anorexia, severe weight loss, and high fever. Infection was lethal in two thirds of the inoculated animals. Histological observation revealed necrotizing bronchiolitis, and moderate to severe alveolitis with edema.

The ferret model has been used to study the airborne transmission efficacy of a series of human 1918-avian H1N1 influenza reassortant viruses. In these studies, the 1918 PB2 protein was found to be both necessary and sufficient for airborne transmission of a virus expressing 1918 HA and NA. Also, it was found that influenza viruses that were able to transmit efficiently in ferrets were able to replicate efficiently at a lower temperature (33 ºC) found in the environment of the mammalian airway. These findings demonstrate that the adaptation of the HA and PB2 proteins is critical for the development of pandemic influenza strains from avian influenza viruses [39].

In a ferret pathogenesis and transmission model, the 2009 pandemic influenza H1N1 virus was found to be more pathogenic than the seasonal H1N1 virus, with more extensive virus replication occurring in the respiratory tract [40]. Replication of the seasonal H1N1 virus was confined to the nasal cavity of ferrets, while the 2009 pandemic H1N1 virus also replicated in the trachea, bronchi, and bronchioles. Virus shedding was more abundant from the upper respiratory tract for the 2009 pandemic H1N1 virus when compared with the seasonal virus, and transmission via aerosol or respiratory droplets was equally efficient [41]. The ferret was described to be a good model for testing vaccines and drugs against the 2009 pandemic H1N1 virus, and if the pandemic unfolds, for testing viral mutations with a suspected higher virulence or easier transmission [30, 40].

According to the WHO guidelines for the production and quality control of human influenza pandemic vaccines, a standard procedure for animal experiments using the ferret is provided [42-43]. In the procedure, safety testing of the candidate vaccines in ferrets is to be done with outbred ferrets aged 4-12 months that are seronegative for currently circulating influenza A and B viruses. Ferrets have been used extensively in research and are a good indicator of influenza virus virulence in humans [33]. In terms of predicting a highly pathogenic human infection or an infection that is attenuated in humans, in the absence of human data, the ferret is described as being the best available model [42].

The disadvantages of using the ferret include the expense, special housing requirements, the limited number of suppliers, the difficulty of obtaining animals that are seronegative for the influenza virus, the exquisite sensitivity of these animals to other respiratory pathogens, and their ease of acquiring infections from their handlers. In addition, there is a lack of species-specific reagents for ferrets; however, this last point does not present an obstacle for the evaluation of HAI or neutralization antibody response [7]. Casing can also be a problem because most animal facilities require that 1-3 ferrets be housed in a rabbit-style cage system [31]. Ferrets have a relatively passive temperament, although they can become fairly aggressive after being exposed to invasive procedures [31]. In addition, the high body temperature of ferrets (average temperature of 38.8 ºC) may limit their utility in the evaluation temperature-sensitive live attenuated influenza vaccines [7].

4. MOUSE MODELS

Mice have been used for influenza research since the earliest days of influenza virus biology. Shortly after the first human influenza was isolated from ferrets in 1933, it was discovered that human influenza could cause disease in mice only if the virus was first adapted to the species by serial passages in the lung. This was subsequently found to be true for all human influenza virus isolates [7, 44].

One of the most commonly used human influenza viruses in mouse studies is influenza A/Puerto Rico/8/34 (PR8) strain, an H1N1 virus with a complex history of several passages in ferrets, and hundreds of passages in eggs and mice [7, 45]. This virus is well adapted in mice and causes lethal infection. The need for adaptation through serial passage of human influenza viruses is one of the major drawbacks of using mice in influenza research because many mutations can arise during adaptation to the murine host that can alter the replication kinetics, resulting in the ability of the virus to escape innate immune responses. PR8 can, however, be used as a challenge virus to evaluate the efficacy of vaccines. Influenza viruses that cause disease and are lethal in mice can provide a useful endpoint for vaccine efficacy studies.

A number of parameters can be used to monitor influenza virus infection in mice. These include changes in body weight, decline in arterial oxygen saturation, lung weight, lung viral titer, pathology score, and mean time to death [31]. Irrespective of whether an influenza virus induces morbidity or mortality in mice, the level of replication of viruses in the lung is the most informative endpoint for efficacy studies, since even a modest reduction in titer of infectious virus in the lung can be associated with survival from lethal infection. Mice immunized with influenza viruses develop serum HAI and neutralizing antibodies, which correlates with protection from subsequent challenge.

A recent advance in influenza A modeling is the finding that the reconstructed 1918 pandemic influenza virus causes lethality in mice [46]. Isolates of the H5N1 virus recovered from human patients can cause lethal disease in BALB/c mice without prior adaptation [47].

In BALB/c mice, the 2009 pandemic H1N1 virus that have been tested replicate more efficiently than the currently circulating human H1N1 virus. Using BALB/c as a model, the 2009 pandemic H1N1 virus was found to be sensitive to both approved and experimental antiviral drugs, suggesting that these compounds can function as a first line of defense against the pandemic [40].

Murine models can be also used in the veterinary field [48] for studying equine H7N7 influenza viruses [49] and avian H9N2 influenza viruses [50].

The mouse remains the primary model for developing influenza antiviral therapy due to the low cost of purchasing and housing the animals and the general fidelity of the illness in mice to the human disease [51, 52]. Many reagents are

available to study the effect of virus replication and treatment on the mouse immune system. However, immunologically, the lack of a functional Mx gene in standard laboratory mouse strains presents a disadvantage in using this model for studies in which the innate immune response to infection is important [53-55]. Nevertheless, the variety of genetic backgrounds and targeted genetic defects make the mouse an attractive animal model for influenza research.

5. COTTON RAT MODELS

The cotton rat (*Sigmodon hispidus*) has been described as being susceptible to a wide range of infectious diseases since its use for paralytic poliovirus infection in 1939. Recently, this rodent was reported to be susceptible to many human pathogens [56], especially respiratory viral infections [57]. Because influenza A virus can be replicated and induces symptoms in the cotton rat model without adaptation of the virus [58], usefulness of this model for influenza research must be addressed.

Influenza A causes nasal and pulmonary infections in adult inbred cotton rats. Animals infected intranasally with doses of a recently isolated H3N2 influenza strain developed increased breathing rates, accompanied by weight loss and decreased body temperature [58].

Evidence of a cross-protective immune response to influenza A was demonstrated in the cotton rat model. Cross-protection from heterosubtypic influenza A challenge in cotton rats is characterized by enhanced viral clearance, protection from tachypnea, a vigorous early cellular recall response, and a reduction in bronchiolar epithelial cell damage. Identification of the mechanisms that contribute to such cross-protection in cotton rats may lead to the development of influenza vaccine strategies that are broadly protective [59].

Immunologically, cotton rats possess an intact immune system that includes an intact Mx gene, which is advantageous over the mouse model [60]. This is important for vaccine evaluation because intact immunity is required for the model to be successful. Interestingly, the pattern of macrophage cells in cotton rats is similar to that of humans in terms of NO production, which is different from other rodents [61] and means that the cotton rat has the potential for being a good rodent model for evaluating human pathogens.

The disadvantages of the cotton rat are primarily low animal availability and the aggressiveness of the species, regardless of gender. When compared with mice, there is a lack of species-specific reagents for cotton rats. Information from cross-reactive monoclonal antibodies or from measuring mRNA by real-time PCR will help with analyzing the immune response of this species.

The average temperature of the cotton rat, as measured using the telemetry system, is about 38.0 ºC, and in each individual, the body temperature fluctuates daily between about 37 and 39 ºC (Haga, Morita, Murayama, unpublished data). Temperature-sensitive live attenuated vaccines could be evaluated in the cotton rat model. These include the measles virus after intramuscular inoculation [62], and respiratory syncytial virus after intranasal administration [63]. This information may help in the design of temperature-sensitive live attenuated influenza vaccines.

6. NON-HUMAN PRIMATE MODELS

There is renewed interest in the use of non-human primates for pandemic influenza research. Non-human primates, however, have not been used extensively. Although Old and New World monkeys were evaluated as models for human influenza infection, in the early days of influenza virus biology it was determined that non-human primates were not susceptible to human influenza viruses in the way their human relatives were [7].

Cynomolgus macaques (*Macaca fascicularis*) infected with influenza virus A/Hong Kong/156/97 (H5N1) developed acute respiratory distress syndrome and fever associated with necrotizing interstitial pneumonia. Reverse transcription PCR, virus isolation, and immunohistochemistry showed that the respiratory tract is the major target of the virus [64]. Pathological observation suggests that the cynomolgus monkey is a suitable animal model for studying the pathogenesis of human H5N1 virus infection [65].

Rhesus macaques have been used to test the replication of avian H5N1 influenza viruses that were isolated from an outbreak of infection among wild birds at Qinghai Lake in China in 2005. The results of intranasal inoculation varied depending on the influenza virus isolate that was used. The Qinghai Lake viruses did not replicate efficiently in monkeys producing no evidence of disease other than transient fever, whereas the duck virus replicated in multiple organs and caused symptoms of respiratory illness [66].

The 1918 pandemic H1N1 virus causes a highly pathogenic respiratory infection in a cynomolgus macaque model, culminating in acute respiratory distress and death. Furthermore, infected animals mounted an immune response that was characterized by dysregulation of the antiviral response, which was insufficient for protection. This indicates that atypical host innate immune responses may contribute to lethality. The ability of influenza viruses to modulate host immune responses, such as that demonstrated with the avian H5N1 influenza viruses, may be a feature shared by virulent influenza viruses [67].

One of the first US isolates of 2009 pandemic H1N1 reported to replicate efficiently in cynomolgus macaques caused more severe pathological lesions in the lungs than the currently circulating human H1N1 virus [40].

From a practical standpoint, these animals are expensive and technically more demanding. However, because non-human primates are much more closely related to humans than small animals typically used to study influenza, they can be good models in the context of translational studies for evaluating therapeutic and prophylactic strategies.

7. CONCLUDING REMARKS

Each animal species provides unique features as an animal model in terms of studying influenza. None of them, however, fully reproduces human infection. When considering the evaluation of antiviral drugs in any animal model, species differences in drug pharmacology and metabolism

must be taken into account. Hence, the intelligent use of each of these models is essential. Because influenza viruses exist in nature in a variety of hosts, it is also important to accumulate data on pathogen-host relationships from both natural and experimental infections. Information obtained from animal models contributes further towards understanding the pathogenicity and control of the influenza virus, and is expected to improve the lives of both humans and animals in the future.

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