

# Pigs, Poultry, and Pandemic Influenza: How Zoonotic Pathogens Threaten Human Health

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## 1 Introduction

Emerging infections have an enormous impact on human health, food supply, economics, and the environment. Examples of pathogens causing emerging infections in humans in recent decades are Chikungunya virus, human immunodeficiency virus type 1, Ebola virus, hantavirus pulmonary syndrome virus, Hendravirus, highly pathogenic avian influenza (HPAI) H5N1 virus, Nipah virus, and SARS-coronavirus [1]. Animals, and wild animals in particular, are considered to be the source of more than 70% of all emerging infections in humans [2]. Therefore, understanding how these zoonotic pathogens—pathogens of nonhuman vertebrate animals that may be transmitted to humans under natural circumstances—emerge in the human population is critical for containing and eradicating these infections.

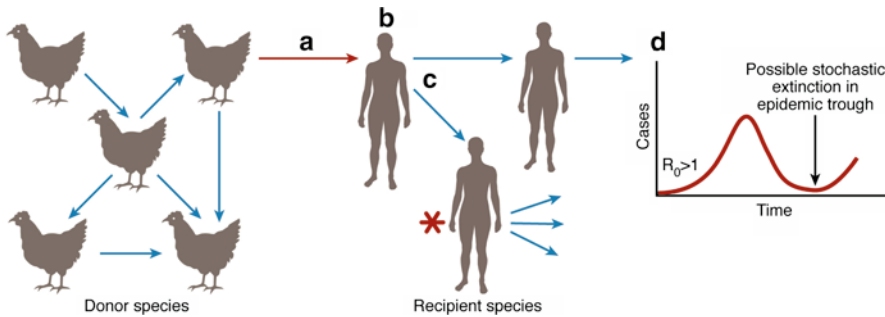
Zoonotic pathogens need to cross the host species barrier in order to become capable of infecting and being maintained in the human population. The host species barrier is not a simple concept: it consists of the interaction of factors that collectively limit the transmission of an infection from a donor species to a recipient species [3]. The process of crossing the host species barrier can be divided into four phases (Fig. 1): interspecies host-host contact between donor species and recipient species; pathogen-host interactions within an individual host of the recipient species, allowing replication and shedding of the pathogen; intraspecies host-host contact in the recipient species, allowing pathogen spread; and persistence in the recipient species population even during epidemic troughs [3].

A zoonotic pathogen that has successfully crossed the host species barrier to humans is avian influenza A virus. Wild waterbirds are considered to be the original reservoir for all influenza A viruses in poultry and mammals, including humans [4] (Fig. 2). From this reservoir, novel strains or even novel subtypes of influenza A virus may cross the host species barrier to humans. This may occur either directly from birds or indirectly via an intermediate host such as domestic swine. If such an influenza virus adapts sufficiently to its new human host to be efficiently transmitted, a pandemic may occur.

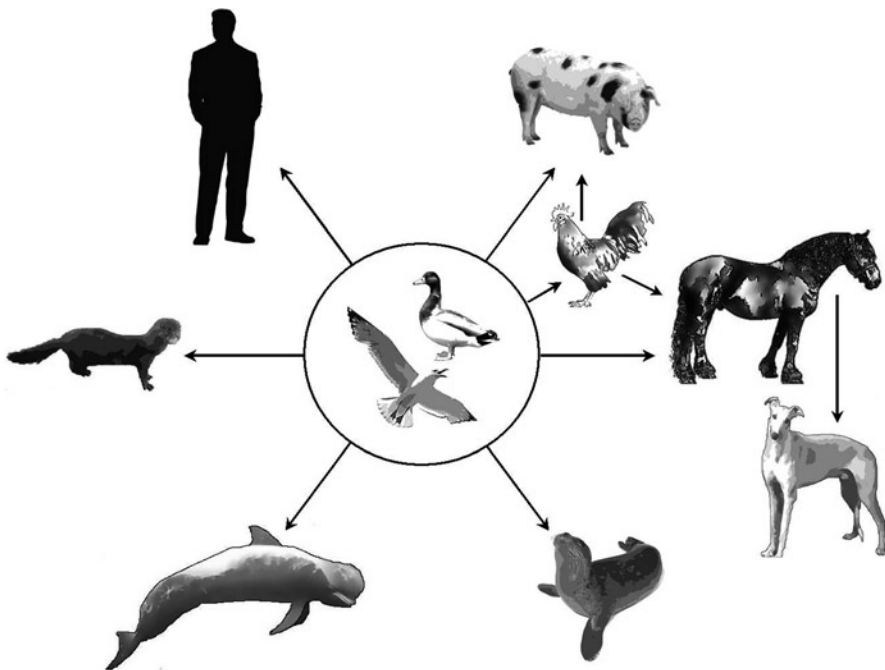
Until 2009, the two influenza A viruses circulating endemically in the human population were human influenza viruses of the subtypes H1N1 and H3N2, which caused annual epidemics every winter season. Two influenza viruses that successfully spread from animals to humans are HPAI H5N1 virus, originating from poultry, and pandemic H1N1 influenza (H1N1) virus, thought to originate from domestic swine. However, these two influenza viruses differ in a critical aspect: their

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**Fig. 1** Schematic illustrating phases in overcoming species barriers. (a) Interspecies host-host contact must allow transmission of virus from donor species to recipient species. (b) Virus-host interactions within an individual of recipient species affect the likelihood of the virus replicating and being shed sufficiently to infect another individual of recipient species. (c) Intraspecies host-host contact in recipient species must allow viral spread ( $R_0 > 1$ ) in the presence of any pre-existing immunity. Superspreader events (red asterisk) early in the transmission chain can help this process. (d) The pathogen must persist in the recipient species population even during epidemic troughs (after most susceptible individuals have had the disease) so that subsequent epidemics can be seeded: If few susceptibles are left, the virus may (stochastically) go extinct in epidemic troughs. Viral variation and evolution can aid invasion and persistence, particularly by affecting host-virus interactions. Reprinted from *Science* [3] with permission from the American Association for the Advancement of Science



**Fig. 2** Schematic representation of known events involving cross-species transmission of avian influenza viruses to mammals besides humans. Cross-species transmission of avian influenza viruses to swine, horses, harbour seals, whales and mink. The source of infection is not precisely known but is thought to be wild bird reservoirs (Anseriformes, such as ducks, and Charadriiformes, such as gulls). Poultry can become infected with avian influenza viruses and may transmit the viruses to swine and horses, when reared together. Horses have transmitted equine influenza H3N8 virus to domestic dogs. Reprinted from *Scientific and Technical Review* [11] with permission from the World Organisation for Animal Health (OIE)

human-to-human transmissibility. Between 1997 and 2010, HPAI H5N1 virus has caused 507 documented infections in humans, of whom 302 have died ([www.who.int/csr/disease/avian\\_influenza/country/cases\\_table\\_2010\\_10\\_18/en/index.html](http://www.who.int/csr/disease/avian_influenza/country/cases_table_2010_10_18/en/index.html)), but is not transmitted efficiently among humans [5]. In contrast, human-to-human transmission of pH1N1 virus is efficient: within months of the emergence of pH1N1 virus in Mexico at the beginning of 2009, it had been reported world-wide, resulting in the first human influenza pandemic of the twenty-first century.

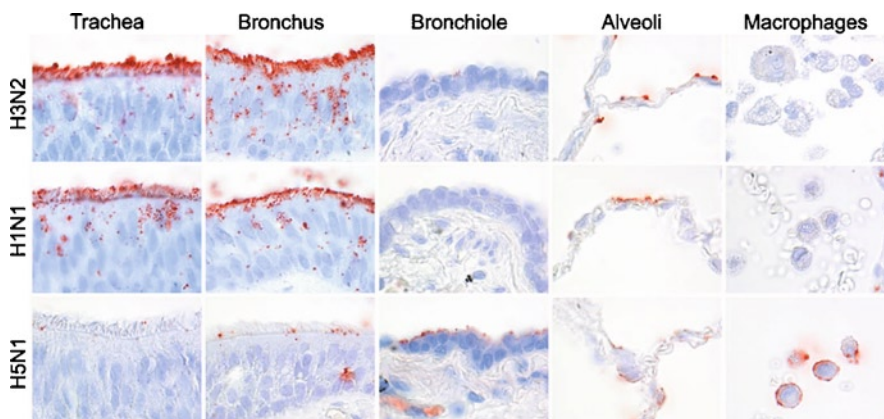
In the past 14 years, we have studied different aspects of the host species barrier for HPAI H5N1 virus and pH1N1 virus. In this review, we will discuss the host species barrier for these viruses, concentrating on three questions: how does HPAI H5N1 virus transmit from birds to humans; what are the within-host dynamics of HPAI H5N1 virus and pH1N1 virus in humans and other mammals; and what determines transmission of influenza viruses among humans.

## 2 How does HPAI H5N1 Virus Transmit from Birds to Humans?

Before HPAI H5N1 virus emerged in humans in 1997 in Hong Kong, it was generally thought that avian influenza viruses had little affinity for the human respiratory tract [6]. This was based on experimental avian influenza virus infections in humans and on studies of the human trachea. How, then, could human infection with HPAI H5N1 virus, which was genetically confirmed to be completely of avian origin, be explained?

Attachment of influenza virus to its host cell is the first step in the virus replication cycle. Because virus attachment is an important determinant of the host range that a virus can infect, we determined the pattern of HPAI H5N1 virus attachment to the human lower respiratory tract (LRT). The LRT starts at the trachea, divides progressively into bronchi and bronchioles, and ends at the pulmonary alveoli. We used a technique which we coined “virus histochemistry”: we incubated formalin-fixed, paraffin-embedded tissue sections with formalin-inactivated fluorescein isothiocyanate (FITC)-labeled influenza virus and detected virus with a peroxidase-labeled rabbit antibody to FITC that was amplified with a tyramide signal amplification system [7].

As expected from previous research, HPAI H5N1 virus did not attach to epithelial cells of the human trachea [7] (Fig. 3). However, HPAI H5N1 virus did attach to epithelial cells of bronchi and bronchioles. The virus also attached to type II pneumocytes and alveolar macrophages in the



**Fig. 3** Attachment of human (H3N2 and H1N1) and avian (highly pathogenic H5N1) influenza viruses in human lower respiratory tract. Reprinted from *American Journal of Pathology* [8] with permission from the American Society for Investigative Pathology

pulmonary alveoli. The pattern of virus attachment of HPAI H5N1 virus contrasted with that of the seasonal human influenza viruses of the subtypes H1N1 and H3N2 [8]. These viruses attached abundantly to epithelial cells of the human trachea and bronchi, and less abundantly to epithelial cells of bronchioles. Within the pulmonary alveoli, they attached predominantly to type I pneumocytes and not to alveolar macrophages.

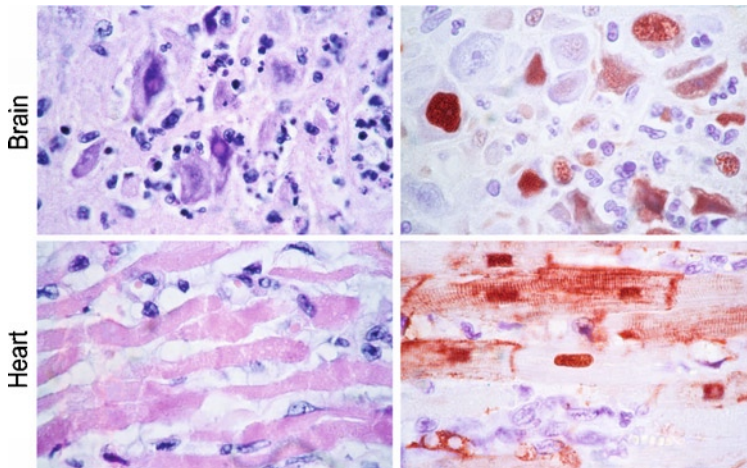
The results from these studies demonstrated for the first time that an avian influenza virus was able to attach to cells of the human respiratory tract, a prerequisite for virus infection. Also, the difference in pattern of virus attachment between HPAI H5N1 virus and seasonal human H1N1 and H3N2 influenza viruses fits with the difference in primary disease presentation: tracheo-bronchitis for seasonal human influenza viruses, and diffuse alveolar damage for HPAI H5N1 virus infection.

### **3 What are the Within-Host Dynamics of HPAI H5N1 Virus and pH1N1 Virus in Humans and Other Mammals?**

Knowledge of the within-host dynamics of an emerging pathogen in a new host species are not only important to understand virus shedding, but also to improve diagnosis and to assess pathogenicity. The within-host dynamics of HPAI H5N1 virus infection were of specific interest because of the high case fatality rate and unusual clinical presentation of human cases, as well as the wide range of mammalian host species in which HPAIV H5N1 virus infection causes severe disease. The within-host dynamics of pH1N1 virus infection were of specific interest to assess the severity of the influenza pandemic.

HPAI H5N1 virus infection in humans is unusual because it can cause different clinical symptoms than expected from a virus traditionally associated with respiratory tract infections. This is perhaps best illustrated by a case report of a child who died of HPAI H5N1 virus infection in Vietnam [9]. This child presented with severe diarrhea but no apparent respiratory illness, followed by rapidly progressive coma, leading to a clinical diagnosis of acute encephalitis. HPAI H5N1 virus was isolated from cerebrospinal fluid, fecal, throat, and serum specimens. The sibling of this child died of a similar illness, although the lack of clinical specimens did not allow diagnosis. Separately, another patient with HPAI H5N1 virus was described with an initial presentation of fever and diarrhea alone [10]. These cases emphasize that clinical surveillance of HPAI H5N1 virus infections should focus not only on respiratory illnesses, but also on clusters of unexplained deaths or severe illnesses of any kind [9].

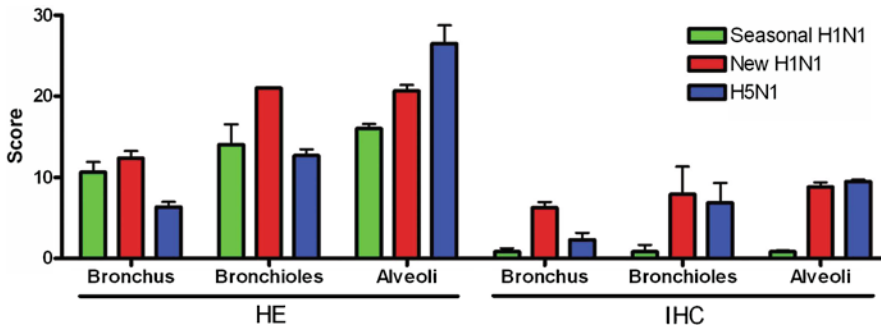
HPAI H5N1 virus infection in mammal species other than humans also cause extra-respiratory disease. Other mammal species that have been reported dead from natural HPAI H5N1 virus infection include tigers (*Panthera tigris*), leopards (*P. pardus*), domestic cats, domestic dogs, Owston's palm civets (*Chrotogale owstoni*), a stone marten (*Mustela foinea*), and an American mink (*M. vison*) [11]. At necropsy of these animals, evidence for lesions associated with HPAI H5N1 virus infection were found not only in the lungs but also in multiple extra-respiratory organs, including brain and liver [11]. In order to examine the extra-respiratory spread of HPAI H5N1 virus infection in mammals more carefully, we experimentally infected domestic cats with HPAI H5N1 virus and examined them by virological and pathological assays 7 days after inoculation [12, 13]. Severe necrosis and inflammation were present in lungs, brain, heart, kidneys, liver, and adrenal glands (Fig. 4). The presence of these pathological changes co-localized with the expression of influenza virus antigen in parenchymal cells of these organs (Fig. 4). (Interestingly, experimental infections of domestic cats with other influenza viruses, HPAI H7N7 virus [14] or pH1N1 virus [15], did not extend beyond the respiratory tract.) This study demonstrates that HPAI H5N1 virus causes systemic disease in domestic cats, with results corresponding in part to the findings of the above-described non-typical cases of fatal HPAI H5N1 virus infection in humans.



**Fig. 4** Cats infected with influenza A virus (H5N1) have lesions associated with virus replication in multiple tissues, including brain and heart. Necrotizing and inflammatory changes are seen (*left* column). Serial sections of these tissues (*right* column) show that these lesions are closely associated with the expression of influenza virus antigen. Tissues were stained either with hematoxylin and eosin (*left* column) or by immunohistochemistry using a monoclonal antibody against the nucleoprotein of influenza A virus as a primary antibody (*right* column). Reprinted from *American Journal of Pathology* [13] with permission from the American Society for Investigative Pathology

When pH1N1 virus emerged in Mexico early in 2009 and started spreading in humans across the world, there was major concern about the level of disease burden and mortality that it would cause. In order to assess the pathogenesis and pathogenicity of pH1N1 virus, we performed experimental infections in ferrets, which are considered one of the most suitable laboratory animal species to model influenza in humans. In these ferrets, we compared the within-host dynamics of pH1N1 virus with that of seasonal human H1N1 virus, which is well-adapted to its human host, and of HPAI H5N1 virus, which is known to cause a high case fatality rate in humans [16]. Our results showed seasonal human H1N1 virus and pH1N1 virus were restricted mainly to the respiratory tract, while HPAI H5N1 virus also replicated extensively in extra-respiratory tissues. Also, we found that the severity of pneumonia and cumulative mortality rate of ferrets infected with pH1N1 virus was intermediate between that for seasonal H1N1 virus and HPAI H5N1 virus infection. Interestingly, influenza virus antigen expression in the pH1N1 virus group was high at all 3 levels of the lower respiratory tract (alveoli, bronchioles, and bronchi) (Fig. 5). This was associated with histopathological changes of diffuse alveolar damage, bronchiolitis, and bronchitis, respectively (Fig. 5). This contrasted with the influenza virus antigen expression in the HPAI H5N1 virus group, which was highest in alveoli, and lower in bronchioles and bronchi; and with that in the seasonal H1N1 virus group, which was low at all three levels. The results of this study suggest that pH1N1 virus has the intrinsic ability to cause more severe pneumonia than seasonal H1N1 virus. This corresponds with the results of other experimental studies in mice, ferrets, and macaques [17–19].

The pathogenesis of pH1N1 virus infection as described in above studies with laboratory animals corresponded with the pathogenesis of pH1N1 virus infection in humans. In a pathological study of 100 fatal human cases of pH1N1 virus infection, marked differences in viral tropism and tissue damage were observed compared with seasonal influenza virus infection and HPAI H5N1 virus infection [20]. In that study, patients with fatal pH1N1 virus infection not only had diffuse alveolar damage associated with the presence of viral antigen in the alveoli, a pattern somewhat similar to patients with fatal HPAI H5N1 virus infection, but also had viral localization along with inflammation and other histopathological changes in trachea, bronchi, and bronchioles, a pattern more commonly seen in severe or fatal cases of seasonal influenza.



**Fig. 5** Histological and immunohistochemical scoring in the lungs of ferrets inoculated with different influenza viruses. Histological scoring of samples stained with hematoxylin and eosin (HE) showed that the alveolar lesions in the new H1N1 virus (synonym for pH1N1 virus) group were intermediate in severity between those of the seasonal H1N1 virus group and the highly pathogenic avian influenza (HPAI) H5N1 virus group, and that the bronchiolar lesions in the new H1N1 virus group were the most severe of all three groups. Scoring of the immunohistochemical analysis (IHC) showed that influenza virus antigen expression in the new H1N1 virus group was high in alveoli, bronchioles, and bronchi. In the HPAI H5N1 virus group, the scores were highest for alveoli and lower in bronchioles and bronchi. In the seasonal H1N1 virus group, scores were low at all three levels. Reprinted from *Journal of Infectious Diseases* [16] with permission from Oxford University Press

#### 4 What Determines Transmission of Influenza Viruses Among Humans?

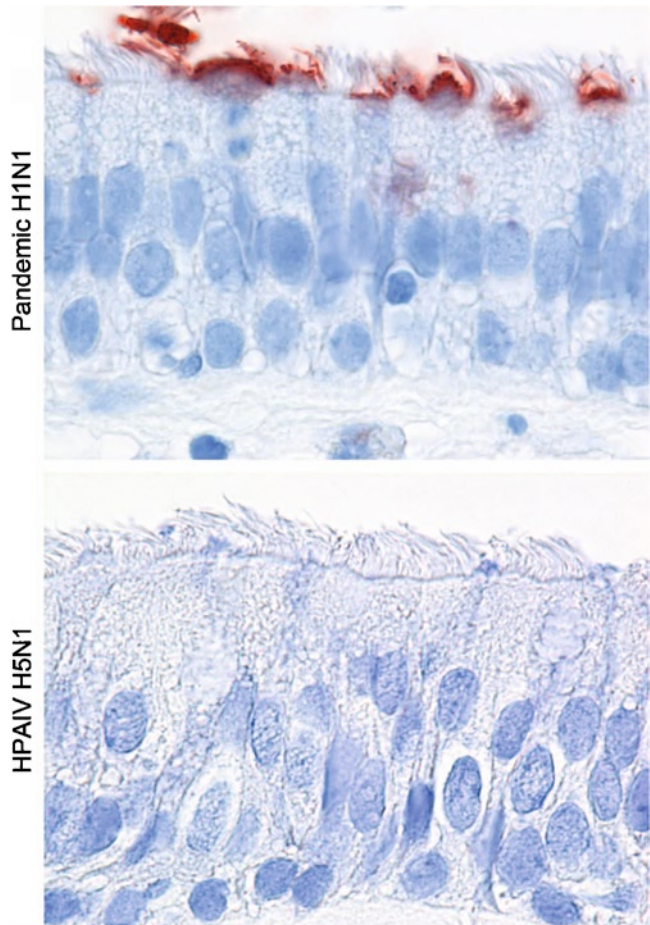
To cause a pandemic, a zoonotic influenza virus must not only be able to cross the species barrier from animals to humans, but also to transmit efficiently among humans. pH1N1 virus, which is thought to originate from domestic swine, is transmitted efficiently among humans; in contrast, HPAIV H5N1, originating from poultry, is not. The factors determining these differences in transmission are poorly understood. One factor that may be important is tropism of the influenza virus for the human upper respiratory tract (URT) [8, 21, 22]. However, there is no consensus on this [23].

To address this question, we used virus histochemistry to compare the pattern of attachment to human URT of influenza viruses with inefficient or efficient human-to-human transmission. We used avian influenza viruses, including HPAI H5N1 virus, to represent inefficiently transmitted viruses; and seasonal human H1N1 virus, seasonal human H3N2 virus, and pH1N1 virus to represent efficiently transmitted viruses [24]. We found that the seasonal human influenza viruses and pH1N1 virus attached abundantly to epithelial cells throughout the human URT. In contrast, the avian influenza viruses, including HPAI H5N1 virus, attached only rarely [24] (Fig. 6). These results indicate that the ability of an influenza virus to attach to human URT epithelium is a critical factor for efficient transmission in the human population.

#### 5 Conclusions

Populations of both wild and domestic animals form a vast reservoir of influenza A viruses, and provide ample opportunity for these viruses to reassort and mutate. The human population is therefore permanently at risk of becoming infected with new variants of influenza virus from this animal reservoir. To contain and eradicate such infections requires not only strategic virus surveillance of both animal and human populations, but also a better understanding of the hurdles that a zoonotic influenza virus needs to jump over in order to cross the species barrier and cause a human pandemic. Advances in these two areas will allow us to better predict the risk of emergence of zoonotic influenza viruses in the human population.

**Fig. 6** Attachment of pandemic influenza H1N1 virus (*upper panel*) and highly pathogenic avian influenza H5N1 virus (*lower panel*) to ciliated epithelial cells in the human upper respiratory tract. Reprinted from *American Journal of Pathology* [24] with permission from the American Society for Investigative Pathology



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