



## The pig as an intermediate host for influenza A viruses between birds and humans

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### Abstract

Swine influenza (SI) was first observed at the time of the pandemic in humans in 1918, and since that time, subtypes H1N1 and H3N2 have been widely reported in pigs, frequently associated with respiratory disease. These include classical swine H1N1, avian-like H1N1 and human- and avian-like H3N2 viruses. Swine husbandry practices influence directly the evolution of SI viruses through reduced immune pressure and constant availability of susceptible hosts. The pig has been the leading contender for the role of intermediate host for reassortment of influenza A viruses, since they are the only mammalian species which are susceptible to, and allow productive replication of avian and human influenza viruses due to the presence of receptors for both virus types ( $\alpha 2,3$  and  $\alpha 2,6$ , respectively), and this can result in modification of the receptor binding specificities of avian influenza viruses from  $\alpha 2,3$  to  $\alpha 2,6$  linkage, thereby providing a potential link from birds to humans. Several independent introductions of avian viruses to pigs have occurred, primarily involving H1N1 that has led to the establishment of stable lineages, but more recently with H4N6 and H9N2 viruses. Although H3N2 viruses related closely to early human strains continue to circulate long after their disappearance from the human population, there have also been frequent transmissions of the prevailing human strains and these may also be able to persist. In addition, reassortant viruses of H3N2 and H1N2 subtype derived from mixed lineages and usually containing genes encoding surface glycoproteins from human viruses have been reported widely in pigs. Numerous transmissions of virus from pigs to humans have occurred but without apparent secondary spread. Pigs may be important in the generation of 'new' strains, some of which may have the potential to transmit to other species including humans. Crown Copyright © 2001 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Swine; Reassortment; Transmission

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## 1. The potential role of the pig

Given the worldwide interaction between humans, pigs, birds and other mammalian species, there is a high potential for cross-species transmission of influenza viruses in nature. Pigs are an important host in influenza virus ecology since they are susceptible to infection with both avian and human influenza A viruses, often being involved in interspecies transmission, facilitated by regular close contact with humans or birds. Pigs serve as major reservoirs of H1N1 and H3N2 influenza viruses, and the maintenance of these viruses in pigs and the frequent introduction of viruses from other species may be important in the generation of ‘new’ strains of influenza, some of which may have the potential to transmit to other species including humans.

The pig has been the leading contender for the role of intermediate host for influenza A viruses. Pigs are the only mammalian species, which are domesticated, reared in abundance and are susceptible to, and allow productive replication of avian influenza viruses. This susceptibility is due to the presence of both  $\alpha 2,3$ - and  $\alpha 2,6$ -galactose sialic acid linkages in cells lining the pig trachea which can result in modification of the receptor binding specificities of avian influenza viruses from  $\alpha 2,3$  to  $\alpha 2,6$  linkage [1], which is the native linkage in humans, thereby providing a potential link from birds to humans.

It has been shown that humans occasionally contract influenza viruses from pigs. The internal protein genes of human influenza viruses share a common ancestor with the genes of some swine influenza viruses. A number of authors have proposed the nucleoprotein (NP) gene as a determinant of host range which can restrict or attenuate virus replication [2–4], thereby controlling the successful transmission of virus to a ‘new’ host. These observations support the potential role of the pig as a mixing vessel of influenza viruses from avian and human sources. The pig appears to have a broader host range in the compatibility of the NP gene in reassortant viruses [2] than both humans and birds. Studies by Kida et al. [5], investigating experimentally the growth potential of a wide diversity of avian influenza viruses in pigs, indicate that these viruses (including representatives of subtypes H1 to H13), with or without HA types known to infect humans, can be transmitted to pigs. Therefore, the possibility for the introduction of avian influenza virus genes to humans via pigs could occur. Furthermore, these studies showed that avian viruses, which do not replicate in pigs, can contribute genes in the generation of reassortants when coinfecting pigs with a swine influenza virus.

## 2. Pigs in the ‘influenza epicentre’

The majority of pandemic strains has apparently originated in China raising the possibility that this region is an influenza epicentre. In China, influenza viruses of all subtypes are prevalent in ducks and in water frequented by ducks. This region accounts for over 60% of the world pig population, and agricultural practices provide that there is close contact between wild aquatic birds, domestic ducks, pigs and humans, thereby presenting the opportunity for interspecies transmission and genetic exchange among influenza viruses, with the pig acting as an intermediary between domestic ducks and humans. However, the interface between pigs and other species,

particularly humans, is also significant in many other parts of the world (see pig to human transmission).

### **3. Transmission of avian influenza viruses to pigs**

In Eurasia, there have been several introductions of avian H1N1 viruses to pigs that have led to the establishment of stable lineages. These viruses have spread widely in pigs in this region and are often associated with disease epizootics [6,7]. Recently, H9N2 viruses have apparently been introduced to pigs in South-East Asia, possibly from poultry, although the potential of these viruses to spread and persist in pigs remain unknown. In 1999, an avian H4N6 virus was isolated from pigs in Canada with respiratory symptoms. There was local spread on the farm, which was located next to an area of open water where waterfowl congregate. The genotype of the virus was entirely avian, although there were some modifications in the receptor binding pocket on the HA gene which may have facilitated binding to receptors with  $\alpha 2,6$  linkage [8].

### **4. Genetic reassortment in the pig**

#### *4.1. Eurasia*

Evidence for the pig as a mixing vessel of influenza viruses of non-swine origin has been demonstrated in Europe by Castrucci et al. [9], who detected reassortment of human and avian viruses in Italian pigs. Phylogenetic analyses of human H3N2 viruses circulating in Italian pigs revealed that genetic reassortment had been occurring between avian and ‘human-like’ viruses since 1983 [9]. The unique co-circulation of influenza A viruses within European swine may lead to pigs serving as a mixing vessel for reassortment between influenza viruses from mammalian and avian hosts with unknown implications for both humans and pigs. It would appear that human H1 viruses are able to perpetuate in pigs following genetic reassortment. Furthermore, these viruses may be maintained in pigs long after one or both of the progenitor viruses have disappeared from their natural hosts. Reassortant viruses of H1N2 subtype derived from human and avian viruses [10] or H1N7 subtype derived from human and equine viruses [11] have been isolated from pigs in Great Britain. The H1N2 viruses derived from a multiple reassortant event, spreading widely within pigs in Great Britain and subsequently to other parts of Europe. Viruses of H1N2 subtype have also been derived from genetic reassortment of strains endemic in pigs, and have been established in pigs in Japan since 1978 [12,13]. Similar viruses were detected in France but apparently failed to become established [14].

#### *4.2. North America*

Since 1998, H3N2 viruses isolated from pigs in the USA have contained combinations of human, swine and avian genes. Furthermore, the HA gene of these viruses was derived from a human virus circulating in the human population in 1995 [15]. These

newly emerged H3N2 viruses appear to be established in pigs in North America and are able to reassort with classical H1N1 viruses producing another unique genotype of H1N2 virus [16].

## **5. Transmission between pigs and humans**

Transmission of influenza A viruses from pigs to humans occurs on a regular basis, however, subsequent transmission of these viruses within the human population is very rare. Serological studies of people having occupational contact with pigs support frequent transmissions through the detection of antibodies to swine influenza viruses. Pigs were implicated as the source of infection when an H1N1 virus was isolated from a soldier who had died of influenza at Fort Dix, NJ, USA. The virus was identical to viruses isolated from pigs in the USA. Furthermore, five other servicemen were shown to be infected by virus isolation, and serological evidence suggested that some 500 personnel at Fort Dix were, or had been, infected with the same virus [17]. Subsequently, there have been several reports of classical swine H1N1 influenza virus being isolated from humans with respiratory illness, occasionally with fatal consequences. All cases examined followed contact with sick pigs. Perhaps of greater significance for humans is a report of two distinct cases of infection of children in the Netherlands during 1993 with H3N2 viruses whose genes encoding internal proteins were of avian origin [18]. Genetically and antigenically related viruses had been detected in European pigs [9], raising the possibility of potential transmission of avian influenza virus genes to humans following genetic reassortment in pigs. These concerns were substantiated further by the results of serological studies in Italy, which indicated that these ‘swine’ H3N2 viruses had apparently been transmitted to young, immunologically naive persons [19].

The prevailing strains in the human population transmit to pigs frequently but it would appear that only H3N2 viruses can establish stable lineages without genetic reassortment. Human H3N2 viruses are endemic in most pig populations worldwide, where they persist many years after their antigenic counterparts have disappeared from humans, and therefore, present a reservoir of virus which may in the future infect a susceptible human population. However, there is no apparent evidence of pigs being infected with this subtype prior to the pandemic in humans in 1968.

## **6. Adaptation of ‘new’ influenza viruses to pigs**

Following interspecies transmission and/or genetic reassortment, an influenza virus may undergo many pig-to-pig transmissions because of the continual availability of susceptible pigs. The mechanisms whereby an avian virus is able to establish a new lineage in pigs remain unclear, although following the introduction of an avian virus into European pigs in 1979, the virus was relatively unstable for approximately 10 years [20], but the mutation rate of this virus did not subsequently increase [21]. Furthermore, adaptation of this virus to pigs resulted in the virus acquiring altered receptor specificity, preferentially recognizing receptors with  $\alpha 2,6$  linkage [1], the native linkage in humans

(see Role of the pig). It would appear that the adaptive processes can take many years as occurred, following transmission of both avian H1N1 and human H3N2 viruses to pigs. Following new introductions of influenza A virus to pigs, close monitoring of the epizootiology of SI in a population is essential to determine the rate of change, which, if elevated, may facilitate further transmissions across the species barrier with potential implications for disease control in a range of other species including humans.

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