

THE MOLECULAR EPIDEMIOLOGY AND EVOLUTION OF INFLUENZA VIRUSES IN PIGS

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Introduction

Swine influenza (SI) is a highly contagious acute viral disease of the respiratory tract in pigs that is distributed worldwide. The disease is economically damaging, primarily due to weight loss and reduced weight gain. In the UK the financial loss resulting from reduced weight gain in finishing pigs alone due to SI has been estimated at approximately £7 per pig, equivalent to a total loss in the UK per annum of £60 million. Increasingly SI is considered one of the most important primary pathogens of swine respiratory disease worldwide. There are significant variations in virus epidemiology in different continents and regions together with approaches to swine husbandry and disease control.

Reservoirs of influenza A viruses

Influenza A viruses infect a large variety of animal species including humans, pigs, horses, sea mammals and birds. Aquatic birds are known to be the source of all influenza viruses for other species. 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. Phylogenetic studies of influenza A viruses have revealed species-specific lineages of viral genes. The evolution of influenza genes in species-specific gene lineages is an invaluable characteristic in studying influenza virus epidemiology.

The methods of swine husbandry make it likely that the virus is maintained by continual passage to young susceptible pigs. Swine husbandry practises influence directly the evolution of influenza viruses in pigs leading generally to reduced genetic drift, particularly in the genes encoding haemagglutinin (HA) and neuraminidase (NA), compared to those of similar viruses in the human population.

Epidemiology

Influenza A viruses of subtypes H1N1, H3N2 and H1N2 have been reported widely in pigs, associated frequently with clinical disease. These include classical swine H1N1, 'avian-like' H1N1 and 'human'- or 'avian-like' H3N2 viruses and H1N2 viruses of numerous origins. These viruses have remained largely endemic in pig populations worldwide. Although usually regarded as an endemic disease, epidemics may result when influenza infection occurs in an immunologically naive population (which can be linked to significant antigenic drift) or through exacerbation by a variety of factors such as poor husbandry, secondary bacterial or viral infections and cold weather. Serosurveillance results in Great Britain indicated that more than half of adult pigs in the national population had been infected with one or more influenza A viruses during their lifetime [1].

Classical H1N1

Classical swine influenza viruses, or their antibodies, have been reported from many parts of the world including North and South America, Europe, Asia, and Africa [reviewed 2].

'Human-like' viruses

Infections of pigs with the prevailing human subtypes also occur under natural conditions. Since 1984, outbreaks of clinical influenza in pigs due to these viruses have been observed throughout Europe with infections frequently characterised by high seroprevalence. In 1998 a novel H3N2 virus emerged in the USA, following genetic reassortment of avian, human and swine viruses and spread widely [3].

'Avian-like' viruses

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. Since 1979 these viruses have become the dominant strain in European pigs. In addition, some of the H3N2 viruses isolated from pigs in Asia since the 1970's have been entirely 'avian-like'.

H1N2 viruses

Influenza A H1N2 viruses, derived from classical swine H1N1 and 'human-like' swine H3N2 viruses have been isolated in Japan and France but viruses of this genotype have not persisted within European pigs. Subsequently an H1N2 influenza virus (*see* Genetic reassortment) related antigenically to human and 'human-like' swine viruses has emerged and become endemic in pigs in Europe [4,5]. In North America another unique genotype of H1N2 virus has appeared and become established in recent years [6].

Emergence and variation of influenza viruses in pigs

Emergence of new strains or modifications to existing viruses has occurred in pigs by three mechanisms (i) an influenza A virus from another species transmitting *in-toto* to pigs. (ii) an influenza virus undergoing antigenic change or drift as a result of accumulating mutations with time in the genes encoding the major viral antigens (iii) co-infection of a pig with two unrelated influenza A viruses can result in the production of a new virus derived by genetic mixing of the progenitor strains leading to the potential emergence of a new virus with different antigenic and genetic characteristics.

Interspecies transmission of virus to pigs

In Europe, avian H1N1 viruses that were transmitted to pigs established a stable lineage, spread widely causing significant economic losses. All of the gene segments of the prototype viruses were of avian origin [7] indicating that transmission of a whole avian virus into pigs had occurred. Phylogenetic analysis of the genes of these viruses has revealed that they have retained an entirely avian genetic composition throughout their maintenance

in pigs. Influenza A viruses of H3N2 subtype related closely originally to a human strain from 1973, continue to circulate widely in pigs long after their disappearance from the human population [1,2]. The appearance of a H3N2 subtype variant strain in the pig population of a country appears to coincide with the epidemic strain infecting the human population at that time. Recently, H9N2 viruses have apparently been introduced to pigs in South-East Asia, most probably from land based poultry [8]. Currently there is no clear evidence supporting their independent maintenance in pigs through pig to pig transmission. In 1999, an avian H4N6 virus was isolated from pigs in Canada with respiratory symptoms but there was no apparent spread [9]. The potential of avian viruses novel to pigs including H9N2 or H4N6, to spread and persist within pigs remains unknown, substantiating the need for good surveillance of swine populations worldwide.

Genetic reassortment

The pig has been the leading contender for the role of intermediate host for influenza A viruses. Pigs are the only mammalian species that are domesticated, reared in abundance and are susceptible to, and allow productive replication of avian and human influenza viruses. 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. Continued co-circulation of influenza A viruses in pigs can result in the production of new reassortant viruses. This is an ongoing process with frequent genetic exchange between co-circulating variants of the same virus that may give rise to 'new' viral genotypes with the potential for spread including to other species. Evidence for the pig as a mixing vessel of influenza viruses of non swine origin was first demonstrated by Castrucci et al. [10], who detected reassortment of human and avian viruses in Italian pigs. Further evidence for the emergence of new strains that are able to spread widely in pigs following genetic reassortment was the appearance of H1N2 virus in Great Britain in 1994 before apparent spread to the rest of Europe. The H1N2 viruses derived from a multiple reassortant event over a number of years involving human H1N1, 'human-like' swine H3N2 and 'avian-like swine' H1N1 [11]. Since 1998 H3N2 viruses isolated from pigs in the USA have contained combinations of human, swine and avian genes. Strains have possessed significant antigenic and genetic heterogeneity due to the acquisition of HA genes from the prevailing human H3N2 viruses [12] rather than through independent genetic drift in swine viruses. These newly emerged triple reassortant H3N2 viruses are now well established in pigs in North America and have reassorted with classical H1N1 viruses producing another unique genotype of H1N2 virus that has subsequently spread within pigs in this region [6].

Genetic variation

Following transmission to pigs influenza virus genes evolve in the pathway of the host of origin but diverge forming a separate sublineage. Genes that code for the surface proteins HA and NA, are subjected to the highest rates of change. Current epidemic strains are clearly

distinguishable from the prototype strains. The HA gene of both the classical and 'avian-like' swine H1N1 viruses is undergoing genetic drift, being more marked in the latter. The more limited antigenic variation in the HA gene of swine viruses is probably due to the lack of significant immune selection in pigs because of the continual availability of nonimmune pigs.

'Human-like' swine H3N2 viruses appear to be evolving independently in different lineages to those of human and avian strains [13,14]. The rates of genetic drift in HA and NA genes is equivalent to those of H3N2 viruses in the human population but in contrast to the latter the changes are not generally associated with antigenic sites.

Adaptation of 'new' influenza viruses to pigs

The mechanisms whereby a virus from another host species is able to establish a new lineage in pigs remains unclear, although following the introduction of an avian virus into European pigs in 1979 the virus was relatively unstable for approximately ten years. Furthermore, adaptation of this virus to pigs resulted in the virus acquiring altered receptor specificity, preferentially recognising receptors with $\alpha 2,6$ linkage [15], the native linkage in humans. The avian H4N6 and H9N2 viruses detected recently in pigs had some modifications in the receptor binding pocket on the HA gene which may have facilitated binding to receptors with $\alpha 2,6$ linkage [8,9]. Furthermore, the continual genetic exchange between viruses is likely to result in the emergence of 'genetic variants' with a higher fitness and therefore potential selective advantage. 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.

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