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Veterinary Microbiology 74 (2000) 29–46

veterinary microbiology

www.elsevier.com/locate/vetmic

The epidemiology and evolution of influenza viruses in pigs

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Abstract

Pigs serve as major reservoirs of H1N1 and H3N2 influenza viruses which are endemic in pig populations world-wide and are responsible for one of the most prevalent respiratory diseases in pigs. The maintenance of these viruses in pigs and the frequent exchange of viruses between pigs and other species is facilitated directly by swine husbandry practices, which provide for a continual supply of susceptible pigs and regular contact with other species, particularly humans. The pig has been a contender for the role of intermediate host for reassortment of influenza A viruses of avian and human origin since it is the only domesticated mammalian species which is reared in abundance and is susceptible to, and allows productive replication, of avian and human influenza viruses. This can lead to the generation of new strains of influenza, some of which may be transmitted to other species including humans. This concept is supported by the detection of human-avian reassortant viruses in European pigs with some evidence for subsequent transmission to the human population. Following interspecies transmission to pigs, some influenza viruses may be extremely unstable genetically, giving rise to variants which could be conducive to the species barrier being breached a second time. Eventually, a stable lineage derived from the dominant variant may become established in pigs. Genetic drift occurs particularly in the genes encoding the external glycoproteins, but does not usually result in the same antigenic variability that occurs in the prevailing strains in the human population. Adaptation of a 'newly' transmitted influenza virus to pigs can take many years. Both human H3N2 and avian H1N1 were detected in pigs many years before they acquired the ability to spread rapidly and become associated with disease epidemics in pigs.

Keywords: Pigs; Influenza; Epidemiology; Evolution

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0378-1135/00/$ – see front matter
PII: S0378-1135(00)00164-4

1. Reservoirs of influenza A viruses

Influenza A viruses infect a large variety of animal species (Alexander, 1982; Webster et al., 1992) including humans, pigs, horses, sea mammals and birds. Given the world-wide interaction between humans, pigs, birds and other mammalian species, there is a high potential for cross-species transmission of influenza viruses in nature. Phylogenetic studies of influenza A viruses have revealed species-specific lineages of viral genes and have demonstrated that the prevalence of interspecies transmission depends on the animal species (Webster et al., 1992). 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. Following the transmission and independent spread of avian or human influenza A viruses to pigs, these viruses are generally referred to as 'avian-like' swine or 'human-like' swine, reflecting their previous host, and following genetic reassortment with other influenza A viruses, some of the genes of these viruses may be maintained in the resulting progeny viruses. Therefore, the evolution of influenza genes in species-specific gene lineages is an invaluable characteristic in studying influenza virus epidemiology.

2. Early history of swine influenza

Swine influenza (SI) was first observed in 1918 in the US, Hungary and China (Chun, 1919; Koen, 1919; Beveridge, 1977). It coincided with an influenza pandemic in humans, which was the most severe of modern times, accounting for at least 20 million deaths world-wide. Those who first noticed the disease in pigs, recognised similarities between the porcine and human disease and suggested they had a common aetiology. Later, retrospective serological investigations confirmed that the disease in humans and pigs had been caused by closely related influenza A viruses in both cases. The causative agent was an H1N1 influenza A virus which had possibly derived from a common ancestor (Gorman et al., 1991; Kanegae et al., 1994; Reid et al., 1999). Genetic sequencing studies of the haemagglutinin (HA) gene of the human virus revealed that the virus most probably spread from humans to pigs and is supported by observations from veterinarians who did not describe the disease in pigs until just after its appearance in humans. Although the disease in pigs was described during the following years (Dorset et al., 1922; McBryde, 1927), it was not until 1930 that the virus was isolated and identified (Shope, 1931).

3. Epidemiology

Influenza A viruses of subtypes H1N1 and H3N2 have been reported widely in pigs, associated frequently with clinical disease. These include classical swine H1N1, 'avian-like' H1N1 and 'human'- and 'avian-like' H3N2 viruses (see Table 1). These viruses have remained largely endemic in pig populations world-wide and have been responsible for one of the most prevalent respiratory diseases in pigs. Although usually regarded as an

Table 1
Phenotypes of influenza A viruses infecting pigs endemically world-wide

Subtype	Location	Comments
H1N1	North America, Europe, Asia, South America	'Classical' virus, first isolated in 1930 in North America
	Europe	'Avian-like' virus, first isolated in 1979
	Asia	'Avian-like' virus, first isolated in 1993
H3N2	Asia, Europe, North America, South America, Africa	'Human-like' virus, first isolated in 1970 in Asia
	Asia	'Avian-like' virus, first isolated in 1978
H1N2	Asia	Classical/'human-like' reassortant in Japan
	Europe	Human/'human-like' reassortant in Great Britain

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, including 14% of pigs which had been infected with influenza viruses of both human and swine origin (Brown et al., 1995b).

3.1. Classical H1N1

Following the reported occurrence of influenza in pigs at the time of the 1918 pandemic, SI was for a long time apparently confined to the north and mid west of the US, where, after its first appearance, annual outbreaks occurred during the winter months. These and viruses related closely are termed classical viruses. Elsewhere SI was observed much later with the situation being frequently complicated by the association of other agents with respiratory disease. However, classical swine influenza viruses, or their antibodies, have been reported from many parts of the world including Canada (Morin et al., 1981) Brazil (Cunha et al., 1978), Hong Kong (Yip, 1976), Japan (Yamane et al., 1978), India (Das et al., 1981), China, Taiwan (Shortridge and Webster, 1979), Kenya (Scott, 1957) and Iran (Samadieh and Shakeri, 1976). In Europe, virus isolations were made in the UK (Blakemore and Gledhill, 1941a, b) and Czechoslovakia (Harnach et al., 1950), whilst at this time antibodies to H1N1 influenza viruses were demonstrated in pigs in the Federal Republic of Germany (Kaplan and Payne, 1959). After these episodes the virus apparently disappeared from these countries and there was no evidence of infections in Europe for nearly 20 years, until 1976 when classical swine influenza virus was isolated from disease outbreaks in northern Italy. The viruses isolated were related closely to classical swine influenza virus from the US (Nardelli et al., 1978), and it is probable

that the virus was introduced via imported pigs from the US. The infection was limited to northern Italy until 1979, when SI caused by classical swine influenza virus was reported from Belgium (Bironi et al., 1980; Vandeputte et al., 1980) and France (Gourreau et al., 1980). The disease spread rapidly to other European countries and has been reported from The Netherlands (Masurel et al., 1983), Germany (Ottis and Bachmann, 1981; Witte et al., 1981), Denmark (Sorensen et al., 1981), Sweden (Martinsson et al., 1983; Abusugra et al., 1987) and the UK (Roberts et al., 1987). This virus became endemic in pigs throughout Europe with a seroprevalence of 20–25% (Zhang et al., 1989; Brown et al., 1995b) but following the emergence of 'avian-like' H1N1 virus its continued circulation throughout Europe is uncertain.

Easterday (1980a) considered that the natural history of SI has remained largely stable for a period of at least 60 years, the virus being maintained relatively unchanged both antigenically and genetically through this period of time. Serological studies of pigs in the US have shown that classical swine H1N1 influenza virus was prevalent throughout the pig population, with approximately 25% of fattening pigs having evidence of infection (Hinshaw et al., 1978), whilst amongst the longer lived breeding population this figure rises to 45% (Easterday, 1980b). Marked regional variation in prevalence has been demonstrated and in north-central US an average prevalence of 51% has been reported (Chambers et al., 1991). In Asia, classical H1N1 viruses are apparently the predominant influenza virus infecting pigs (Guan et al., 1996).

3.2. Human-like viruses

Infections of pigs with the prevailing human subtypes also occur under natural conditions. Shope (1938) presented serological evidence that human to pig transmission could occur, but it was not until the isolation of Hong Kong H3N2 virus from pigs in Taiwan in 1970 (Kundin, 1970) that investigations began to examine the potential transmission of human strains to pigs. Although no disease was reported among infected pigs, in the next several years H3N2 viruses were isolated regularly from pigs (Tumova et al., 1976; Shortridge and Webster, 1979; Nerome et al., 1981; Ottis et al., 1982) and/or antibody was demonstrated (Harkness et al., 1972; Tumova et al., 1976; Arikawa et al., 1979) in swine populations throughout the world. H3N2 influenza A viruses related to a human strain from 1973, continued to circulate in European pig populations long after their disappearance from the human population. Since 1984, outbreaks of clinical influenza in pigs due to a H3N2 influenza A virus, related antigenically to human strains from the early to mid 1970's, have been observed throughout Europe (Aymard et al., 1985; Haesebrouck et al., 1985; Pritchard et al., 1987) with infections frequently characterised by high seroprevalence (Tumova et al., 1980; Lange et al., 1984; Roberts et al., 1987). This apparently high level of H3N2 infections in Europe is in sharp contrast to the low prevalence in pigs in North America which suggests that the virus is not established in the American swine populations, but occurs only by infrequent introduction from infected humans (Easterday, 1980a). Until recently, virus has been isolated rarely from pigs in the US (Chambers et al., 1991) and only since 1990 from pigs in Canada (Bikour et al., 1994) which might reflect different epizootiological patterns in different areas world-wide.

Human H1N1 viruses can also infect pigs, but although pig to pig transmission has been demonstrated under experimental conditions (Kundin and Easterday, 1972), most strains are not readily transmitted among pigs in the field (Hinshaw et al., 1978). Serological surveillance studies world-wide suggest that the prevailing human H1N1 strains are readily transmitted to pigs (Roberts et al., 1987; Brown et al., 1995b) and have resulted occasionally in the isolation of virus (Katsuda et al., 1995a), but are not apparently maintained in pigs independently of the human population.

3.3. Avian-like viruses

Since 1979 the dominant H1N1 viruses in European pigs have been 'avian-like' H1N1 viruses which are antigenically and genetically distinguishable from North American classical swine H1N1 influenza viruses, but related closely to H1N1 viruses isolated from ducks (Pensaert et al., 1981; Scholtissek et al., 1983). All of the gene segments of the prototype viruses were of avian origin (Schultz et al., 1991) indicating that transmission of a whole avian virus into pigs had occurred and, as a result, have been implicated as the possible precursors of the next human pandemic virus (Ludwig et al., 1995). These 'avian-like' viruses appear to have a selective advantage over classical swine H1N1 viruses which are related antigenically, since in Europe they have replaced classical swine influenza virus (Bachmann, 1989; Campitelli et al., 1997). Within two years of the introduction of 'avian-like' viruses into pigs in Great Britain, classical swine H1N1 apparently disappeared as a clinical entity. More recently, an independent introduction of H1N1 virus from birds to pigs has occurred in southern China and these viruses have been detected in pigs in south east Asia since 1993 (Guan et al., 1996) where they are currently cocirculating with classical H1N1 viruses. Phylogenetic analysis of the genes of these viruses has revealed that they form an Asian sublineage of the Eurasian avian lineage. In addition, some of the H3N2 viruses isolated from pigs in Asia since the 1970's have been entirely 'avian-like' (Kida et al., 1988) and have been introduced apparently from ducks, although their association with epizootics of respiratory disease in pigs is unproven.

3.4. H1N2 viruses

Influenza A H1N2 viruses, derived from classical swine H1N1 and 'human-like' swine H3N2 viruses have been isolated in Japan (Sugimura et al., 1980) and France (Gourreau et al., 1994). In Japan, these viruses appear to have spread widely within pigs and are associated frequently with respiratory epizootics (Ouchi et al., 1996). Subsequently an H1N2 influenza virus (see Section 6) related antigenically to human and 'human-like' swine viruses has emerged and become endemic in pigs in Great Britain (Brown et al., 1995a) often in association with respiratory disease.

4. Outbreak course and persistence in pigs

Swine husbandry practises influence directly the evolution of influenza viruses in pigs leading generally to reduced genetic drift, particularly in the genes encoding HA and

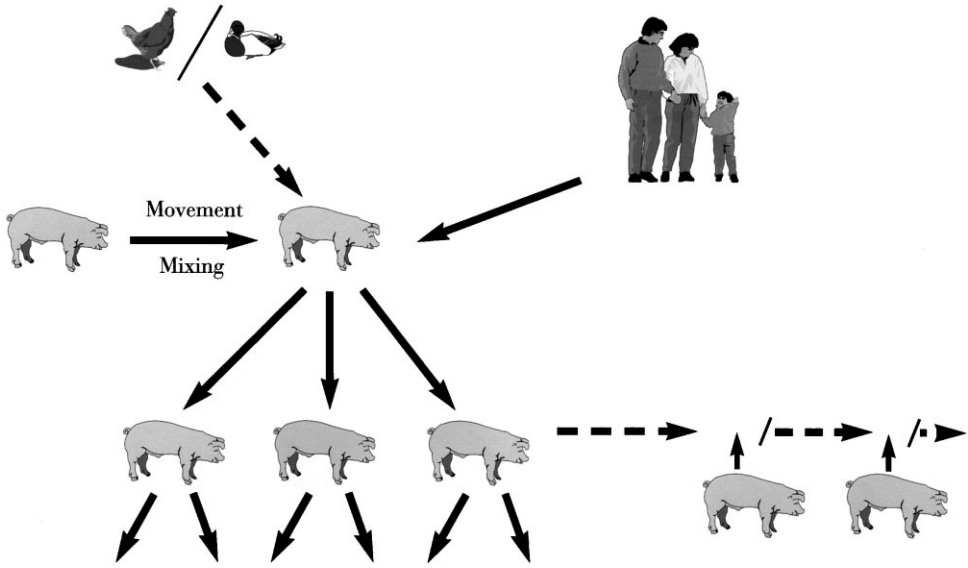

Fig. 1. Origin and perpetuation of influenza A viruses in pigs. Influenza generally appears with the introduction of infected pigs into a herd, either through the movement or mixing of infected pigs with susceptible animals. Transmission from humans to pigs occurs occasionally, but only rarely from avian species. Once a herd is infected with a virus which is able replicate, irrespective of its origin, the virus persists through the production of young susceptible pigs and the introduction of new stock, often leading to the herd becoming infected endemically. Following the introduction of an influenza virus, well adapted and pathogenic to pigs, into a fully susceptible herd, there may be clinical symptoms in affected pigs and rapid spread. Transmission of virus from another species to pigs may lead eventually to the establishment of a new virus lineage in pigs.

neuraminidase (NA), compared to those of similar viruses in the human population. SI generally appears with the introduction of new pigs into a herd, thereby being related to the movement of animals from infected to susceptible herds. Once a herd is infected, the virus is likely to persist through the production of young susceptible pigs and the introduction of new stock (Fig. 1). Complete depopulation is the only effective measure for eliminating the disease. Once a herd becomes infected with SI, it is likely to be maintained with annual episodes of acute disease. There may be differences in the epizootiology of SI between the US and Europe. In Europe many herds may harbour virus without showing clinical signs (Bachmann, 1989).

The disease often appears simultaneously on several farms within an area. Such multicentric outbreaks are not necessarily the result of recent movement of infected animals, but due to the widespread distribution of the virus among herds in an area. The primary route of virus transmission is through pig to pig contact via the nasopharyngeal route, the virus being shed in nasal secretions and disseminated through droplets or aerosols. Close contact between pigs (often enhanced through husbandry practices), stressful situations, meteorological and environmental factors are conducive to the spread of influenza viruses.

Outbreaks of disease occur throughout the year, but usually peak in the colder months (Easterday, 1980a). Infection with swine influenza H1N1 virus is frequently subclinical and typical signs are seen often in only 25 to 30% of a herd. Blaskovic et al. (1970) showed that swine influenza H1N1 virus was excreted from one infected pig for over four months, although 7 to 10 days is typical. Continuous circulation of influenza viruses within a herd without the apparent need for an intermediate host has been shown by the isolation of virus from a herd the year round (Nakamura et al., 1972). Furthermore, swine influenza H1N1 virus has been recovered from pigs with no signs of disease (Hinshaw et al., 1978).

The interepizootic survival and the potential existence of reservoirs for the virus have been studied extensively. There are no clear data to support or reject the existence of a long-term true carrier state of influenza viruses in pigs, but the widespread occurrence of the virus in pigs themselves and the methods of swine husbandry make it likely that the virus is maintained by continual passage to young susceptible pigs.

5. Transmission between pigs and other species

Pigs serve as major reservoirs of H1N1 and H3N2 influenza viruses and are often involved in interspecies transmission of influenza viruses. The maintenance of these viruses in pigs and the frequent introduction of new viruses from other species could be important in the generation of pandemic strains of human influenza.

5.1. Transmission between humans and pigs

Early theories suggesting that the transmission of virus from pigs to humans resulted in the 1918 pandemic were at the time speculative; it was not until 1976 that further evidence for such transmissions became available. 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 (Hodder et al., 1977; Top and Russell, 1977).

The Fort Dix incident cannot be regarded as evidence of zoonosis; although it was likely that pigs were the source of the virus, this was never established. However, there is considerable evidence that transmission from pigs to humans does occur; antibodies to swine H1 viruses were found in people who had close contact with pigs (Kluska et al., 1961; Schnurrenberger et al., 1970). Final confirmation of the zoonotic nature of H1N1 influenza viruses from pigs came in 1976, when following an influenza epizootic in pigs, viruses isolated from the pigs and a human contact were shown to be both antigenically and genetically identical swine H1N1 influenza viruses (Hinshaw et al., 1978; Easterday, 1980b). Subsequently, there have been several reports from North America of swine virus being isolated from humans with respiratory illness (Easterday, 1978; Dasco et al., 1984), occasionally with fatal consequences (Rota et al., 1989; Wentworth et al., 1994). All cases examined followed contact with sick pigs and were

due to viruses related most closely to classical swine H1N1 influenza virus. 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 (Claas et al., 1994). Genetically and antigenically related viruses had been detected in European pigs (Castrucci et al., 1993) raising the possibility of potential transmission of avian influenza virus genes to humans following genetic reassortment in pigs.

Influenza viruses of subtype H3N2 are ubiquitous in animals and endemic in most pig populations world-wide, where they persist many years after their antigenic counterparts have disappeared from humans (Shortridge et al., 1977; Haesebrouck et al., 1985; Wibberley et al., 1988; Brown et al., 1995b) and therefore present a reservoir of virus which may in the future infect a susceptible human population. There is no apparent evidence of pigs being infected with this subtype prior to the pandemic in humans in 1968. Indeed 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 (Aymard et al., 1980; Nerome et al., 1981; Brown et al., 1995b).

Further evidence of the spread of influenza viruses from humans to pigs was the appearance in pigs of H1N1 viruses (or antibodies to H1N1) related to those circulating in the human population since 1977 (Aymard et al., 1980; Nerome et al., 1982; Goto et al., 1992; Brown et al., 1995b). Genetic analysis of two strains of H1N1 virus isolated from pigs in Japan revealed that the HA and NA genes were most closely related to those of human H1N1 viruses circulating in the human population at that time (Katsuda et al., 1995a). In addition, reassortant viruses with some characteristics of human H1 viruses have been isolated from pigs in England (Brown et al., 1995a; Brown et al., 1998).

5.2. Transmission between pigs and birds

The probable introduction of classical swine H1N1 influenza viruses to turkeys from infected pigs has been reported from North America (Mohan et al., 1981; Pomeroy, 1982; Halvorson et al., 1992) and in some cases influenza-like illness in pigs has been followed immediately by disease signs in turkeys. Serological studies have revealed antibodies to classical swine H1 influenza virus in both turkeys and pigs. Genetic analyses of H1N1 viruses from turkeys in the US has revealed a high degree of genetic exchange and reassortment of influenza A viruses from turkeys and pigs, in the former species (Wright et al., 1992). Hinshaw et al. (1983) report the isolation of swine H1N1 virus from turkeys and the subsequent transmission to a laboratory technician who displayed fever, respiratory illness, virus shedding and seroconversion. These findings raise the possibility that viruses from pigs, humans, turkeys and ducks may serve as source of virus for the other three. In Europe, avian H1N1 viruses were transmitted to pigs (see 'avian-like' H1N1 viruses), established a stable lineage and have subsequently been reintroduced to turkeys from pigs, causing economic losses (Ludwig et al., 1994; Wood et al., 1997). Recently, H9N2 viruses have been introduced to pigs in south east Asia apparently from poultry (Shortridge, 1999, personal communication), although their potential to spread and persist in pigs remains unknown.

6. Genetic reassortment

6.1. The potential role of the pig as a reassortment vessel

The pig has been the leading contender for the role of intermediate host for reassortment of influenza A viruses. It is the only domesticated mammalian species which is reared in abundance and is susceptible to, and allows productive replication of avian (Hinshaw et al., 1981; Schultz et al., 1991) and human (Chambers et al., 1991) influenza viruses. This susceptibility is due to the presence of both α-2,3- and α-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 α-2,3 to α-2,6 linkage (Ito et al., 1998), which is the native linkage in humans, thereby providing a potential link from birds to humans.

The ability of an influenza virus to cross between species is controlled by the viral genes, and the prevalence of transmission will depend on the animal species. The success of interspecies transmission of influenza viruses depends on the viral gene constellation. Successful transmission between species can follow genetic reassortment, with a progeny virus containing a specific gene constellation having the ability to replicate in the new host. Reassorted viruses with other gene constellations may have a relatively low fitness and will not be able to perpetuate in the new host (Webster et al., 1992).

It has been shown that humans occasionally contract influenza viruses from pigs (see Section 5). 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 (Scholtissek et al., 1985; Tian et al., 1985; Snyder et al., 1987) 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 (Scholtissek et al., 1985) than both humans and birds. Recent studies by Kida et al. (1994), 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.

Evidence for the pig as a mixing vessel of influenza viruses of non-swine origin has been demonstrated in Europe by Castrucci et al. (1993), 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 (Castrucci et al., 1993). 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 (Brown et al., 1998) or H1N7 subtype derived from human and equine viruses (Brown et al., 1994) have been isolated from pigs in Great Britain. The H1N2 viruses derived from a multiple reassortant event and spread widely within pigs in Great Britain. The H1N7 virus comprised six genes from a human H1N1 virus which circulated in the human population during the late 1970's and two genes (NA and M) derived from an equine H7N7 virus which has not been isolated from horses since 1980, although there is serological evidence that this virus may be circulating in horses at marginal levels in some parts of the world (Mumford and Wood, 1992; Madic et al., 1996). Genetic analyses of the HA and NA indicated a low rate of antigenic drift following transmission to pigs in contrast to the higher rate in the natural hosts. Other studies of influenza viruses isolated from pigs in North America (Wright et al., 1992) and Southern China (Shu et al., 1994) failed to detect any reassortant viruses containing internal protein gene segments of non swine origin, although genetic heterogeneity of the HA of swine H3 influenza viruses occurs in nature in China (Kida et al., 1988).

7. Virus adaptation and pathogenesis

Pigs infected with human H1N1 or H3N2 influenza virus readily develop antibodies to these viruses. As a result, the transmission of human influenza viruses to pigs has been studied widely and monitored using serosurveillance methods. However, pigs transiently infected with some avian influenza viruses may not always produce a detectable antibody response (Hinshaw et al., 1981; Kida et al., 1994). These findings are of importance in studying the epidemiology of influenza virus in pigs; serosurveillance may not be suitable for the detection of some reassortant or 'new' influenza viruses in pigs. Natural and experimental infection of pigs with an H1N7 human-equine reassortant virus did not induce detectable humoral antibody but the virus was able to transmit between pigs (Brown et al., 1994). These findings demonstrate the potential value of monitoring pigs using virus isolation.

Successful cross-species transmission is dependent on both host and virus genetic factors and subsequent spread within the new host population requires a period of adaptation of the virus to the new host (Webster et al., 1992). It is possible that following the transmission of an avian H1N1 virus to pigs in continental Europe in 1979 (Pensaert et al., 1981), subsequent infection of pigs was usually subclinical, since the virus was not well adapted to its new host. The introduction from continental Europe of an 'avian-like' swine H1N1 virus well adapted to its new host (Brown et al., 1997), into an immunologically naive pig population, such as found in Great Britain in 1992, may partly explain the rapid spread of the virus and its association with disease outbreaks (Brown et al., 1993); this was consistent with the epidemiology of the virus in pigs in Europe as a whole. Interestingly, the widespread prevalence of antigenically related classical swine H1N1 viruses in pigs in Great Britain (Brown et al., 1995b) and continental Europe (Bachmann, 1989) apparently failed to prevent infection with 'avian-like' swine H1N1 viruses.

The evolution and adaptation of human H3N2 viruses in pigs following transmission in the early 1970's appeared similar to that of avian H1N1 viruses. In Europe, the presence of these human H3N2 viruses in pigs was for at least 10 years based on antibody detection and it was not until 1984 that the virus was first associated directly with outbreaks of respiratory disease in pigs (Haesebrouck et al., 1985) and such occurrences became increasingly more frequent thereafter (Wibberley et al., 1988; Castrucci et al., 1994). Locally in many parts of Europe 'swine adapted' human H3N2 viruses became the predominant epidemic strain and still remain so, for example in the 'Low countries' (De Jong et al., 1999; Van Reeth, personal communication). Interestingly, H3N2 viruses circulating in pigs in Italy since 1983 all contain internal protein genes of avian origin, having replaced H3N2 viruses whose genotype is entirely human (Campitelli et al., 1997), suggesting that the acquisition of internal protein genes from an avian virus adapted to pigs afforded a selection advantage to these reassorted viruses.

The results of serosurveillance studies have indicated that the prevailing human viruses of both H1N1 and H3N2 subtypes are transmitted to pigs, but fail to persist. The frequent close contact between humans and pigs would facilitate the transmission of virus from humans to pigs. It is not clear why these viruses fail to persist in pigs, but since immune selection is not considered important in pigs, strains with different antigenic characteristics may be disadvantaged compared to the 'highly-adapted' established viruses which continually circulate within a large susceptible population. However, the recent detection of an H1N2 influenza virus in pigs in Great Britain whose HA is related most closely to that of a human H1 virus from the early 1980's, suggests that the genes of human viruses may persist after reassortment with one or more influenza viruses endemic in pigs, and following adaptation to pigs may often be associated with outbreaks of respiratory disease (Brown et al., 1998).

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 unknown, although following the introduction of an avian virus into European pigs in 1979, the mutation rate of this virus did not subsequently increase (Stech et al., 1999). These processes can take many years as occurred following transmission of both avian H1N1 and human H3N2 viruses to pigs. In future studies it would be desirable to characterise all the gene segments of viruses isolated to detect changing genotypes with potential implications for pathogenicity to pigs and/or other species.

8. Genetic variation

Phylogenetic analyses of influenza virus genes have revealed that they have evolved broadly in five major host-specific pathways comprising early and late equine viruses, human/classical swine viruses, H13 gull viruses and all other avian viruses. Geographic patterns of evolution occur amongst bird populations forming sublineages relating to North America, Eurasia and Australasia. Following transmission to pigs, influenza virus genes evolve in the pathway of the host of origin but diverge forming a separate

sublineage (Gorman et al., 1991; Scholtissek et al., 1993; Nerome et al., 1995). All of the genes of human and classical swine viruses form a sister group since they share a common ancestor and the comparable rate of change in some genes such as NP is very similar (Gorman et al., 1991). However, analyses of the genes of avian viruses following their transmission to pigs in Europe revealed the highest evolutionary rates for influenza genes for a period of approximately 10 years, and may be due to the virus possessing a mutator mutation in the polymerase complex (Ludwig et al., 1995).

Genes that code for the surface proteins HA and NA are subjected to the highest rates of change. The HA gene of both the classical and 'avian-like' swine H1N1 viruses is undergoing genetic drift, being more marked in the latter. However, genetic drift in the HA gene of swine H1N1 viruses is confined generally to regions unrelated to antigenic sites (Luoh et al., 1992; Brown et al., 1997), which is in marked contrast to genetic drift in the HA gene of human H1N1 viruses (Xu et al., 1993). The 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. The HA genes of classical swine H1N1 influenza virus isolates in North America have remained conserved both genetically and antigenically (Sheerar et al., 1989; Luoh et al., 1992; Bikour et al., 1995) over a period of at least 25 years, but viruses distinguishable antigenically, although closely related, have been reported by Olsen et al. (1993) and Wentworth et al. (1994). In addition, Rekik et al. (1994) reported antigenic drift in the HA gene of recent isolates of swine H1N1 influenza virus in Canada associated with altered pathogenesis termed proliferative and necrotising pneumonia (Dea et al., 1992). Following new introductions of influenza A virus to pigs, as occurred in south east Asia in 1993, 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.

Influenza viruses of H3N2 subtype continue to circulate widely in pigs world-wide. The majority of these viruses are antigenically related closely to early human strains such as A/Port Chalmers/1/73. The limited immune selection in pigs facilitates the persistence of these viruses, which may in future transmit to a susceptible human population. However, some viruses although related closely to the prototype human viruses have antigenic differences in the surface glycoproteins and may cocirculate with the former strains. (Haesebrouck and Pensaert, 1988; Kaiser et al., 1991; Brown et al., 1995a). 'Human-like' swine H3N2 viruses appear to be evolving independently in different lineages to those of human and avian strains (Castrucci et al., 1994; De Jong et al., 1999). 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 (Nerome et al., 1995). However, marked genetic drift resulting in considerable antigenic variation in the HA gene of 'human-like' H3N2 viruses in European pigs, has led to an apparent increase in epizootics attributable to this virus (De Jong et al., 1999). In addition, the prevailing epidemic strains in the human population are transmitted frequently to pigs (Nerome et al., 1995; Katsuda et al., 1995b; Shu et al., 1996) and these viruses are clearly distinguishable antigenically from the early human viruses established in pigs.

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