Genetics, Evolution, and the Zoonotic Capacity of European Swine Influenza Viruses

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Abstract The European swine influenza virus lineage differs genetically from the classical swine influenza viruses and the triple reassortants found in North America and Asia. The avian-like swine H1N1 viruses emerged in 1979 after an avian-to-swine transmission and spread to all major European pig-producing countries. Reassortment of these viruses with seasonal H3N2 viruses led to human-like swine H3N2 viruses which appeared in 1984. Finally, human-like swine H1N2 viruses emerged in 1994. These are triple reassortants comprising genes of avian-like H1N1, seasonal H1N1, and seasonal H3N2 viruses. All three subtypes established persistent infection chains and became prevalent in the European pig population. They successively replaced the circulating classical swine H1N1 viruses of that time and gave rise to a number of reassortant viruses including the pandemic (H1N1) 2009 virus. All three European lineages have the capacity to infect humans but zoonotic infections are benign.

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1 Introduction

Swine influenza was first recognized as a disease of pigs during the great pandemic in autumn 1918. At that time, John S. Koen, who worked as a hog cholera inspector for the U.S. Bureau of Animal Industry in Fort Dodge, Iowa, observed a striking similarity between the clinical presentation of diseased humans and pigs: "*Last fall and winter we were confronted with a new condition, if not a new disease. I believe I have as much to support this diagnosis in pigs as the physicians have to support a similar diagnosis in man. The similarity of the epidemic among people and the epidemic in pigs was so close, the reports so frequent, that an outbreak in the family would be followed immediately by an outbreak among the hogs and* vice versa*, as to present a most striking coincidence if not suggesting a close relation between the two conditions. It looked like "flu", it presented the identical symptoms of "flu", it terminated like "flu", and until proved it was not "flu", I shall stand by that diagnosis.*" (Koen 1919). The etiologic agent of "flu", influenza A virus, was first isolated by Richard E. Shope (Shope 1931a).

Influenza viruses are members of the family *Orthomyxoviridae* which comprises five genera: *Influenza virus A*, *B*, and *C*, *Thogotovirus*, and *Isavirus* (Kawaoka et al. 2005). Each influenza virus genus includes one species (also designated as *influenza A virus*, *influenza B virus*, and *influenza C virus*; abbreviated FLUAV, FLUBV, FLUCV).

Influenza A viruses are enveloped negative-stranded RNA viruses. The RNA genome is segmented (Duesberg 1968) and associated with the viral nucleoprotein

(NP) and the viral polymerase complex. The eight RNA segments vary in their sizes (ranging from 890 to 2,341 nucleotides) and encode 11 proteins. Expression of viral genes occurs after transcription of genomic RNA with the help of the viral RNA-dependent RNA polymerase.

New isolates of influenza A virus predominantly have a filamentous structure with a diameter of approximately 80–120 nm and a length ranging from 2 to 200 μm (Chu et al. 1949). After adaptation to cell culture, the virion tends to have a spherical or pleomorph appearance. The viral envelope is composed of the cell membrane lipids but the majority of surface proteins are provided by the viral hemagglutinin (HA) and neuraminidase (NA). A third viral membrane protein is the M2 proton channel. The inner surface of the envelope is coated with the matrix protein (M1). The virion contains eight nucleocapsids; these are complexes of RNA and viral protein. Electron micrographs show helical rod-like structures with a terminal loop. The width ranges from 10 to 15 nm and the length from 30 to 120 nm. They were interpreted to represent backfolded and twisted ribonucleoproteins (Compans et al. 1972). The nucleocapsids are associated with the envelope by matrix proteins (Noda et al. 2006).

2 Influenza Virus Ecology

Influenza A viruses have a broad host range (Webster et al. 1992). The main reservoir hosts are aquatic birds of the orders *Anseriformes* (geese and ducks) and *Charadriiformes* (waders and gulls), but numerous other bird species may also be infected (Munster et al. 2007). Reassortment of 16 HA and nine NA types allows the formation of maximal 144 HA/NA combinations of which more than 110 types have been already isolated from birds. Whether all theoretical combinations exist in nature is unknown. In mammals, stable infection chains are observed only for certain subtypes (Table 1). Important mammalian host species include: humans, pigs, and horses. Dogs, domestic cats, and felid carnivores (tiger, leopard) as well as several mustelid carnivores (ferret, stone marten, mink), marine mammals (whales, seals), the camel, the muskrat, civet, racoon dog, pika, and giant anteater were described as accidental hosts without establishment of stable infection chains.

Influenza virus ecology is strongly influenced by virus adaptation to its host. One major determinant of the host range is the receptor molecule on the surface of the host cell. Influenza A virus binds to sialic acid (N-acetylneuraminic acid) which is linked by an α-glycosidic bond to the terminal galactose residues of carbohydrate chains of glycoproteins and glycolipids (Rogers and Paulsen 1983). Both species and tissue-specific expression of receptor molecules determine host range and tropism of influenza A viruses (Ito et al. 1998; Ito and Kawaoka 2000). Whereas avian influenza viruses bind to α-2,3-linked sialic acid, seasonal influenza virus strains of humans recognize α-2,6-linked sialic acid. Airway epithelia of the upper respiratory tract of pigs express both receptors. Thus, pigs are susceptible to

Table 1 Mammalian host species of influenza A virus

Host	Stable infection chain	Incidental zoonotic infection or reassorted isolate	Extinct
Human	H1N1, H3N2, pandemic (H1N1) 2009 virus	H1N2, H5N1, H7N1, H7N2, H7N3, H7N7, H9N2	H2N2
Pig	H1N1, H1N2, H3N2	H1N7, H2N3, H3N1, H3N3, H4N6, H5N1, H5N2, H9N2, pandemic (H1N1) 2009 virus	
Horse	H3N8	H1N8, H3N3	H7N7
Dog		H3N8, H5N1	
Racoon dog (*Nyctereutes procyonoides*)		H5N1	
Mink		H3N2, H10N4	
Stone marten		H5N1	
Ferret		H1N1	
Seal (*Phoca vitulina*)		H3N3, H4N5, H7N7	
Whale		H1N3, H13N2, H13N9	
Camel		H1N1	
Giant anteater		H1N1	
Tiger, leopard		H5N1	
Domestic cat		H5N1, pandemic (H1N1) 2009 virus	
Civet		H5N1	
Pika (*Ochotona spec.*)		H5N1	
Muskrat (*Ondatra zibethicus*)		H4N6	

influenza viruses which are adapted either to birds or to humans and can serve as intermediate hosts after trans-species infections (Ito et al. 1998). Due to their receptor configuration, pigs were considered as mixing vessels for the reassortment of human and avian influenza viruses (Scholtissek et al. 1985). Other host determining factors are the nucleoprotein (Scholtissek 1990) and polymerase subunit PB2. Amino acid position 627 of PB2 was shown to be critical for virus replication (Subbarao et al. 1993). The tissue-specific expression of host proteases, however, contributes to virulence or pathogenicity but not to the host range (Webster et al. 1992; Steinhauer 1999). Recent genome-wide RNAi screening studies revealed the involvement of hundreds of host factors that are required for efficient influenza virus replication (König et al. 2010; Karlas et al. 2010). It remains to be elucidated which of these factors establishes the host range.

3 Genetic Drift and Reassortment: Two Mechanisms for the Generation of Genetic Variability of Influenza viruses

A typical property of influenza A viruses is their great variability which is mainly caused by two mechanisms. Genetic drift is the continuous accumulation of nucleotide substitutions over time. The substitution rate of influenza viruses ranges from 10^{-5} to 10^{-6} substitutions/site/replication cycle depending on the experimental setup (e.g., Stech et al. 1999; Nobusawa and Sato 2006; Parvin et al. 1986). According to the genome size of appr. 13,600 nucleotides, between 1 and 10 % of the progeny virus has substitutions. Older estimations determined even higher substitution rates (e.g., Yewdell et al. 1979; Holland et al. 1982). The most base substitutions are neutral, this means they do not cause changes of the amino acid sequence or—if so—a substitution does not seem to influence the fitness of the progeny virus. The reason is that the majority of amino acid residues of influenza virus proteins are negatively selected (purifying selection). Substitutions of such amino acids would decrease the viral fitness and are only endured as long as certain selection pressures act on the virus. A host change could induce such a selection pressure. Only very few sites are positively selected. A positive selection increases the heterogeneity of the gene pool, it is also designated as 'diversifying selection'. One example of positive selection is the gradual changing of the antigenic sites of the hemagglutinin known as 'antigenic drift'. Substitutions that result in immune escape variants have an increased probability to infect hosts with preimmunity. Eighteen codons of the hemagglutinating HA1 domain were identified to be positively selected (Bush et al. 1999a, b). One driver of antigenic drift is the receptor binding avidity of the viral hemagglutinin (Hensley et al. 2009). Though antigen drift of the hemagglutinin may be striking and can be investigated by serological means, substitutions of all influenza virus genes occur with the same frequency. Accordingly, the whole genome is subjected to a genetic drift rather than antigenic drift of the HA gene only. Due to the preponderance of either positive or negative selection acting on each gene, the relation of synonymous and non-synonymous substitutions of the eight gene segments differs.

The second important mechanism of influenza virus variability is reassortment or the exchange of one or more gene segments. Reassortments are of biological importance as they lead to novel combinations of genome segments which have been evolved by negative or positive selection. This mechanism greatly enhances evolutionary rates and accounts for rapid viral adaptation to changing environmental conditions. Reassortments occur naturally or can be induced experimentally (Kilbourne 1968). They are accomplished by a segmented virus genome and by double or multiple infections of a host with virus strains of different subtypes or genetic lineages. Reassortment events leading to exchanges of HA and NA genes are of special importance as they can lead to an antigen shift. Shift variants exhibit major differences of antigenic epitopes and less cross-reactivity with pre-existing antibodies of a host. Circulation of two or more subtypes within a population at the same time can lead to reassortments which are not associated with an antigen shift.

In addition, reassortments may occur after incidental zoonotic infections. Such events introduce genes into a virus population that are adapted to other species. Beside the HA and NA genes, other gene segments can also reassort but were in the dark for long time due to a lack of sequence data. Such reassortants are serologically inconspicuous.

4 The Concept of Genetic Influenza A Virus Lineages

Genetic drift did not only contribute to the evolution of the known HA and NA subtypes, but led to the formation of distinct genetic lineages of all genome segments (Webster et al. 1992). The genetic configuration of an influenza virus is defined by its genotype (Lu et al. 2007), which describes a virus with greater accuracy than subtyping by the HA and NA types only. Precise genotyping requires complete genome sequences but greatly enhanced our understanding of influenza virus ecology and evolution. Whereas sequence comparisons of different HA types yield nucleotide identities of roughly 56 % on average, sequences of a given lineage have nucleotide identities greater than 90 %. Two factors determine the evolution of genetic lineages: host species barriers (Kuiken et al. 2006) and geographic isolation. Starting with the pandemic of 1918, two stable infection chains of H1N1 were established in humans and pigs which lead to new, distinct genetic lineages: the seasonal H1N1 of humans and the classical swine H1N1 lineage (Fig. 1). Such lineages can be demonstrated for all eight genome segments. There are distinct lineages for birds, humans, pigs, and horses. It appears that some lineages became extinct but the significance of this observation is yet unclear due to a lack of sufficient sequence data. Viruses of a lineage are adapted to their host species. Trans-species infections occasionally occur, but virus replication is less efficient and infection chains may disrupt after a few generations. Stable infection chains will establish rarely. Besides the pandemic virus of 1918, the 'avian-like' swine influenza viruses in Europe are another example for a successful establishment of a stable infection chain. However, after some 30 years of circulation the latter viruses have not yet accumulated sufficient substitutions to establish distinct genetic lineages for each of their genes. Their H1 HA gene, for example, is presently considered as a sublineage or clade of lineage 1C (Fig. 1). The genes of the present pandemic (H1N1) 2009 virus have the potential to lead to new genetic lineages. Among the 16 HA types at least 69 genetic lineages were described; the nine NA types comprise altogether 46 lineages and each of the internal segment has 7–11 lineages (Lu et al. 2007).

In addition to host species barriers, geographic isolation can induce the development of genetic lineages. As a result of different flyways of migratory birds, American and Eurasian lineages of influenza virus genes evolved (Olsen et al. 2006). As the evolution of such lineages is promoted by isolation rather than host-specific barriers, trans-species infections are not uncommon (Krauss et al. 2007; Wallensten et al. 2005; zu Dohna et al. 2009). They occur frequently in

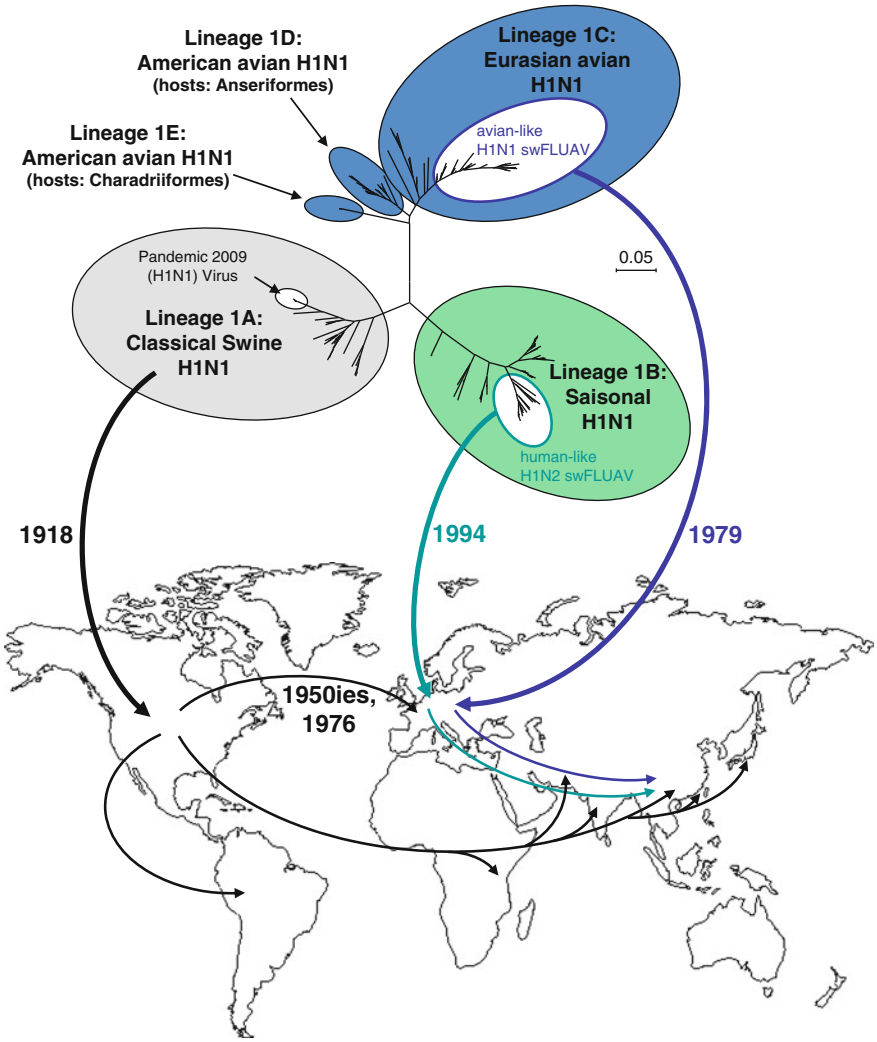


Fig. 1 Genetic lineages and sublineages of the HAH1 gene and their geographic distribution. Adaptation to host species and geographic isolation lead to the evolution of five HAH1 lineages (*top*). Genetic data suggest that the seasonal H1N1 viruses of humans emerged around 1918 from an American avian ancestor, either as a whole or as a reassortant (Anhlan et al. 2011), and spread worldwide (pandemic of 1918, not indicated). Classical swine H1N1 viruses also emerged around that time in the USA, probably after zoonotic infections of pigs. In several waves classical swine viruses were translocated to South America, numerous Asian countries, East Africa, and Europe. In Europe, they vanished after appearance of the avian-like H1N1 swine viruses in Belgium and Germany in 1979. Human-like H1N2 swine influenza viruses emerged in 1994 in the UK and spread to the European continent. After 2000, avian-like H1N1 and human-like H1N2 arrived in Asia where they co-circulate with the classical swine strains. Numerous reassortants indicate a dynamic influenza activity in Asia

overlapping breeding grounds, for example around the Arctic Beringia Sea (Wahlgren et al. 2008) or after translocation of infected birds (Makarova et al. 1999). Emergence of new lineages or subtypes may lead to the extinction of previously circulating types, a phenomenon that was repeatedly observed but cannot be sufficiently explained yet. For example, seasonal H1N1 viruses were superseded by pandemic H2N2 viruses in 1957. Likewise, circulating classical swine H1N1 viruses were replaced by avian-like swine H1N1 viruses after 1979 in Europe.

Despite the generation of thousands of sequence entries in the GenBank in recent years, our present understanding of the dynamics of the influenza virus epizootiology of birds and non-human mammals is still fragmentary.

5 The Disease

Swine influenza was originally described as a disease of autumn and early winter which occurred in annual epizootics (Shope 1931b). In many regions with dense pig populations the disease became enzootic and nowadays infections occur all year-round. The main symptoms of swine influenza are sudden onset of the disease, fever, anorexia, coughing, nasal discharge, sneezing, dyspnoea, exhaustion, and apathy. In general, infections with the virus cause a mild disease with a benign outcome. The morbidity within an affected herd is high (up to 100 %); the mortality is low but depends on the virus strain and other factors such as mixed infections. Usually the disease lasts 2–6 days and in most cases animals completely recover. Affected pigs develop an acute bronchitis with swollen mucosa, abundant mucus, hyperemia, and enlarged local lymph nodes. Inflammation surrounds bronchi and bronchioles. Sometimes secondary bacterial lobular pneumonia exacerbates the disease and may lead to death. Koen (1919) estimated influenza-associated mortality with "*1 per cent, at any rate less than 2 per cent*"; Shope with 1–4 % (Koen 1919; Shope 1931b).

In Europe, swine influenza is caused by three virus subtypes that are genetically distinct from the classical swine H1N1 viruses. In regions with enzootic persistence, the clinical signs are less marked and the virus circulates throughout the year. The avian-like swine H1N1 viruses generally induce less severe symptoms than human-like swine H3N2 viruses and natural H1N1 infections are sometimes unrecognized. The European H1N2 strains differ regarding their virulence. For example, the German strains which appeared in 2000 are more virulent than the Belgian strains. Changes in epizootiology may have several reasons. One reason is that swine husbandry practices changed in the past decades. A short fattening period of only 6 months leads to a rapid turnover of the swine population in a herd which requires purchase of piglets from various suppliers. This increases the chance of a virus to infect naive pigs and accomplishes gathering of influenza viruses from several distinct sources. Less marked clinical signs of enzootic viruses, varying levels of maternal antibodies, and preexisting immunities of older

pigs constitute selection pressures which are at the molecular level yet undefined and prepare the ground for the reassortment of novel virus combinations.

6 European Swine Influenza Viruses

There is evidence that influenza viruses have been introduced several times into European pigs. Stable infection chains, however, were first established in the 1970s. Prior to this, seasonal and classical swine influenza viruses have been detected in serological studies and occasional virus isolations. In this review we distinguish between zoonotic viruses of human and avian origin isolated from pigs on the one hand and the "*human-like*" and "*avian-like*" viruses on the other hand. Whereas the former viruses occur occasionally and become extinct after a few replication cycles, the latter viruses have defined genetic settings which developed distinct clades in phylogenetic trees. Such clades indicate stable infection chains over many virus generations. Moreover, these viruses exhibit evolutionary changes as a result of genetic drift and/or reassortment events which will be reviewed here. Previous authors (e.g., Brown 2000; Kuntz-Simon and Madec 2009) used the terms "*human-like*" and "*avian-like*" primarily to indicate the previous host.

6.1 Early Descriptions of Swine Influenza in Europe

Several early reports describe the influenza of pigs in Europe. Soon after the first isolation of swine influenza virus by Richard E. Shope, K. Köbe published the isolation of the etiologic agent of "enzootic pneumonia" of piglets, a condition that he named "Ferkelgrippe" (Köbe 1933). Köbe had observed that the histopathological lesions of the lungs were like "American swine influenza", but "Ferkelgrippe" in Germany showed enzootic rather than epizootic transmission and occurred in piglets younger than those of Shope's experiments. In analogy to Shope's work on swine influenza, Köbe believed that "Ferkelgrippe" was the result of a mixed infection; experimental pneumonia was induced only after coinfection of piglets with a filtrable virus that he had isolated from dispersed lung tissue of affected pigs and a bacterium that he designated *Bacterium influenzae suis*. The virus alone induced only mild symptoms and was distinct from the classical swine fever virus (hog cholera virus). It is unknown whether Köbe tried to propagate the virus in ferrets or mice. Unfortunately, the virus became lost in the past decades. Köbe's mentor Otto Waldmann confirmed the findings of his associate and commented that the observed differences in age incidence could be due to different husbandry practices in Germany and the USA (Waldmann 1933). Shortly thereafter, Gerhard Elkeles in Berlin, Germany, infected 2–6-week-old piglets with human influenza virus and could induce a mild flu-like disease in pigs; experimental coinfection of piglets with the human virus and either human or

porcine strains of *Haemophilus influenzae* resulted in a more severe disease (Elkeles 1934). These were the first experiments demonstrating the susceptibility of pigs to human influenza virus strains. Later they were confirmed by Shope and Francis (1936), but these authors used older pigs (6–14 weeks). With regard to the findings of Köbe and Elkeles, they discussed a different natural susceptibility of European and American pigs. Further work was published by Lamont (1938) and Blakemore and Gledhill (1941) who described outbreaks of swine influenza in Northern Ireland and England. Interestingly, Blakemore and Gledhill (1941) observed one outbreak on an Essex farm with cases of chronic disease (for 8 weeks), and—like O. Waldmann—concluded that husbandry conditions may have an influence on the course of the disease. Both Lamont and Blakemore handed over tissue specimens of several outbreaks to R. E. Glover in Cambridge who together with C. H. Andrewes succeeded to isolate three influenza virus strains after serial passages in ferrets and mice (Glover 1941). Serological characterization revealed that these isolates differed from Shope's swine influenza virus but resembled the human strains. Later, three of Glover's virus strains of that time were (partially) sequenced and were shown to cluster with A/WS/1933 and with A/Puerto Rico/8/1934 (Gorman et al. 1991; Neumeier and Meier-Ewert 1992; Neumeier et al. 1994; Yoshioka et al. 1994).

There is no hard evidence that classical swine influenza virus entered Europe in the 1930s or 1940s. European strains of classical swine influenza virus were first isolated in the 1950s in the former Czechoslovakia (Harnach et al. 1950). A serological survey conducted in 1957 by the W.H.O. revealed antibodies in pigs to classical swine H1N1 in Czechoslovakia and Germany (Kaplan and Payne 1959). After this episode, classical swine viruses disappeared in the 1960s in Europe, but were reintroduced in 1976 in Italy (Nardelli et al. 1978). These viruses spread to several European countries, including Belgium (Biront et al. 1980; Vandeputte et al. 1980), Germany (Sinnecker et al. 1983), France (Gourreau et al. 1980), England (Roberts et al. 1987), and Sweden (Martinsson et al. 1983). After the emergence of avian-like H1N1 swine viruses they disappeared again. The last European strain of classical swine H1N1 was isolated in 1993 in England soon after the first detection of avian-like H1N1 on the British Isles (Brown et al. 1997b).

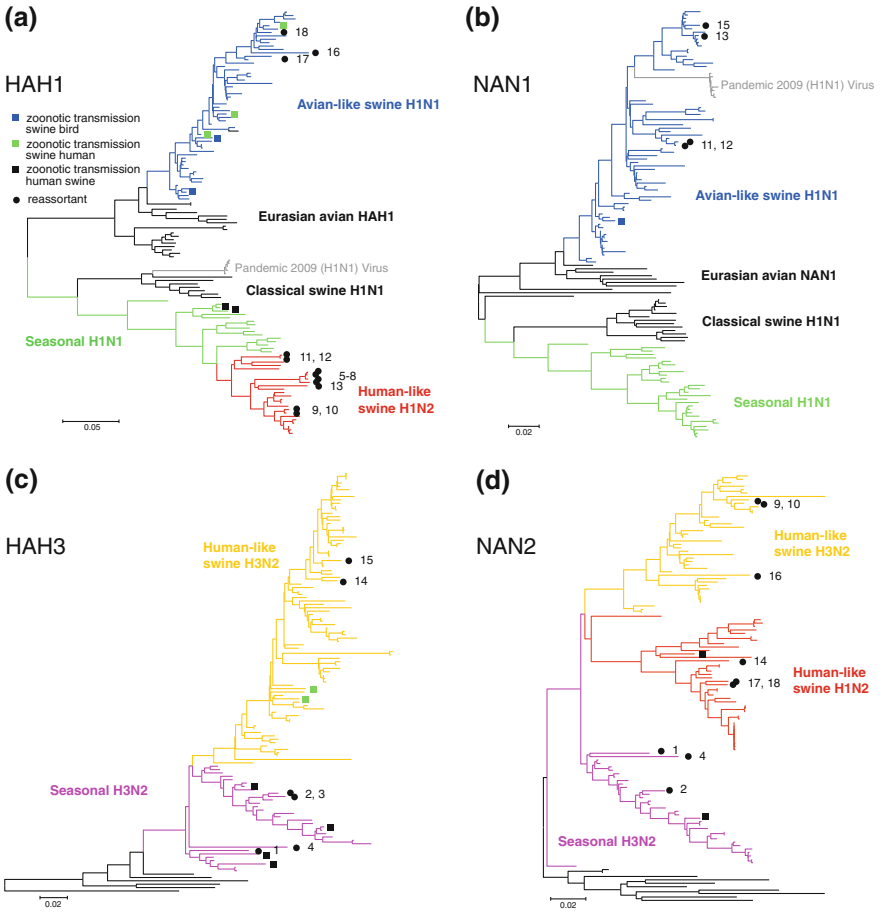
6.2 *Stable Establishment of Influenza Viruses in European Pigs: Avian-Like Swine H1N1*

A distinct sublineage of European H1N1 swine influenza viruses emerged in January 1979 in Belgium (Pensaert et al. 1981). These viruses differed serologically from classical swine viruses but showed relationship to avian viruses. Some virulent strains induced clinical symptoms which were typical for swine influenza. In winter 1979/80, similar viruses appeared in Germany and France (Witte et al. 1981; Ottis et al. 1981; Gourreau et al. 1981). Retrospective serological analyses

revealed that the majority of infections were asymptomatic. The molecular characterization revealed that all segments derived from an avian H1N1 influenza virus (Scholtissek et al. 1983). The phylogenetic comparison (Fig. 2a, b) demonstrates that the hemagglutinin of the swine viruses is most closely related to virus isolates from German ducks (A/duck/Bavaria/1/1977, A/duck/Bavaria/2/1977) which were the first avian H1N1 viruses detected in Europe (Ottis and Bachmann 1980). The hemagglutinin of the so-called "avian-like" swine H1N1 viruses shows a considerable cross-reaction with the classical swine H1N1; therefore, one of the commercially available vaccines (Gripovac™) for pigs includes A/New Jersey/8/1976 (H1N1). Further characterization revealed the avian origin of all segments (Schultz et al. 1991; Castrucci et al. 1993; Campitelli et al. 1997; Brown et al. 1997b). In very short time the avian-like H1N1 swine viruses established a stable infection chain and spread to all major swine-producing countries in Europe. They succeeded to replace the previous circulating classical swine strains. After 30 years of circulation, the avian-like swine H1N1 are endemic in the major pig-producing European countries. However, the seroprevalence varies considerably. In 2002/2003 it was highest in Belgium and Germany (80.8, 70.8 %); prevalence was lower in Italy and Spain; (46.4, 38.5 %) and low in the Czech Republic, Ireland, and Poland (>18 %) (Van Reeth et al. 2008). A more recent study conducted as a cross-sectional survey in Spain in 2008–2009 revealed a striking increase in the H1N1 seroprevalence (Simon-Grifé et al. 2010). Likewise, a German study indicates a similar annual variability suggesting fluctuations in the prevalence of swine influenza viruses over time (R. Dürrwald, personal communication).

6.3 Emergence of Human-Like H3N2 in European Pigs

The first "human-like" swine H3N2 virus emerged in Germany in 1982 (Schrader and Süss 2004). The HA and NA surface proteins of strain A/swine/Potsdam/35/1982 were derived from an A/Port Chalmers/1/1973-like seasonal H3N2 virus (Fig. 2c, d), whereas an avian-like H1N1 swine virus served as donor for the internal segments (M-segment: Schmidtke et al. 2006; Krumbholz et al. 2009; PB1-segment: Zell et al. 2007; PB2, PA, NP, NS segments: R. Zell unpublished). This virus disappeared soon. Another virus with a very similar genetic composition reemerged in 1984 and achieved to establish a persistent infection chain. The viruses were designated as "human-like swine H3N2" (Fig. 2c, d) due to their antigenic similarity to human H3N2. They spread rapidly in the European pig population. Epizootics were reported in Belgium (Haesebrouck et al. 1985; Haesebrouck and Pensaert 1988), France (Madec et al. 1984), and Germany (Zhang et al. 1989), Italy (Castrucci et al. 1993), the Netherlands (Loeffen et al. 1999), and Spain (Castro et al. 1988; Yus et al. 1992). The molecular analysis of these viruses revealed avian-like internal genes and human A/Port Chalmers/1/1973-like HA and NA genes (Campitelli et al. 1997; Marozin et al. 2002), but this



(e)

		PB2	PB1	PA	HA	NP	NA	M	NS
	Seasonal (human) H1N1	A	A	B	1B	A	1A	B	1A
	Seasonal (human) H3N2	A	D	B	3A	A	2A	B	1A
	avian-like swine H1N1: A/swine/Arnsberg/6554/1979-like	F	G	I	1C	F	1F	F	1E
	human-like swine H3N2: A/swine/Gent/1/1984-like	F	G	I	3A	F	2A	F	1E
	human-like swine H1N2: A/swine/Scotland/410440/1994-like	F	G	I	1B	F	2A	F	1E
1	A/swine/Potsdam/35/1982 (H3N2)	F	G	I	3A	F	2A	F	1E
2	A/swine/Karrenzien/2/1987 (H3N2)	F	G	I	3A	F	2A	F	1E
3	A/swine/Jena/3765/1989 (H3N2)	/	/	/	3A	/	/	/	/
4	A/swine/Leipzig/145/1992 (H3N2)	F	G	I	3A	F	2A	F	1E
5	A/swine/Italy/19447/2006 (H1N2)	/	/	/	1B	/	2A	/	/
6	A/swine/Italy/19498/2006 (H1N2)	/	/	/	1B	/	2A	/	/
7	A/swine/Italy/23255/ (H1N2)	/	/	/	1B	/	2A	/	/
8	A/swine/Italy/30073-5/2006 (H1N2)	/	/	/	1B	/	2A	/	/
9	A/swine/Ötitingen/IDT4735/2005 (H1N2)	F	G	I	1B	F	2A	F	1E
10	A/swine/Cloppenburg/IDT4777/2005 (H1N2)	F	G	I	1B	F	2A	F	1E
11	A/swine/Cotes d'Armor/60293/2001 (H1N1)	F	G	I	1B	F	1F	F	1E
12	A/swine/Cotes d'Armor/98574/2001 (H1N1)	F	G	I	1B	F	1F	F	1E
13	A/swine/Cotes d'Armor/0190/2006 (H1N1)	F	G	I	1B	F	1F	F	1E
14	A/swine/Nordkirchen/IDT1993/2003 (H3N2)	F	G	I	3A	F	2A	F	1E
15	A/swine/Italy/66945/2006 (H3N1)	F	G	I	3A	F	1F	F	1E
16	A/swine/Sweden/1021/2009 (H1N2)	F	G	I	1C	F	2A	F	1E
17	A/swine/Italy/2064/1999 (H1N2)	F	G	I	1C	F	2A	F	1E
18	A/swine/Germany/SEk1178/2000 (H1N2)	/	/	/	1C	F	2A	F	1E

Fig. 2 Phylogenetic trees. 125 representative sequences were aligned and used to infer the evolutionary relationships using the neighbor-joining method. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007). Avian-like swine influenza virus sequences are indicated in *blue*, human-like H3N2 in *ochre*, human-like H1N2 in *red*, seasonal H1N1 in *green*, and seasonal H3N2 in *pink*. Strain designations were omitted for clarity. **a** HAH1 sequences, **b** NAN1 sequences, **c** HAH3 sequences, **d** NAN2 sequences, **e** genetic composition of seasonal H1N1, H3N2 lineages, the prevalent European swine H1N1, H1N2, and H3N2 sublineages and 18 reassortants. Genotyping was done according to Lu et al. 2007

parental human H3N2 virus was clearly distinct from that of the previous German strain (Fig. 2c, d; see also Schrader and Süss 2004). In most European countries the seroprevalence in 2002/2003 of the human-like swine H3N2 is lower than that of avian-like H1N1. Human-like swine H3N2 is (almost) absent in Poland and the Czech Republic, very low in Ireland (4.2 %), and below 60 % in Belgium and Germany. Only in Italy and Spain H3N2 prevalences are as high as H1N1 prevalences (Van Reeth et al. 2008; Simon-Grifé et al. 2010).

6.4 Emergence of Human-Like H1N2 in European Pigs

Swine H1N2 viruses that became prevalent in Europe were first isolated in Great Britain in 1994 (Brown et al. 1995; 1998). Available sequence data indicate that these H1N2 viruses resulted from repeated reassortment events involving a seasonal A/Chile/1/1983-like H1N1 virus (donor of HA) and a seasonal H3N2 virus (donor of NA) (Fig. 2a, d). Apparently, human H3N2 viruses circulated in pigs unrecognized for several years, as one member of this clade was already isolated in 1991 (A/swine/UK/119404/1991) (compare Zell et al. 2008b, therein Fig. 1b). Since the avian-like swine H1N1, but no human-like H3N2 viruses, circulated among British pig during that time, it has to be concluded that the former viruses were the donor of the internal segments. Three years later, the human-like swine H1N2 viruses spread to the European mainland: France (1997), Italy (1998), Belgium (1999), and Germany (2000) (Marozin et al. 2002; Van Reeth et al. 2000; Schrader and Süss 2003). In 2002/2003, the seroprevalences of H1N2 in Belgium and Spain exceeded that of human-like H3N2; it was low in Germany (32.1 %) and Italy (13.8 %) and very low in the Czech Republic (3 %) and Ireland (0.6 %) (Van Reeth et al. 2008).

The evolution of the three prevalent sublineages of the European swine influenza viruses is schematically depicted in Fig. 3.

6.5 Other Reassortant Swine Influenza Viruses Isolated in Europe

Sooner or later co-circulation of two or more influenza virus types within a population leads to reassortant viruses, but such reassortants may have little chance to replace either parent virus. The three prevalent European swine influenza viruses gave rise to three groups of reassortants. The first group comprises reassortants of seasonal human H3N2 and swine influenza viruses (Fig. 4). The strains A/swine/Potsdam/35/1982, A/swine/Karrenzien/2/1987, and A/swine/Leipzig/145/1992 (Schrader and Süss 2004) are examples of swine H3N2 viruses which emerged independently of each other in Germany. They have the six internal gene segments

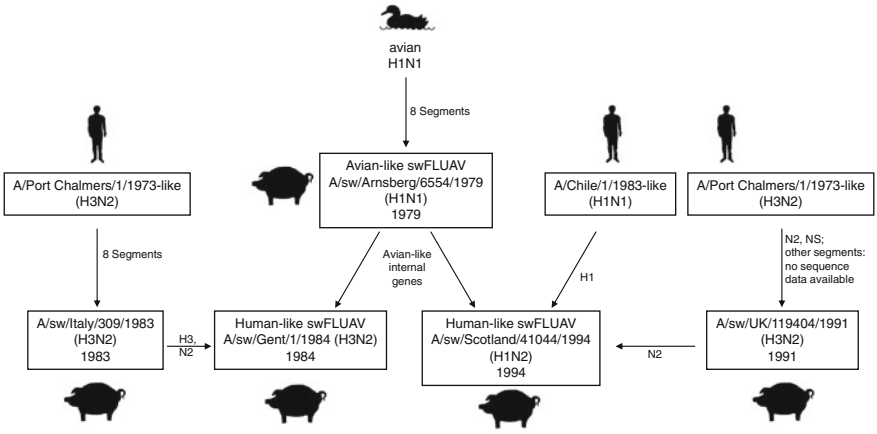


Fig. 3 Evolution of three prevalent sublineages of European swine influenza viruses

of avian-like H1N1 swine viruses and human HA and NA genes. These genes, however, branch independently of A/swine/Gent/1/1984-like viruses in phylogenetic trees (compare Fig. 2c, d) and are evidence of repeated 2 + 6 reassortments in pigs. Although only partial sequence data are available, further strains e.g., A/swine/Jena/3765/1989 (H3N2), A/swine/Leipzig/663/1992 (H3N2), and A/swine/Leipzig/318/1993 (H3N2), belong to this group and indicate that such reassortants may have circulated for 2–3 years. The second group of reassortants emerged in Italy. The preliminary characterization revealed a 7 + 1 reassortment between human H3N2 and swine H1N2 influenza viruses. These viruses have a neuraminidase gene of seasonal H3N2 viruses and seven segments (HA, internal genes) of human-like swine H1N2 viruses (Chiapponi et al. 2007). They circulated in Italy between 2003 and 2006.

The third group comprises reassortants between the prevalent sublineages of European swine influenza viruses. The three sublineages allow six HA/NA combinations and all of them have been detected in recent years. The compilation of Fig. 4 illustrates that several of these reassortments occurred repeatedly at different places and times: Some of the reassortants were published (Gourreau et al. 1994; Balint et al. 2009; Zell et al. 2008a, b), for others only preliminary reports are available (Chiapponi et al. 2007; Franck et al. 2007; Hjulsager et al. 2006). Such reassortants do not constitute antigenic shift mutants and failed to establish persistent stable infection chains yet.

Another rather unusual reassortant was isolated from pigs in England (Brown et al. 1994). The strain A/swine/England/191974/1992 (H1N7) was reported to comprise six segments of a human H1N1 virus (PB2, PB1, PA, HA, NP, NS) and the NA and M segments of an equine H7N7 virus (Brown et al. 1997a). Sequence data of the HA, NP, NA, and M segments are available in the GenBank. Although this virus represents an interesting reassortment, it has to be considered with some caution as the NA and M genes have a striking similarity to A/equine/Prague/1/

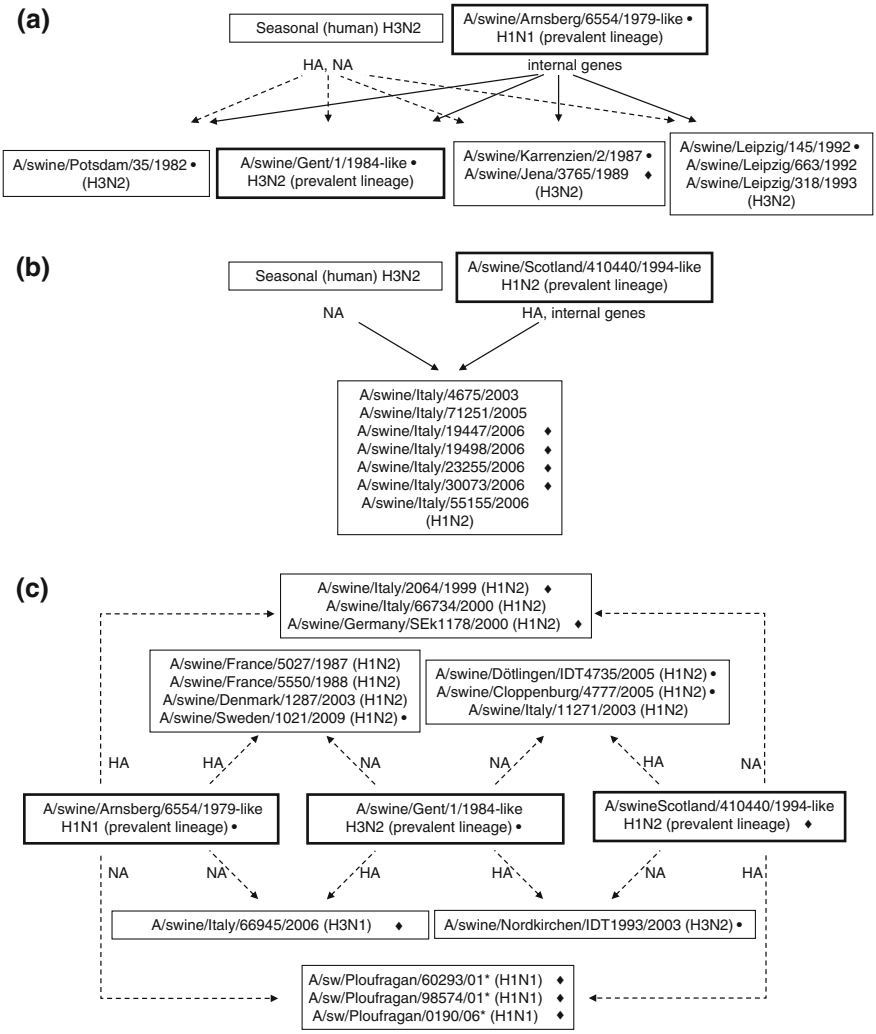


Fig. 4 Three groups of reassortant swine influenza viruses emerged in Europe. **a** 6 + 2 reassortants of avian-like swine H1N1 and human H3N2 influenza viruses. **b** 7 + 1 reassortants of human-like swine H1N2 and human H3N2 viruses. **c** Six different reassortants of the three prevalent European swine influenza virus lineages. The prevalent swine influenza virus lineages are boxed with bold lines. *Filled circles* (•) indicate the availability of complete sequence data in the GenBank, *filled diamonds* (◆) indicate partial sequence data. * Strains designations follow those of Franck et al. (2007); these strains were renamed when their sequences were deposited in the GenBank

1956 (H7N7). All other equine H7N7 sequences available from the GenBank (isolates of 1966–1977) show synonymous substitutions as a consequence of genetic drift and therefore differ significantly from A/equine/Prague/1/1956.

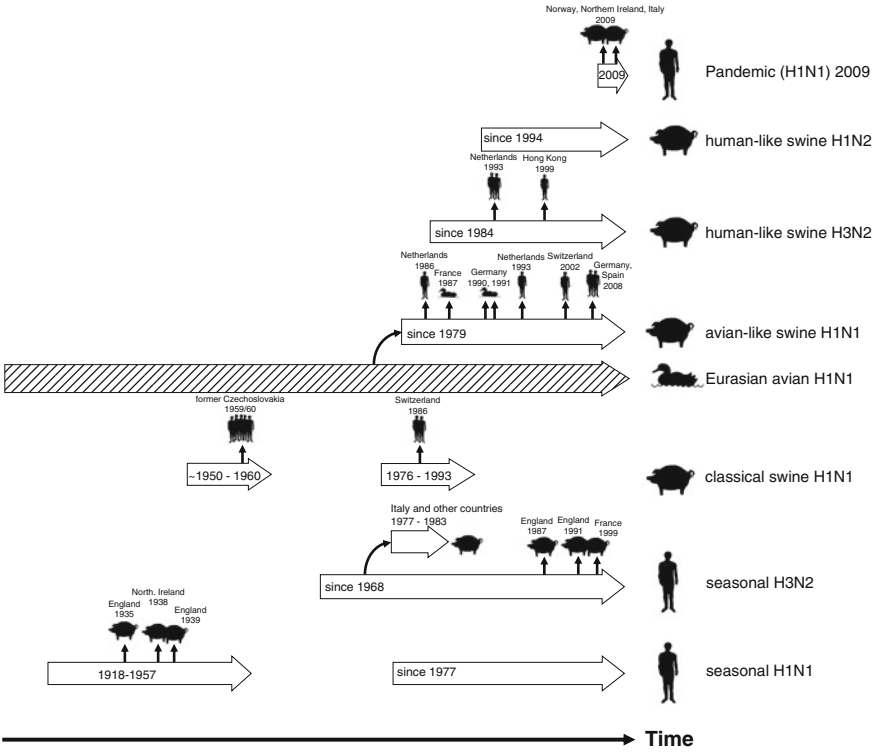


Fig. 5 Zoonotic infections of influenza viruses in Europe over time. Indicated are the relevant lineages of human, avian, swine influenza viruses in Europe and characteristic virus isolates

Equine H7N7 viruses disappeared around 1977 and it is quite astounding that an A/equine/Prague/1/1956-like virus should have persisted in an unknown reservoir for 36 years without accumulation of synonymous substitutions. Therefore, the biological significance of this reassortant should be scrutinized.

7 Zoonotic Infections

7.1 Human→Swine Infections

Human H1N1 influenza viruses have only a limited capacity to productively infect pigs (Hinshaw et al. 1978). However, there are several swine isolates of human origin from the 1930s which were isolated from clinically ill pigs (Lamont 1938; Blakemore and Gledhill 1941). Serological similarity of these strains to contemporary human strains was already observed by Glover (1941). Evidence for zoonotic infections of pigs by human H1N1 was also presented by Shope (1938). Thereafter,

human–swine infections with H1N1 have not been documented in Europe, especially not after reemergence of H1N1 in 1977 (Fig. 5). However, there is indirect evidence that such infections may have occurred: (i) the emergence of H1N2 reassortants in swine in Europe (Brown et al. 1998; Marozin et al. 2002), and (ii) the observed antibody prevalence to human H1N1 in pig sera (Aymard et al. 1980). In Japan and China, several studies demonstrate the transmission of seasonal H1N1 to pigs as shown by virus isolation and seroprevalence studies (Goto et al. 1988; Katsuda et al. 1995; Nerome et al. 1982; Yu et al. 2007).

The human H2N2 viruses have never been isolated from pigs after natural infection, although there is one study that showed antibodies against H2N2 in four pigs in the former Czechoslovakia (Kaplan and Payne 1959). In principle, pigs are susceptible to these viruses as experimental infection of pigs with A/Singapore/1/1957 (H2N2) was successful (Patocka et al. 1958).

Seasonal H3N2 viruses were frequently detected in pigs in Europe and elsewhere. This is documented in several serological studies from Germany, the UK, France, Romania, and the Czech Republic (Sandow and Wildfuhr 1970; Harkness et al. 1972; Popovici et al. 1972; Aymard et al. 1980; Tumova et al. 1980; Pospisil et al. 2001). Occasionally, human H3N2 viruses were isolated from pigs (A/swine/England/163266/1987, A/swine/United Kingdom/119404/1991; Brown et al. 1998). In Italy, A/England/42/1972-like H3N2 viruses persisted from 1977–1983 in pigs (Ottis et al. 1982; Castrucci et al. 1993). Moreover, zoonotic infections with seasonal H3N2 gave rise to numerous reassortant viruses: the European human-like swine H3N2 lineage and the American tripple reassortants are the most prominent representatives (Castrucci et al. 1993; Olsen 2002; Karasin et al. 2000c). Figure 5 shows a compilation of zoonotic influenza virus infections in Europe.

The pandemic (H1N1) 2009 virus was repeatedly transmitted to pigs, first in Canada (Pasma and Joseph 2010), later in Norway, Northern Ireland, Italy (Hofshagen et al. 2009; Welsh et al. 2010; Moreno et al. 2010), and other European countries. The available reports indicate that this new reassortant induces a mild disease (Brookes et al. 2010; Itoh et al. 2009; Lange et al. 2009) and that many infections may be unrecognized. In addition, pandemic (H1N1) 2009 virus exhibits a significant cross-reaction to antibodies against avian-like swine H1N1 which impedes serological distinction (Kyriakis et al. 2010; Dürrwald et al. 2010). On the other hand, this cross-reactivity may hinder establishment of the pandemic virus in regions with high prevalence of avian-like swine H1N1.

7.2 Bird→Swine Infections

Although an initial bird→swine infection gave rise to the avian-like swine H1N1 sublineage, no discrete infections with avian influenza viruses have been documented in Europe. A similar observation was previously made in North America, when the genetic origin of 73 swine isolates (1976–1990) was investigated and no

entry of avian genes could be detected (Wright et al. 1992). Later studies, however, revealed several of such infections in Asia, America, and Africa (Guan et al. 1996; El-Sayed et al. 2010; Karasin et al. 2000a, b; 2004; Yu et al. 2007, 2008; Peiris et al. 2001; Lee et al. 2009).

7.3 Swine→Bird Infections

In the 1980s and early 1990s, at least three swine-origin strains were isolated from birds (Andral et al. 1985; Ludwig et al. 1994; Wood et al. 1997). Partial genetic analyses revealed a reintroduction of avian-like swine H1N1 viruses into turkey farms (Ludwig et al. 1994). Improved hygiene in poultry husbandry and advanced adaptation of the swine H1N1 to its pig host may explain the failure of virus isolation in recent years in Europe. Human-like swine H3N2 and H1N2 strains have not been isolated from birds yet. Absence of α-2,6-linked sialic acids in poultry may be the main reason for the inability of human-like H3N2 and H1N2 sublineages to replicate in birds. The chapter by Yassine and colleagues on "Interspecies transmission of Influenza A viruses between swine and poultry" in this book described these interspecies infections in more detail.

7.4 Swine→Wild Boar Infections

In principle, wild boars should be susceptible to influenza viruses of swine and avian origin and may serve as a reservoir for such viruses. Although they have contacts to feral birds, the possibility of a transmission of avian influenza viruses to feral pigs is only insufficiently investigated in Europe. However, several serological studies searched for antibodies to swine influenza viruses in wild boars (recently reviewed in Kuntz-Simon and Madec 2009). Antibodies to avian-like swine H1N1 influenza viruses in feral pigs were detectable in Spain, Poland, and Croatia but not in Slovenia, Russia, and Ukraine. Another recent study demonstrated antibodies to avian-like swine H1N1 and human-like swine H3N2 viruses in Germany (Kaden et al. 2008). Two virus isolates described in that study [A/wild boar/WS169/2006 (H3N2), A/wild boar/WS188/2006 (H3N2)] should be considered with caution. The sequences of both isolates are identical and the published sequences of five different gene segments (HA, NP, NA, M, NS) show a sequence identity of nearly 100 % to A/swine/Bakum/909/1993 (H3N2) which was used as a H3N2 control in this study. Since influenza viruses exhibit a genetic drift due to the accumulation of synonymous and non-synonymous substitutions, one would expect some genetic variation in the course of several hundred virus generations (1993–2006). The wild boar isolates obviously lack this genetic drift.

Table 2 Zoonotic infection

No.	Country	Years	No. of patients	Type	Designation	Reference
1	Former Czechoslovakia	1959	6	Classical swine H1N1	No information available	Kluska et al. (1960)
2	Switzerland	1986	2	Avian-like swine H1N1	No information available	de Jong et al. (1986)
3	The Netherlands	1986	1	Avian-like swine H1N1	A/Netherlands/386/1986	de Jong et al. (1986); Rimmelzwaan et al. (2001)
4	The Netherlands	1993	1	Avian-like swine H1N1	A/Netherlands/477/1993	Rimmelzwaan et al. (2001)
5	The Netherlands	1993	2	Human-like swine H3N2	A/Netherlands/5/1993, A/Netherlands/35/1993	Claas et al. (1994)
6	Hong Kong	1999	1	Human-like swine H3N2	A/Hong Kong/1774/1999	Gregory et al. (2001)
7	Switzerland	2002	1	Avian-like swine H1N1	A/Switzerland/8808/2002	Gregory et al. (2003)
8	Germany	2007	1	Avian-like swine H1N1	A/Niedersachsen/58/2007	Schweiger et al. (2008)
9	Spain	2008	1	Avian-like swine H1N1	A/Aragon/RR3218/2008	Adiego Sancha et al. (2009)

7.5 Swine→Human Infections

Swine-to-human transmissions of classical swine H1N1 influenza viruses were first observed in Czechoslovakia in the 1950s (Kluska et al. 1961). Since then, sporadic infections were repeatedly demonstrated by virus isolation in the United States, Europe, and the Asian part of the former Soviet Union (reviewed in Myers et al. 2007). Several incidents of human infection with the European avian-like H1N1 and human-like H3N2 swine influenza viruses have been reported so far (Table 2) (Adiego Sancho et al. 2009; Claas et al. 1994; de Jong et al. 1986; Gregory et al. 2001, 2003; Rimmelzwaan et al. 2001; Schweiger et al. 2008). Apparently, zoonotic infections with the European swine viruses cause a benign disease with mild flu-like symptoms, whereas infections with classical swine strains may lead to more serious symptoms—few fatalities after infections with the latter viruses were reported (Myers et al. 2007). Despite repeated isolation of swine influenza viruses from human specimens, the prevalence of zoonotic infections in Europe is largely obscure. Previous work demonstrated seropositivity of personnel having contact to diseased pigs (Aymard et al. 1980; Sinnecker et al. 1983). A recent study conducted in Thuringia, Germany, indicates that approximately 15 % of the investigated sera of occupationally exposed humans (pig farmers, slaughterers, veterinarians) exhibit antibodies to the European lineages of swine influenza viruses (Krumbholz et al. 2010).

References

Adiego Sancho B, Omenaca Teres M, Martinez Cuenca S et al (2009) Human case of swine influenza A (H1N1), Aragon, Spain, November 2008. Eurosurveillance 14:1–2

Andral B, Toquin D, Madec F, Aymard M, Gourreau JM, Kaiser C, Fontaine M, Metz MH (1985) Disease in turkeys associated with H1N1 influenza virus following an outbreak of the disease in pigs. Vet Rec 116:617–618

Anhlan D, Grundmann N, Makalowski W, Ludwig S, Scholtissek C (2011) Origin of the 1918 pandemic H1N1 influenza A virus as studied by codon usage patterns and phylogenetic analysis. RNA 17:64–73

Aymard M, Brigaud M, Chastel C, Fontaine M, Tillon JP, Vannier P (1980) Comparison of influenza antibody serologic immunity in man and in pig. Comp Immunol Microbiol Infect Dis 3:111–119

Balint A, Metreveli G, Widen F, Zohari S, Berg M, Isaksson M, Renström LHM, Wallgren P, Belak S, Segall T, Kiss I (2009) The first Swedish H1N2 swine influenza virus isolate represents an uncommon reassortant. Virol J 6:180

Bironf P, Meulemans G, Charlier G, Castrijck F (1980) Isolation of an influenza A virus related to the New Jersey strain (Hsw1N1) in fattening pigs. Vlaams Diergeneeskundig Tijdschrift 49:8–11

Blakemore F, Gledhill AW (1941) Discussion on swine influenza in the British Isles. Proc R Soc Med 34:611–615

Brookes SM, Nunez A, Choudhury B et al (2010) Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in non-immune pigs, PLOS One 5:e9068

Brown IH (2000) The epidemiology and evolution of influenza viruses in pigs. Vet Microbiol 74:29–46

Brown IH, Alexander DJ, Chakraverty P, Harris PA, Manvell RJ (1994) Isolation of an influenza A virus of unusual subtype (H1N7) from pigs in England, and the subsequent experimental transmission from pig to pig. Vet Microbiol 39:125–134

Brown IH, Chakraverty P, Harris PA, Alexander DJ (1995) Disease outbreaks in pigs in Great Britain due to an influenza A virus of H1N2 subtype. Vet Rec 136(13):328–329

Brown IH, Hill ML, Harris PA, Alexander DJ, McCauley JW (1997a) Genetic characterisation of an influenza A virus of unusual subtype (H1N7) isolated from pigs in England. Arch Virol 142:1045–1050

Brown IH, Ludwig S, Olsen CW, Hannoun C, Scholtissek C, Hinshaw VS, Harris PA, McCauley JW, Strong I, Alexander DJ (1997b) Antigenic and genetic analyses of H1N1 influenza A viruses from European pigs. J Gen Virol 78:553–562

Brown IH, Harris PA, McCauley JW, Alexander DJ (1998) Multiple genetic reassortment of avian and human influenza A viruses in European pigs, resulting in the emergence of an H1N2 virus of novel genotype. J Gen Virol 79:2947–2955

Bush RM, Bender CA, Subbarao K, Cox NJ, Fitch WM (1999a) Predicting the evolution of human influenza A. Science 286:1921–1925

Bush RM, Fitch WM, Bender CA, Cox NJ (1999b) Positive selection on the H3 hemagglutinin of human influenza virus A. Mol Biol Evol 16(11):1457–1465

Campitelli L, Donatelli I, Foni E, Castrucci MR, Fabiani C, Kawaoka Y, Krauss S, Webster RG (1997) Continued evolution of H1N1 and H3N2 influenza viruses in pigs in Italy. Virology 232:310–318

Castro JM, del Pozo M, Simarro I (1988) Identification of H3N2 influenza virus isolated from pigs with respiratory problems in Spain. Vet Rec 122:418–419

Castrucci MR, Donatelli I, Sidoli L, Barigazzi G, Kawaoka Y, Webster RG (1993) Genetic reassortment between avian and human influenza A viruses in Italian pigs. Virology 193:503–506

Chiapponi C, Barbieri I, Manfredi R, Zanni I, Barigazzi G, Foni E (2007) Genetic diversity among H1N1 and H1N2 swine influenza viruses in Italy: preliminary results. In: Markowska-Daniel I (ed) 5th international symposium on emerging and re-emerging pig diseases. Krakow, Poland, p 261

Chu CM, Dawson IM, Elford WJ (1949) Filamentous forms associated with newly isolated influenza virus. Lancet 253:602–603

Claas ECJ, Kawaoka Y, De Jong JC, Masurel N, Webster RG (1994) Infection of children with avian-human reassortant influenza virus from pigs in Europe. Virology 204:453–457

Compans RW, Content J, Duesberg PH (1972) Structure of the ribonucleoprotein of influenza virus. J Virol 10(4):795–800

de Jong JC, de Ronde-Verloop JM, Bangma PJ, van Kregten E, Kerckhaert J, Paccaud MF, Wicki F, Wunderli W (1986) Isolation of swine-influenza-like A(H1N1) viruses from man in Europe. Lancet 328(8519):1329–1330

Duesberg PH (1968) The RNA's of influenza virus. Proc Natl Acad Sci USA 59:930–937

Dürrwald R, Krumbholz A, Baumgarte S, Schlegel M, Vahlenkamp TW, Selbitz HJ, Wutzler P, Zell R (2010) Swine influenza A vaccines, pandemic (H1N1) 2009 virus, and cross-reactivity. Emerg Inf Dis 16:1029–1030

Elkeles G (1934) Experimentelle Untersuchungen zur Aetiologie der Influenza (in German). Mededeelingen uit het Instituut voor Praeventieve Geneeskunde 1934:60–79

El-Sayed A, Awad W, Fayed A, Hamann HP, Zschöck M (2010) Avian influenza prevalence in pigs. Egypt. Emerg Inf Dis 16:726–727

Franck N, Queguiner S, Gorin S et al (2007). Molecular epidemiology of swine influenza virus in France: identification of novel H1N1 reassortants. In: Markowska-Daniel I (ed) 5th international symposium on emerging and re-emerging pig diseases, Krakow, Poland, p 250, 24–27 June 2007

Glover RE (1941) Discussion on swine influenza in the British Isles. Proc R Soc Med 34:615–617

Gorman OT, Bean WJ, Kawaoka Y, Donatelli I, Guo Y, Webster RG (1991) Evolution of influenza A virus nucleoprotein genes: implications for the origins of H1N1 human and classical swine viruses. J Virol 65(7):3704–3714

Goto H, Ogawa Y, Hirano T, Miwa Y, Piao FZ, Takai M, Noro S, Sakurada N (1988) Antibody responses of swine to type A influenza viruses during the past ten years in Japan. Epidemiol Infect 100:523–526

Gourreau JM, Kaiser C, Hannoun C, Vaissaire J, Gayot G (1980) First isolation in France of swine influenza virus (Hsw1N1) from a disease outbreak involving different microorganisms. Bulletin de l'Academie Veterinaire de France 53:181–188

Gourreau JM, Hannoun C, Kaiser C (1981) Diffusion du virus de la grippe du porc (Hsw1 N1) en France [in French]. Ann Virol (Inst Pasteur) 132E:287–294

Gourreau JM, Kaiser C, Valette M, Douglas AR, Labie J, Aymard M (1994) Isolation of two H1N2 influenza viruses from swine in France. Arch Virol 135:365–382

Gregory V, Lim W, Cameron K et al (2001) Infection of a child in Hong Kong by an influenza A H3N2 virus closely related to viruses circulation in European pigs. J Gen Virol 82:1397–1406

Gregory V, Bennett M, Thomas Y, Kaiser L, Wunderli W, Matter H, Hay A, Lin YP (2003) Human infection by a swine influenza A (H1N1) virus in Switzerland. Arch Virol 148:793–802

Guan Y, Shortridge KF, Krauss S, Li PH, Kawaoka Y, Webster RG (1996) Emergence of avian H1N1 influenza viruses in pigs in China. J Virol 70(11):8041–8046

Haesebrouck F, Pensaert M (1988) Influenza in swine in Belgium (1969–1986): epizootiologic aspects. Comp Immunol Microbiol Infect Dis 11:215–222

Haesebrouck F, Biront P, Pensaert MB, Leunen J (1985) Epizootics of respiratory tract disease in swine in Belgium due to H3N2 influenza virus and experimental reproduction of disease. Am J Vet Res 46:1926–1928

Harkness JW, Schild GC, Lamont PH, Brand CM (1972) Studies on relationships between human and porcine influenza. Bull WHO 46:709–719

Harnach R, Hubik R, Chivatal O (1950) Isolation of influenza virus in Czechoslovakia. Casopis Ceskoslavenskych Veterinaru 5:289

Hensley SE, Das SR, Bailey AL, Schmidt LM, Hickman HD, Jayaraman A, Viswanathan K, Raman R, Sasisekharan R, Bennink JR, Yewdell JW (2009) Hemagglutinin receptor binding avidity drives influenza A virus antigenic drift. Science 326:734–736

Hinshaw VS, Bean Jr WJ, Webster RG, Easterday BC (1978) The prevalence of influenza viruses in swine and the antigenic and genetic relatedness of influenza viruses from man and swine. Virology 84:51–62

Hjulsager CK, Bragstad K, Botner A, Nielsen EO, Vigre H, Enoe C, Larsen LE (2006). New swine influenza A H1N2 reassortment found in Danish swine. In: Proceedings of the 19th IPVS congress, Copenhagen. Abstract No. 0.55–03, p 265

Hofshagen M, Gjerset B, Er C et al (2009) Pandemic influenza A(H1N1)v: human to pig transmission in Norway? Eurosurveillance 14:pii:19406

Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, VandePol S (1982) Rapid evolution of RNA genomes. Science 215:1577–1585

Ito T, Kawaoka Y (2000) Host-range barrier of influenza A viruses. Vet Microbiol 74:71–75

Ito T, Couceiro JNSS, Kelm S, Baum LG, Krauss S, Castrucci MR, Donatelli I, Kida H, Paulson JC, Webster RG, Kawaoka Y (1998) Molecular Basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol 72(9):7367–7373

Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, Muramoto Y, Tamura D, Sakai-Tagawa Y, Noda T, Sakabe S, Imai M, Hatta Y, Watanabe S, Li C, Yamada S, Fujii K, Murakami S, Imai H, Kakugawa S, Ito M, Takano R, Iwatsuki-Horimoto K, Shimojima M, Horimoto T, Goto H, Takahashi K, Makino A, Ishigaki H, Nakayama M, Okamatsu M, Takahashi K, Warshauer D, Shult PA, Saito R, Suzuki H, Furuta Y, Yamashita M, Mitamura K, Nakano K, Nakamura M, Brockman-Schneider R, Mitamura H, Yamazaki M, Sugaya N, Suresh M, Ozawa M, Neumann G, Gern J, Kida H, Ogasawara K, Kawaoka Y (2009) In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature 460:1021–1025

Kaden V, Lange E, Starick E, Brue W, Krakowski W, Klopries M (2008) Epidemiological survey of swine infuenza A virus in selected wild boar populations in Germany. Vet Microbiol 131:123–132

Kaplan MM, Payne AMM (1959) Serological survey in animals for type A influenza in relation to the 1957 pandemic. Bull WHO 20:465–488

Karasin AI, Brown IH, Carman S, Olsen CW (2000a) Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada. J Virol 74:9322–9327

Karasin AI, Olsen CW, Brown IH, Carman S, Stalker M, Josephson G (2000b) H4N6 influenza virus isolated from pigs in Ontario. Can Vet J 41:938–939

Karasin AI, Schutten MM, Cooper LA, Smith CB, Subbarao K, Anderson GA, Carman S, Olsen CW (2000c) Genetic characterization of H3N2 influenza viruses isolated from pigs in North America, 1977–1999: evidence for wholly human and reassortant virus genotypes. Virus Res 68:71–85

Karasin AI, West K, Carman S, Olsen CW (2004) Characterization of avian H3N3 and H1N1 influenza A viruses isolated from pigs in Canada. J Clin Microbiol 42:4349–4354

Karlas A, Nachuy N, Shin Y, Pleissner KP, Artarini A, Heuer D, Becker D, Khalil H, Ogilvie LA, Hess S, Mäurer AP, Müller E, Wolff T, Rudel T, Meyer TF (2010) Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. Nature 463:818–822

Katsuda K, Sato S, Shirahata T, Lindstrom S, Nerome R, Ishida M, Nerome K, Goto H (1995) Antigenic and genetic characteristics of H1N1 human influenza virus isolated from pigs in Japan. J Gen Virol 76:1247–1249

Kawaoka Y, Cox NJ, Haller O et al (2005) Family orthomyxoviridae. In: Fauquet CM (eds). Virus taxonomy. Eight report of the international committee on taxonomy of viruses, Elsevier Academic Press, Amsterdam, pp 681–693

Kilbourne ED (1968) Recombination of influenza A viruses of human and animal origin. Science 160:74–76

Kluska V, Macku M, Mensik J (1961) Demonstration of antibodies against swine influenza viruses in man (in Czech). Cesk Pediatr 16:408–414

Köbe K (1933) Die Aetiologie der Ferkelgrippe (enzootische Pneumonie des Ferkels) (in German). Zentralbl Bakt 1. Abt 129:161–176

Koen JS (1919) A practical method for field diagnosis of swine diseases. Am J Vet Med 14: 468–470

König R, Stertz S, Zhou Y, Inoue A, Hoffmann HH, Bhattacharyya S, Alamares JG, Tscherne DM, Ortigoza MB, Liang Y, Gao Q, Andrews SE, Bandyopadhyay S, De Jesus P, Tu BP, Pache L, Shih C, Orth A, Bonamy G, Miraglia L, Ideker T, García-Sastre A, Young JA, Palese P, Shaw ML, Chanda SK (2010) Human host factors required for influenza virus replication. Nature 463:813–817

Krauss S, Obert CA, Franks J, Walker D, Jones K, Seiler P, Niles L, Pryor SP, Obenauer JC, Naeve CW, Widjaja L, Webby RJ, Webster RG (2007) Influenza in migratory birds and evidence of limited intercontinental virus exchange. PLoS Pathog 3:e167

Krumbholz A, Schmidtke M, Bergmann S, Motzke S, Bauer K, Stech J, Dürrwald R, Wutzler P, Zell R (2009) High prevalence of amantadine resistance among circulating European porcine influenza A viruses. J Gen Virol 90:900–908

Krumbholz A, Lange J, Dürrwald R, Hoyer H, Bengsch S, Wutzler P, Zell R (2010) Prevalence of antibodies to swine influenza viruses in humans with occupational exposure to pigs, Thuringia, Germany, 2008–2009. J Med Virol 82:1617–1625

Kuiken T, Holmes EC, McCauley J, Rimmelzwaan GF, Williams CS, Grenfell BT (2006) Host species barriers to influenza virus infections. Science 312:394–397

Kuntz-Simon G, Madec F (2009) Genetic and antigenetic evolution of swine influenza viruses in Europe and evaluation of their zoonotic potential. Zoonoses Public Health 56:310–325

Kyriakis CS, Olsen CW, Carman S, Brown IH, Brookes SM, Van Doorsselaere J, Van Reeth K (2010) Serologic cross-reactivity with pandemich (H1N1) 2009 virus in pigs, Europe. Emerg Inf Dis 16:96–99

Lamont HG (1938) The problems of the practitioner in connection with the differential diagnosis and treatment of diseases of young pigs. Vet Rec 50:1377

Lange E, Kalthoff D, Blohm U, Teifke JP, Breithaupt A, Maresch C, Starick E, Fereidouni S, Hoffmann B, Mettenleiter TC, Beer M, Vahlenkamp TW (2009) Pathogenesis and transmission of the novel swine-origin influenza virus A/H1N1 after experimental infection of pigs. J Gen Virol 90:2119–2123

Lee JH, Pascua PN, Song MS, Baek YH, Kim CJ, Choi HW, Sung MH, Webby RJ, Webster RG, Poo H, Choi YK (2009) Isolation and genetic characterization of H5N2 influenza viruses from pigs in Korea. J Virol 83(9):4205–4215

Loeffen WL, Kamp EM, Stockhofe-Zurwieden N, van Nieuwstadt AP, Bongers JH, Hunneman WA, Elbers AR, Baars J, Nell T, van Zijderveld FG (1999) Survey of infectious agents involved in acute respiratory disease in finishing pigs. Vet Rec 145:123–129

Lu G, Rowley T, Garten R, Donis RO (2007) FluGenome: a web tool for genotyping influenza A virus. Nucl Acids Res 35:W275–W279

Ludwig S, Haustein A, Kaleta EF, Scholtissek C (1994) Recent influenza A (H1N1) infections of pigs and turkeys in Northern Europe. Virology 202:281–286

Madec F, Gourreau JM, Kaiser C, Aymard M (1984) Apparition de manifestations grippales chez les porcs en association avec un virus A/H3N2. Bull Acad Vet Fr 57:513–522

Makarova NV, Kaverin NV, Krauss S, Senne D, Webster RG (1999) Transmission of Eurasian avian H2 influenza virus to shorebirds in North America. J Gen Virol 80:3167–3171

Marozin S, Gregory V, Cameron K, Bennett M, Valette M, Aymard M, Foni E, Barigazzi G, Lin Y, Hay A (2002) Antigenic and genetic diversity among swine influenza A H1N1 and H1N2 viruses in Europe. J Gen Virol 83:735–745

Martinsson K, Klingeborn B, Rockborn G (1983) Utbrott av influenza suis i Sverige. (in Swedish). Svensk Vet Tidn 35:537

Moreno A, Di Trani L, Alborali L, Vaccari G, Barbieri I, Falcone E, Sozzi E, Puzelli S, Ferri G, Cordioli P (2010) First pandemic H1N1 outbreak from a pig farm in Italy. Open Virol J 4:52–56

Munster VJ, Baas C, Lexmond P, Waldenström J, Wallensten A, Fransson T, Rimmelzwaan GF, Beyer WEP, Schutten M, Olsen B, Osterhaus ADME (2007) Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS Path 3(5):e61

Myers KP, Olsen CW, Gray GC (2007) Cases of swine influenza in humans: a review of the literature. Clin Infect Dis 44:1084–1088

Nardelli L, Pascucci S, Gualandi GL, Loda P (1978) Outbreaks of classical swine influenza in Italy in 1976. Zentralbl Veterinarmed B 25:853–857

Nerome K, Ishida M, Oya A, Kanai C, Suwicha K (1982) Isolation of an influenza H1N1 virus from a pig. Virology 117:485–489

Neumeier E, Meier-Ewert H (1992) Nucleotide sequence analysis of the HA1 coding portion of the haemagglutinin gene of swine H1N1 influenza viruses. Virus Res 23:107–117

Neumeier E, Meier-Ewert H, Cox NJ (1994) Genetic relatedness between influenza A (H1N1) viruses isolated from humans and pigs. J Gen Virol 75:2103–2107

Nobusawa E, Sato K (2006) Comparison of the mutation rates of human influenza A and B viruses. J Virol 80(7):3675–3678

Noda T, Sagara H, Yen A, Takada A, Kida H, Cheng RH, Kawaoka Y (2006) Architecture of ribonucleoprotein complexes in influenza A virus particles. Nature 439:490–492

Olsen CW (2002) The emergence of novel swine influenza viruses in North America. Virus Res 85:199–210

Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus AD, Fouchier RA (2006) Global patterns of influenza A virus in wild birds. Science 312:384–388

Ottis K, Bachmann PA (1980) Occurrence of Hsw1N1 subtype influenza A viruses in wild ducks in Europe. Arch Virol 63:185–190

Ottis K, Bollwahn W, Bachmann PA, Heinritzi K (1981) Ausbruch von Schweineinfluenza in der Bundesrepublik Deutschland: Klinik, Nachweis und Differenzierung (in German). Tierärztl Umsch 36:608–612

Ottis K, Sidoli L, Bachmann PA, Webster RG, Kaplan MM (1982) Human influenza a viruses in pigs: isolation of a H3N2 strain antigenically related to A/England/42/72 and evidence for continuous circulation of human viruses in the pig population. Arch Virol 73:103–108

Parvin JD, Moscona A, Pan WT, Leider JM, Palese P (1986) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. J Virol 59(2):377–383

Pasma T, Joseph T (2010) Pandemic (H1N1) 2009 infection in swine herds, Manitoba, Canada. Emerg Inf Dis 16:706–708

Patocka F, Schreiber E, Kubelka V, Korb J, John C, Schön E (1958) An attempt ot transmit the human influenza virus strain A-Sing 57 to swine; preliminary report. J Hyg Epidemiol Microbiol Immunol 2(1):9–15

Peiris JS, Guan Y, Markwell D, Ghose P, Webster RG, Shortridge KF (2001) Cocirculation of avian H9N2 and contemporary human H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment? J Virol 75:9679–9686

Pensaert M, Ottis K, Vandeputte J, Kaplan MM, Bachmann PA (1981) Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. Bull WHO 59(1):75–78

Popovici V, Hiastru F, Draghici D, Zilisteanu E, Matepiuc M, Cretescu L, Niculescu I (1972) Infection of pigs with an influenza virus related to the A2-Hong Kong-1-68 strain. Acta Virol 16:363

Pospisil Z, Lany P, Tumova B, Buchta J, Zendulkova D, Cihal P (2001) Swine influenza surveillance and the impact of human influenza epidemics on pig herds in the Czech Republic. Acta Vet Brno 70:327–332

Rimmelzwaan GF, de Jong JC, Bestebroer TM, van Loon AM, Claas ECJ, Fouchier RAM, Osterhaus ADME (2001) Antigenic and genetic characterization of swine influenza A (H1N1) viruses isolated from pneumonia patients in the Netherlands. Virology 282:301–308

Roberts DH, Cartwright SF, Wibberley G (1987) Outbreaks of classical swine influenza in pigs in England in 1986. Vet Rec 121:53–55

Rogers GN, Paulsen JC (1983) Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. Virology 127:361–373

Sandow D, Wildfuhr W (1970) Comparative serological studies of the influenza strains A2 Hongkong-1-68 and A2 DDR-Berlin in pigs. Monatsh Veterinärmed 25:320–322

Schmidtke M, Zell R, Bauer K, Krumbholz A, Schrader C, Süss J, Wutzler P (2006) Amantadine resistance among porcine H1N1, H1N2, and H3N2 influenza A viruses isolated in Germany between 1981 and 2001. Intervirology 49:286–293

Scholtissek C (1990) Pigs as "mixing vessels" for the creation of new pandemic influenza A viruses. Med Princ Pract 2:65–71

Scholtissek C, Bürger H, Bachmann PA, Hannoun C (1983) Genetic relatedness of hemagglutinins of the H1N1 subtype of influenza A viruses isolated from swine and birds. Virology 129:521–523

Scholtissek C, Bürger H, Kistner O, Shortridge KF (1985) The nucleoprotein as a possible major factor in determining host specificity of influenza H3N2 viruses. Virology 147:287–294

Schrader C, Süss J (2003) Genetic characterization of a porcine H1N2 influenza virus strain isolated in Germany. Intervirology 46:66–70

Schrader C, Süss J (2004) Molecular epidemiology of porcine H3N2 influenza A viruses isolated in Germany between 1982 and 2001. Intervirology 47:72–77

Schultz U, Fitch WM, Ludwig S, Mandler J, Scholtissek C (1991) Evolution of pig influenza viruses. Virology 183:61–73

Schweiger B, Heckler R, Biere B (2008) Characterization of a porcine influenza virus isolated from a human sample. In: Programme and abstracts, abstract CLV2, 18th Annual Meeting Gesellschaft für Virologie, 5–8 March 2008, Heidelberg

Shope RE (1931a) The etiology of swine influenza. Science 73:214–215

Shope RE (1931b) Swine influenza: I. Experimental transmission and pathology. J Exp Med 54:349–362

Shope RE (1938) Serological evidence for the occurrence of infection with human influenza virus in swine. J Exp Med 67:739–748

Shope RE, Francis T (1936) The susceptibility of swine to the virus of human influenza. J Exp Med 64:791–801

Simon-Grifé M, Martin-Valls GE, Vilar MJ (2010) Seroprevalence and risk factors of swine influenza in Spain. Vet Microbiol, (in press) (doi: 10.1016/j.vetmic.2010.10.015)

Sinnecker H, Sinnecker R, Zilske E, Strey A, Leopoldt D (1983) Influenzavirus A/swine-Ausbrüche bei Hausschweinen und Antikörperbefunde in Humanseren [in German]. Zentralbl Bakteriol Mikrobiol Hyg A 255(2–3):209–213

Stech J, Xiong X, Scholtissek C, Webster RG (1999) Independence of evolutionary and mutational rates after transmission of avian influenza viruses to swine. J Virol 73(3):1878–1884

Steinhauer DA (1999) Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology 258:1–20

Subbarao EK, London W, Myrphy BR (1993) A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. J Virol 67(4):1761–1764

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tumova B, Stumpa A, Mensik J (1980) Surveillance of influenza in pig herds in Czechoslovakia in 1974–1979. 2. Antibodies against influenza A (H3N2) and A (H1N1) viruses. Zentralbl Veterinärmed B 27:601–607

Van Reeth K, Brown IH, Pensaert M (2000) Isolations of H1N2 influenza A virus from pigs in Belgium. Vet Rec 146(20):588–589

Van Reeth K, Brown IH, Dürrwald R, Foni E, Labarque G, Lenihan P, Maldonado J, Markowska-Daniel I, Pensaert M, Pospisil Z, Koch G (2008) Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002–2003. Influenza Other Respi Viruses 2:99–105

Vandeputte J, Pensaert M, Castryck F (1980) Serological diagnosis and distribution of swine influenzavirus in Belgium. Vlaams Diergeneeskundig Tijdschrift 49:1–7

Wahlgren A, Waldenström J, Sahlin S, Haemig PD, Fouchier RAM, Osterhaus ADME, Pinhassi J, Bonnedahl J, Pisareva M, Grudinin M, Kiselev O, Hernandez J, Falk KI, Lundkvist A, Olsen B (2008) Gene segment reassortment between American and Asian lineages of avian influenza virus from waterfowl in the Beringia area. Vector-borne Zoon Dis 8(6):783–789

Waldmann O (1933) Die Aetiologie des Ferkelkümmerns. Die Ferkelgrippe. (in German). Berl Tierarztl Wochenschr 44:693–697

Wallensten A, Munster VJ, Elmberg J, Osterhaus ADME, Fouchier RAM, Olsen B (2005) Multiple gene segment reassortment between Eurasian and American lineages of influenza A virus (H6N2) in Guillemot (*Uria aalge*). Arch Virol 150:1685–1692

Webster RG, Bean WJ, Gorman WT, Chambers TM, Kawaoka Y (1992) Evolution and ecology of influenza A viruses. Microbiol Rev 56(1):152–179

Welsh MD, Baird PM, Guelbenzu-Gonzalo MP, Hanna A, Reid SM, Essen S, Russell C, Thomas S, Barrass L, McNeilly F, McKillen J, Todd D, Harkin V, McDowell S, Choudhury B, Irvine RM, Borobia J, Grant J, Brown IH (2010) Initial incursion of pandemic (H1N1) 2009 influenza A virus into European pigs. Vet Rec 166:642–645

Witte KK, Nienhoff H, Ernst H, Schmidt U, Prager D (1981) Erstmaliges Auftreten einer durch das Schweineinfluenzavirus verursachten Epizootie in Schweinebeständen der Bundesrepublik Deutschland [in German]. Tierärztliche Umschau 36(9):591–606

Wood GW, Banks J, Brown IH, Strong I, Alexander DJ (1997) The nucleotide sequence of the HA1 of the haemagglutinin of an HI avian influenza virus isolate from turkeys in Germany provides additional evidence suggesting recent transmission from pigs. Avian Pathol 26:347–355

Wright SM, Kawaoka Y, Sharp GB, Senne DA, Webster RG (1992) Interspecies transmission and reassortment of influenza A viruses in pigs and turkeys in the United States. Am J Epidemiol 136:488–497

Yewdell JW, Webster RG, Gerhard WU (1979) Antigenic variation in three distinct determinants of an influenza type A haemagglutinin molecule. Nature 279:246–248 (17 May 1979)

Yoshioka Y, Sugita S, Kanegae Y, Shortridge KF, Nerome K (1994) Origin and evolutionary pathways of the H1 haemagglutinin gene of avian, swine and human influenza viruses: cocirculation of two distinct lineages of swine virus. Arch Virol 134:17–28

Yu H, Zhang GH, Hua RH, Zhang Q, Liu TQ, Liao M, Tong GZ (2007) Isolation and genetic analysis of human origin H1N1 and H3N2 influenza viruses from pigs in China. Biochem Biophys Res Commun 356:91–96

Yu H, Hua RH, Wei TC, Zhou YJ, Tian ZJ, Li GX, Liu TQ, Tong GZ (2008) Isolation and genetic characterization of avian origin H9N2 influenza viruses from pigs in China. Vet Microbiol 131:82–92

Yus E, Sanjuan ML, Garcia F, Castro JM, Simarro I (1992) Influenza A viruses: epidemiologic study in fatteners in Spain (1987–1989). Zentralbl Veterinärmed B 39:113–118

Zell R, Krumbholz A, Eitner A, Krieg R, Halbhuber KJ, Wutzler P (2007) Prevalence of PB1-F2 of influenza A viruses. J Gen Virol 88:536–546

Zell R, Motzke S, Krumbholz A, Wutzler P, Herwig V, Dürrwald R (2008a) Novel reassortant of swine influenza H1N2 virus in Germany. J Gen Virol 89:271–276

Zell R, Bergmann S, Krumbholz A, Wutzler P, Dürrwald R (2008b) Ongoing evolution of swine influenza viruses: a novel reassortant. Arch Virol 153:2085–2092

Zhang XM, Herbst W, Lange-Herbst H, Schliesser T (1989) Seroprevalence of porcine and human influenza A virus antibodies in pigs between 1986 and 1988 in Hassia. Zentralbl Veterinärmed B 36:765–770

zu Dohna H, Li J, Cardona CJ, Miller J, Carpenter TE (2009). Invasions by Eurasian avian influenza virus H6 genes and replacement of the virus' North American clade. Emerg Inf Dis 15:1040–1045