Epidemiological update on swine influenza (H1N1) in pigs

Shiv Chandra Dubey · G. Venkatesh · Diwakar D. Kulkarni

Received: 28 October 2009 / Accepted: 29 October 2009
© Association of Microbiologists of India 2009

Abstract The 2009 H1N1 pandemic has slowed down its spread after initial speed of transmission. The conventional swine influenza H1N1 virus (SIV) in pig populations worldwide needs to be differentiated from pandemic H1N1 influenza virus, however it is also essential to know about the exact role of pigs in the spread and mutations taking place in pig-to-pig transmission. The present paper reviews epidemiological features of classical SIV and its differentiation with pandemic influenza.

Keywords Swine flu · Influenza · H1N1

For the first time, the full repertoire of molecular biology has been applied to a novel form of influenza spreading on a global scale. It appears that rapid molecular analysis and diagnosis coupled to effective clinical and public health methods have at the very least substantially slowed the progress of the outbreak. None of this was possible in 1957 or even in 1977. This provides an important dress rehearsal for potential H5N1 outbreaks. We still cannot stop the arrival of antigenically shifted pandemic influenzas, but in the past we were unaware of their existence until they were already upon us. In future, we are at least likely to see the dust of an approaching pandemic rising in the distance, giving us crucial time to react.

– Derek Gatherer, 2009 (1)

Introduction

A new swine-origin influenza A (H1N1) virus having the capacity of human-to-human (H2H) transmission initially emerged in Mexico and the United States in March–April 2009 and spread worldwide within a few months. There were more than 41,400 laboratory confirmed human cases and over 5000 deaths up to 17 October 2009 in this pandemic [2], which is supposed to be one of the fastest growing and spreading infectious diseases in the history of mankind. Therefore this virus has attracted the most deserved attention from the scientific world. This pathogen was earlier identified by several names; the former designations include swine influenza, novel influenza, swine-origin influenza A [H1N1] virus, Mexican flu, North American flu, etc. On 11 June 2009, the WHO issued a pandemic alert level of six (of six possible levels) indicating that all of the criteria for a pandemic had been met by the pandemic H1N1/2009 virus outbreak [3].

Due to the mention of term ‘swine’ in the initial stages by WHO, there has been some confusion regarding classical swine influenza virus (SIV) and influenza A H1N1 pandemic spreading virus of 2009 (IAV-H1N1). The present paper reviews some epidemiological features of classical SIV and its differential features with IAV-H1N1.

The virus

The swine influenza virus (SIV) belongs to genus *Influenzavirus A* of the *Orthomyxoviridae* family. Influenza A viruses has been isolated from a variety of animals, including humans, pigs, horse, sea mammals and birds [4]. The influenza A virus contains eight single-stranded RNA segments of negative sense and encodes at least 11 proteins. The virus is further classified into subtypes based

S. C. Dubey (✉) · G. Venkatesh · D. D. Kulkarni
High Security Animal Disease Laboratory,
IVRI, Anand Nagar, Bhopal, India - 462021

E-mail: scd_11@yahoo.in

on the antigenic properties of the external glycoproteins hemagglutinin (HA) and neuraminidase (NA). Depending on the antigenicity of these two envelope spikes, influenza A viruses are categorized into 16 H (H1–H16) and nine N (N1–N9) groups, and their combination designates the subtype of the virus [5]. To date, 105 influenza A virus subtypes have been identified, all of which are endemic in water birds. However, some subtypes have adapted to other birds and mammals (e.g. pigs, horses, humans) in species-specific strains [3]. The avian influenza has been reviewed in detail [6]. The standard nomenclature for influenza viruses includes the influenza type, the host of origin (excluding humans), the place of isolation, the strain number, the year of isolation, and finally the influenza A subtype in parentheses e.g. A/Ck/Assam/India/140204/2008 (H5N1).

The HA protein is found on the surface of the influenza virus particle and is responsible for binding to receptors on host cells and initiating infection. HA is also the principal target of the host's immune system. Thus, in order for influenza to spread in a new host, the HA protein must acquire the ability to bind to the new host's cells. In the new host, the HA protein comes under selective pressure for change to evade the host's immune system [7]. The HA and NA are very important for the induction of an antibody response in the host, but they are also highly variable while the "internal" proteins such as the nucleoprotein (NP) and matrix (M) proteins are more conserved between different influenza A viruses. The genome of the virus has been shown to undergo continuous variation by several mechanisms such as rapid point mutation, genetic reassortment and gene recombination that occur between different virus subtypes [8, 9].

The hosts and transmission

The natural reservoir of influenza A viruses are aquatic birds, in which the viruses appear to have achieved an optimal level of host adaptation [10]. Transmission between birds occurs directly or indirectly through contaminated aerosols, water, feed and other materials. From the main reservoir of aquatic birds, viruses are occasionally transmitted to other animals, including mammals and domestic poultry, causing transitory infections and outbreaks. Through adaptation by mutation or genetic reassortment, some of these viruses may establish species-specific permanent lineages of influenza A viruses, and cause epidemics or epizootics in the new host. Transmission of influenza virus is more efficient in winter times, and this determines the seasonality of influenza epidemics. Influenza occurs during the colder months in temperate climates whereas in tropical and subtropical countries, infection occurs throughout the year [11].

Swine influenza virus stays with pigs and is readily transmitted by contact with respiratory secretions from infected pigs and fomites, including contaminated inanimate objects and people moving between infected and uninfected pigs as well as by the air. It is therefore impossible to prevent SIV infections by sanitary measures alone, and the virus is enzootic in most densely swine populated regions [12]. Outbreaks of swine influenza are most commonly associated with the introduction of new animals into a herd. The incubation period is usually 1–3 days. Pigs begin excreting the virus within 24 h of infection, and may shed the virus for seven to ten days. A carrier state can exist for up to 3 months [13].

Clinical picture and epidemic

Swine influenza (SI) virus causes epidemics of acute respiratory disease in pigs characterized by hyperthermia, respiratory distress including coughing, sneezing, nasal discharge and inappetance. Acute swine influenza is characterized by a short incubation period (1–3 days) after which animals appear anorexic, inactive and have the tendency to huddle and pile. Fever ranging from 40.5 to 41.7°C is present at this stage (this is why the animals tend to huddle). If animals are forced to move, respiratory distress becomes more evident. Open mouth abdominal breathing may be observed and movements are accompanied by paroxysms of coughing. Conjunctivitis, nasal discharge and sneezing may also be observed. SIV can also contribute to more chronic, multifactorial respiratory disease problems in combination with other viruses or bacteria [12].

In pigs, morbidity rapidly reaches 100% but mortality is low and usually ranges from 1% to 4%. Generally animals recover within 3–7 days of onset [14]. There is no clear evidence to support or reject the existence of long-term carriers. Recovery of virus from nasal swabs has been successful in the past up to 29 days after initial infection. After 30–45 days and 60 days post-infection, the disease failed to be transmitted to susceptible contact pigs. It is thus believed that the virus is maintained into the pig population because of constant replacement of susceptible animals [15].

Signs of swine flu in pigs include sudden onset of fever, depression, coughing (barking), discharge from the nose or eyes, sneezing, breathing difficulties and eye redness. The virus replicates in epithelial cells of the entire respiratory tract, notably the nasal mucosa, tonsils, trachea and lungs, but almost never enters other tissues. There is a massive infection of epithelial cells of the bronchi, bronchioli and alveoli and virus titres in the lungs may reach up to 10^8 EID₅₀ per g lung tissue. Virus has occasionally been

isolated from the serum of experimentally infected pigs, in barely detectable amounts, but virus isolation from tissues other than respiratory tract is very rare [12].

Origin of pandemic influenza A virus H1N1

In 1918, H1N1 influenza virus caused a pandemic in humans. Since then, this virus has continued to circulate in human population. Along with human pandemic in 1918, H1N1 influenza viruses also caused a pandemic in pigs but the virus was first isolated from swine in 1930 [16]. They have been shown to be antigenically highly similar to a recently reconstructed human 1918 A (H1N1) virus [17] and likely share a common ancestor [18, 19]. Since 1918, H1N1 virus established a swine lineage independent of the human lineage, and circulated in humans until the influenza A (H2N2) pandemic of 1957. During this period there was substantial antigenic drift of A (H1N1) viruses in humans away from the 1918 virus [17, 20]. A (H1N1) influenza viruses from the early 1950s re-emerged in humans in 1977 [4]. These swine H1N1 viruses are named “classical swine H1N1” to distinguish them from other swine influenza viruses that have also been affecting the pig population more recently. Since the swine lineage has been independent of the human lineage, these swine H1N1 are different from the human H1N1s. Analysis of the full genome sequences of representative influenza A (H1N1) viruses from 17 countries and five continents that were sampled between 1918 and 2006 revealed that human H1N1 virus has not acquired new gene segments from avian or other sources [21]. However, several distinct intra-subtype reassortant events were found to have occurred among viruses from various sub-lineages [3].

The origin of IAV-H1N1 has been described in detail [22]. From 1918 to 1997, there have been no major changes among swine influenza viruses in the Americas. These H1 viruses circulated until 1957, only to reappear in 1977 [23]. Except for this short interruption of 20 years, H1 viruses have been circulating in the human population since 1918 till date and have undergone continuous antigenic change (or “antigenic drift”). From 1957 to 1968, influenza viruses of the H2 subtype were prevalent. In 1968, another subtype change occurred when viruses with an H3 HA appeared [24]. While humans experienced two pandemics, viz. H2 and H3, there were no changes in the subtypes of influenza viruses circulating in pigs, which remained as classical H1N1 virus. While classical H1N1 was the dominant virus, human H3N2 viruses were detected by serology at very low levels, but these viruses never became established in the swine population [25]. However, from 1997 onwards, many different new influenza virus genotypes emerged,

and a particular one became prevalent and started to co-circulate with the classical H1N1 viruses. This was a triple reassortant virus, with genes derived from classical swine (NP, M and NS), genes from avian influenza viruses (PB2 and PA) and genes from a human H3N2 virus [26]. These H3N2 viruses have been circulating in pigs since 1997 and established an independent lineage from the human H3N2s. In addition, these viruses have continued to re-assort with classical H1N1 viruses in the pig population, so different genotypes have been established in swine since 1997 [22].

Current global situation

Influenza viruses of three different subtypes – H1N1, H3N2 and H1N2 – are circulating in swine worldwide [12]. Unlike for human influenza viruses, the origin and nature of swine influenza viruses (SIV) differ in different continents. The predominant H1N1 SIV in Europe, for example, is entirely of avian origin and they were introduced from wild ducks into the pig population in 1979. Two types of H1N1 SIV, in contrast, are circulating in the USA: the so-called “classical” H1N1 viruses that have been present since the early 20th century and novel re-assortants with the surface glycoproteins of the classical virus and internal pro-H1N2 SIV. Viruses of both other subtypes, H1N1 and H3N2, also have a different origin in Europe and in the USA and were introduced in the swine population at different times. These differences between SIV in different parts of the world also have implications for the diagnosis and control of SIV. The strains used as antigens in vaccines and diagnostic tests also differ in Europe and in the USA. It is remarkable that most SIV are re-assortants with mixtures of human, avian and swine virus genes [12].

Swine influenza A viruses of the H1N1 subtype currently circulate as two distinct lineages within North American and European swine populations [27, 28]. Independently, a novel lineage of avian-like H1N1 swine IAV emerged in Europe in 1979 that essentially replaced classical swine IAV [27, 29, 30]. This second lineage of swine IAV is enzootic throughout swine-producing regions of Western Europe, where it co-circulates with swine IAVs of the H3N2 and H1N2 subtypes [31, 32]. All eight gene segments of the prototype H1N1 viruses of this lineage are thought to be derived from closely related Eurasian avian IAVs by a stable host switch without re-assortment, and this lineage is phylogenetically and antigenically distinct from the classical swine H1N1 lineage [29, 33–35]. Apart from classical and avian H1N1, the other subtypes of SIV that are most frequently identified in pigs include, reassortant (r) H3N2, and rH1N2. Other subtypes are rH1N7, rH3N1, avian H4N6, avH3N3, and avH9N2 [36]. The H1N1, H1N2 and H3N2 viruses found in Europe

are antigenically and genetically different from those found in America [37–42].

Classical swine flu is now common in North and South America, Europe, parts of Asia and Africa. In 1998, a new triple-reassortant H3N2 virus – comprising genes from classical swine H1N1, North American avian, and human H3N2 (A/Sydney/5/97-like) influenza – was reported as the cause of outbreaks in North American swine, with subsequent establishment in pig populations [37, 43]. Co-circulation and mixing of the triple re-assortant H3N2 with established swine lineages subsequently generated further H1N1 and H1N2 reassortant swine viruses which have caused sporadic human infections in the United States since 2005 [44]. From December 2005 through February 2009, the CDC received 11 notifications of human infection with triple re-assortant swine influenza A (H1) viruses, 8 of which occurred after June 2007 [45]. Consequently, human infection with H1N1 swine influenza has been a nationally notifiable disease in the United States since 2007 [46].

In Europe, an avian H1N1 virus was introduced to pigs ('avian-like' swine H1N1) and first detected in Belgium in 1979 [29]. This lineage became established and gradually replaced classical swine H1N1 viruses, and also re-assorted in pigs with human H3N2 viruses (A/Port Chalmers/1/1973-like) [27]. It is noteworthy that, until now, there has been no evidence of Eurasian avian-like swine H1N1 circulating in North American pigs. In Asia, the classical swine influenza lineage circulates, in addition to other identified viruses, including human H3N2, Eurasian avian-like H1N1, and North American triple reassortant H3N2 [47–49].

The classical swine flu virus can be handled in BSL-2 laboratory, but looking at the nature of infections with the mutated H1N1 spreading so fast in humans, the biosafety regulations need to be reconsidered.

Status in India

The facilities for final confirmation of the pandemic IAV-H1N1 infection in humans are available in National Institute of Virology, Pune and National Institute of Communicable Diseases, New Delhi. Till 24 October 2009, 12,334 laboratory-confirmed human cases were reported with total mortality reaching to 399 [50]. Moreover, classical H1N1 viruses are being regularly isolated from human cases.

In most of the Asian countries, the current status of SIV in organized or backyard pig population is not fully understood and hence there is a need of comprehensive country-wise surveillance programme with cross checking of results between regional laboratories. There is no report of clinical swine influenza in pigs in India. A few studies

were carried out in 1980s to know the presence of human influenza A virus antibodies in pigs. When tested by HI, antibodies were prevalent against the serotypes H3N2, H1N1, H2N2, and H0N1 [51, 52]. However, antibodies against Hsw1N1 were absent in pigs [51]. In view of latest H1N1 pandemic India has initiated a preliminary surveillance program of SIV infection in swine population using serum, tissue and nasal swabs from various states. Consequently, swine influenza diagnostic facility for animals has now been created at High Security Animal Disease Laboratory (HSADL) at Bhopal. This being biocontainment laboratory having BSL-3+ facilities and the "OIE-Recognised Avian Influenza Laboratory", the influenza-suspected samples from all over the country are being tested in this laboratory. The flow chart of the diagnostic protocol given below has been developed by HSADL [53] and is being followed in the ongoing surveillance activity (Fig. 1).

Swine influenza and zoonoses

Involvements of antigenically and genetically indistinguishable swine influenza (H1N1) virus in human cases have also been demonstrated in the past [54]. Webster and co-workers reported serological studies of slaughterhouse workers indicated that swine influenza viruses are transmitted to humans relatively frequently as 20% of workers in 1977 had antibodies to swine influenza virus [4]. The swine virus was occasionally isolated from humans with respiratory illness [55] and occasionally was lethal [56]. Although it is accepted that pandemic strains of human influenza emerge only rarely, the available information indicates that interspecies transmission of influenza viruses may not be so rare, for up to 10% of persons with occupational exposure to pigs develop antibodies to swine influenza virus [57]. The majority of transfers of influenza viruses from pigs to humans are dead-end transfers in that they do not spread efficiently from human to human. Cases and clusters of human infections with swine influenza viruses have been reported sporadically in the United States since 1970s. Worldwide, more than 50 cases of swine influenza virus infection in humans, mostly due to classic swine influenza virus, have been documented in the past 35 years [58, 59]. Whereas serologic studies suggest that people with occupational swine exposure are at highest risk for infection [60].

A herd of pigs in western province of Alberta, Canada had been tested positive for swine flu, apparently after being infected by a farm worker. The worker had travelled and returned from Mexico (the place where the pandemic H1N1 epidemic occurred) during the acute phase of the disease. The infection spread to about 200 pigs in the first week of May 2009 and the herd was quarantined. Both the man

Fig. 1 Flow chart of SIV testing protocol at HSADL, Bhopal
*HI against H1

and the pigs were recovering and the virus did not seem to have spread beyond the farm. However, during the initial quarantine period, the owner culled entire herd (of about 3000 pigs) and thus further transmissibility of the virus to other pigs could not be ascertained [61].

The tracheal epithelium in pigs expresses receptors for both human and avian influenza viruses, and this provides a biological basis for the susceptibility of pigs to both avian and human influenza viruses. The respiratory tract epithelial cells in pigs contain the sialic acid receptors preferred by both avian (α 2, 3-N-acetylneuraminic acid-galactose) and human influenza viruses [47, 62]. Pigs can therefore function as intermediate hosts or “mixing vessels” in establishing new influenza virus lineages by supporting co-infection, replication, and re-assortment among human, avian and swine influenza viruses [27, 63].

Zoonotic infections of humans with swine influenza viruses have been diagnosed in the United States, Europe, New Zealand and Asia [64]. However, the total number of

zoonotic infections that have been described is relatively smaller when compared to the number of people worldwide involved directly or indirectly in swine farming. Swine farm workers are likely to be routinely exposed to and get infected with swine influenza viruses, but only a small percentage of those zoonotic infections are documented. Zoonotic infections may be recognized if information regarding contact with sick pigs is specifically communicated to physician or if a patient is hospitalized or die or if virus isolation is attempted and that yields antigenically atypical virus. In most cases, however, swine influenza virus infections in people may not be clinically distinguishable from routine human influenza virus infections for need of differential diagnostic facilities. Because a relatively small number of zoonotic swine influenza virus infection have been documented by virus isolation, whether infections with swine influenza viruses are clinically different than infections with routine human influenza viruses remains unclear. It is therefore important to pursue virus isolation

aggressively when swine farming people show influenza-like symptoms [65].

The special environment and lifestyle in southern China provide more chances for wild aquatic birds, domestic poultry, pigs and humans to come in contact closely and hence create an opportunity for interspecies transmission and generation of new reassortant influenza viruses. Although, it is virtually impossible to prevent new outbreaks of influenza in human and animals, it is now well-recognized that animal influenza virus surveillance can play a key role in the early recognition of outbreak threats. Therefore it is of great significance to carry out swine influenza virus surveillance [66].

Although zoonotic infections with swine influenza viruses have been documented [28] the results of many studies strongly support the hypothesis that people associated with swine production are infected with swine influenza viruses more regularly than the small number of other zoonotic infections. Previous studies in the 1960s suggested increased rates of infection among persons in contact with pigs or working with swine influenza viruses [67, 55]. In this study, factors related to a person's degree of contact with pigs were specifically associated to seropositivity to swine viruses. The number of hours per day spent in the barn was not a factor of significance, suggesting that the overall frequency of pig contact is a more important consideration than the length of contact at any one time. This lack of significance is consistent with the fact that influenza virus infections in pigs occur sporadically, and pigs generally only shed virus for approximately 7 days after infection [68].

Further agenda

The influenza pandemic of 1918–1919 was a unique event in recorded history, costing around 50 million lives in less than a year [69]. Compared to the 1918 pandemic, the pandemics caused by the 1957 (H2N2) and 1968 (H3N2) viruses were relatively mild, with estimates of one million and half a million deaths worldwide, respectively [24]. The current outbreak indicates that the new H1N1 viruses are able to transmit from human to human, and this distinguishes these viruses from other viruses present in nature. The emergence of pandemic H1N1 influenza provides further evidence of the role of domestic pigs in the ecosystem of influenza A. As reported recently, all three pandemics of the twentieth century seem to have been generated by a series of multiple re-assortment events in swine or humans, and to have emerged over a period of years before pandemic recognition. Despite widespread influenza surveillance in humans, the lack of systematic swine surveillance allowed for the undetected persistence and evolution of this potentially

pandemic strain for many years. These findings underscore the need for close communication and collaboration between human and animal health agencies for ongoing surveillance, investigation, research, prevention, and control efforts.

In the context of current reports of epidemic swine-origin influenza A (H1N1) viruses and global concern regarding the emergence of a human influenza pandemic of animal-influenza origin, epidemiologic and laboratory surveillance of interspecies transmission of influenza viruses should be increased, especially in environments in which humans and swine are routinely exposed to each other. Cases of infection in persons who have been exposed to pigs may be sentinels for early zoonotic transmission of novel triple re-assortant swine influenza A (H1) viruses to humans. Consequently, surveillance in settings involving pigs might facilitate early identification and joint responses of public health and animal health agencies to contain potential outbreaks before widespread community transmission occurs [70].

References

1. Gatherer D (2009) The 2009 H1N1 influenza outbreak in its historical context. *J Clin Virol* 45(3):174–8
2. WHO (2009) Pandemic (H1N1) 2009 - update 71; 17 Oct 2009 Available at: http://www.who.int/csr/don/2009_10_23/en/index.html. Accessed 23 Oct 2009
3. Michaelis M, Doerr HW, Cinatl Jr. J (2009) An Influenza A H1N1 Virus Revival – Pandemic H1N1/09 Virus. *Infection* Published online: Sep 18, 2009
4. Webster RG, Bean WJ, Gorman OT, Chamber TM and Kawaoka Y (1992) Evolution and ecology of Influenza A viruses. *Microbiol Rev* 56:152–179
5. Fouchier RA, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, Rimmelzwaan GF, Olsen B and Osterhaus ADME (2005) Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol* 79:2814–22
6. Dubey SC, Nagarajan S, Tosh C, Bhatia S, Lal Krishna (2009) Avian influenza: A long-known disease and its current threat. *Ind J Anim Sci* 79:113–140
7. Murphy B and Webster R (1996) Orthomyxoviruses. In: Fields B, Knipe D and Howley P (eds). *Fields Virology* Lippincott, Philadelphia, pp. 1397–1445
8. Lin YP, Shu LL, Wright S, Bean WJ, Sharp GB, Shortridge KF and Webster RG (1994) Analysis of the influenza virus gene pool of avian species from southern China. *Virology* 198:557–566
9. Xu X, Lindstrom SE, Shaw MW, Smith CB, Hall HE, Mungall BA, Subbarao K, Cox NJ and Klimov A (2004) Reassortment and evolution of current human influenza A and B viruses. *Virus Res* 103:55–60
10. Webster RG, Bean WJ, Gorman OT, Chamber TM and Kawaoka Y (1992) Evolution and ecology of influenza A virus of seals. *N Engl J Med* 304:91

11. Hampson AW and Mackenzie JS (2006) The influenza viruses. *Med J Aust.* 185:S39–S43
12. Reeth KV (2007) Avian and swine influenza viruses: our current understanding of the zoonotic risk. *Vet. Res* 38:243–260
13. AVMA (2009). Swine Influenza Backgrounder, Amer. Vet. Med. Assoc. 29 April 2009 Available at www.avma.org/public_health/influenza/swine_bgnd.asp. Accessed 23 Oct 2009
14. OIE (2009) Swine Influenza Technical Disease Card. Available at: http://www.oie.int/eng/maladies/Swine%20Influenza_OIE%20TDC_FNL.pdf. Accessed 23 Oct 2009
15. Vannier P, Gourreau JM, and Kaiser C (1985) Experimental infection of specific pathogen free pigs with a swine flu strain (H5w1N1) and study of the viral shedding period. *Can Vet J* 26:138–143
16. Shope RE and Lewis P (1931) Swine influenza. I. Experimental transmission and pathology. *J Exp Med* 54:349–359
17. Tumpey TM, Garcia-Sastre A, Taubenberger JK, Palese P, Swayne DE and Basler CF (2004) Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci USA* 101:3166–3171
18. Gorman OT, Bean JW, Kawaoka Y, Donatelli IL, Guo Y, and Webster RG (1991) Evolution of influenza A virus nucleoprotein genes: implications for the origins of H1N1 human and classical swine viruses. *J Virol* 65:3704–3714
19. Reid AH and Taubenberger JK (2003) The origin of the 1918 pandemic influenza virus: a continuing enigma *J Gen Virol* 84: 2285–2292
20. Kilbourne ED, Smith C, Brett I, Pokorny BA, Johansson B and Cox N (2002) The total influenza vaccine failure of 1947 revisited: Major intrasubtypic antigenic change can explain failure of vaccine in a post-World War II epidemic. *Proc Natl Acad Sci USA* 99:10748–10752
21. Nelson MI, Viboud C, Simonsen L, Bennett RT, Griesemer SB, St George K, Taylor J, Spiro DJ, Sengamalay NA, Ghedin E, Taubenberger JK, Holmes EC (2008) Multiple reassortment events in the evolutionary history of H1N1 influenza A virus since 1918. *PLoS Pathog* 4: e1000012
22. Chang LY, Shih SR, Shao PL, Huang DTN and Huang LM (2009) Novel Swine-origin Influenza Virus A (H1N1): The First Pandemic of the 21st Century. *J Formos Med Assoc* 108: 526–532
23. Nakajima K, Desselberger U and Palese P (1978) Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. *Nature* 274:334–339
24. Palese P, Tumpey TM and Adolfo GS (2006) What Can We Learn from Reconstructing the Extinct 1918 Pandemic Influenza Virus? *Immunity* 24:121–124
25. Chambers TM, Hinshaw VS, Kawaoka Y, Easterday BC and Webster RG (1991) Influenza viral infection of swine in the United States 1988–1989. *Arch Virol* 116:261–265
26. Zhou NN, Senne DA, Landgraf JS, Swenson SL, Erickson G, Rossow K, Liu L, Yoon K, Krauss S and Webster RG (1999) Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J Virol* 73:8851–56
27. Brown IH (2000) The epidemiology and evolution of influenza viruses in pigs. *Vet Microbiol* 74:29–46
28. Olsen CW, Brown IH, Easterday BC and Reeth KV (2006) Swine influenza. In: Straw BE, Zimmerman JJ, D’Allaire S and D. J. Taylor (eds). *Diseases of swine*, 9th ed. Blackwell Publishing Professional, Ames, IA, pp. 469–482
29. Pensaert M, Ottis K, Vandeputte J, Kaplan MM and Bachmann PA (1981) Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. *Bull World Health Organ* 59:75–78
30. Scholtissek C, Burger H, Bachmann PA and Hannoun C (1983) Genetic relatedness of hemagglutinins of the H1 subtype of influenza A viruses isolated from swine and birds. *Virology* 129:521–523
31. Maldonado J, Reeth KV, Riera P, Sitja M, Saubi N, Espuna E and Artigas C (2006) Evidence of the concurrent circulation of H1N2, H1N1 and H3N2 influenza A viruses in densely populated pig areas in Spain. *Vet J* 172:377–381
32. Dunham EJ, Dugan VG, Kaser EK, Perkins SE, Brown IH, Holmes EC and Taubenberger JK (2009) Different evolutionary trajectories of European avian-like and classical swine H1N1 influenza A viruses. *J Virol* 83(11):5485–94
33. Brown IH, Ludwig S, Olsen CW, Hannoun C, Scholtissek C, Hinshaw VS, Harris PA, McCauley JW, Strong I and Alexander DJ (1997). Antigenic and genetic analyses of H1N1 influenza A viruses from European pigs. *J Gen Virol* 8:553–562
34. Donatelli I, Campitelli L, Castrucci MR, Ruggieri A, Sidoli L and Oxford JS (1991) Detection of two antigenic subpopulations of A(H1N1) influenza viruses from pigs: antigenic drift or interspecies transmission? *J Med Virol* 34:248–257
35. Schultz U, Fitch WM, Ludwig S, Mandler J and Scholtissek C (1991) Evolution of pig influenza viruses. *Virology* 183:61–73
36. OIE (2008) Swine influenza. OIE Terrestrial Manual, Ch. 2.8.8: 1128–1138 Available at: http://www.oie.int/eng/normes/mmanual/2008/pdf/2.08.08_SWINE_INFLUENZA.pdf. Accessed 7 Jan 2008
37. Brown IH, Harris PA, McCauley JW and 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
38. Castrucci MR, Donatelli I, Sidoli L, Barigazzi G, Kawaoka Y and Webster RG (1993) Genetic reassortment between avian and human influenza A viruses in Italian pigs. *Virology* 193: 503–506
39. Choi YK, Goyal SM, Farnham MW and Joo HS (2002) Phylogenetic analysis of H1N2 isolates of influenza A virus from pigs in the United States. *Virus Res* 87:173–179
40. Done SH and Brown IH (1997) Swine influenza in the United Kingdom, past and present. *Large Anim Pract* 2:20–28
41. Noble S, McGregor MS, Wentworth DE and Hinshaw VS (1993) Antigenic and genetic conservation of the haemagglutinin in H1N1 swine influenza viruses. *J Gen Virol* 74:8243–8251
42. Sheerar MG, Easterday BC, and Hinshaw VS (1989) Antigenic Conservation of H1N1 Swine Influenza Viruses. *J Gen Virol* 70:3297

43. Webby RJ, Swenson SL, Krauss SL, Gerrish PJ, Goyal SM, and Webster RG (2000) Evolution of swine H3N2 influenza viruses in the United States. *J Virol* 74:8243–8251
44. Newman AP, Reisdorf E, Beinemann J, Uyeki TM, Balish A, Shu B, Lindstrom S, Achenbach J, Smith C and Davis JP (2008) Human case of swine influenza A (H1N1) triple reassortant virus infection, Wisconsin. *Emerg Infect Dis* 14:1470–1472
45. Shinde V, Bridges CB, Uyeki TM, Shu B, Balish A, Xu X, Lindstrom S, Gubareva LV, Deyde V, Garten RJ, Harris M, Gerber S, Vagasky S, Smith F, Pascoe N, Martin K, Dufficy D, Ritger K, Conover C, Quinlisk P, Klimov A, Bresee JS and Finelli L (2009) Triple-reassortant swine influenza A (H1) in humans in the United States, 2005–2009. *N Engl J Med* 360: 2616–2625
46. CDC 2009. Novel influenza A virus infections – 2007 – case definition. Available at: www.cdc.gov/ncphi/diss/nndss/casedef/novel_influenzaA.htm. Accessed 23 Oct 2009
47. Peiris JSM, Guan Y, Markwell D, Ghose P, Webster RG and Shortridge KF (2001) Co-circulation of avian H9N2 and contemporary "human" H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment? *J Virol* 75:9679–9686
48. Jung K and Song DS (2007) Evidence of the cocirculation of influenza H1N1, H1N2 and H3N2 viruses in the pig population of Korea. *Vet Rec* 161:104–105
49. Smith GJD., Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghvani J, Bhatt S, Peiris JSM, Guan Y and Rambaut A (2009) *Nature* 459:1122–1125
50. Press Information Bureau Press Release (2009). Consolidated Status of Influenza A H1N1- Ministry of Health and Family Welfare, Govt. of India, 24 th Oct 2009
51. Rao BL, Khan FU, Bhat HR, Kadam SS (1983) Zoonotic studies on influenza in pigs and birds, India, 1980–81. *Int J Zoonoses*. 10(1):40–4
52. Chatterjee S, Mukherjee KK, Mondal MC, Chakravarti SK and Chakraborty MS. (1995) A serological survey of influenza a antibody in human and pig sera in Calcutta. *Folia Microbiol (Praha)*. 1995;40(3):345–8
53. Dubey SC (2009) Report on swine influenza (H1N1) in animals. Paper presented at OIE Meeting at Paris, France on 24.06.2009
54. Hinshaw VS, Bean WJ Jr., Webster RG and 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
55. Dacso CC, Couch RB, Six HR, Young JF, Quarles JM. and Kasel JA (1984) Sporadic occurrence of zoonotic swine influenza virus infections. *J Clin Microbiol* 20:833–835
56. Rota PA, Rocha EP, Harmon M W, Hinshaw VS, Sheerar MG, Kawaoka Y, Cox NJ and Smith TF (1989) Laboratory characterization of a swine influenza virus isolated from a fatal case of human influenza. *J Clin Microbiol* 27:1413–1416
57. Schnurrenberger PR, Woods GT and Martin RJ (1970) Serologic evidence of human infection with swine influenza virus. *Am Rev Respir Dis* 102:356–361
58. CDC (2009). Swine influenza A (H1N1) infection in two children — southern California, March–April 2009 *Morb Mortal Wkly Rep*. 58: 400–402 Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5815a5.htm>. Accessed 23 Oct 2009
59. Wentworth DE, McGregor MW, Macklin MD, Neumann V, Hinshaw VS (1997) Transmission of swine influenza virus to humans after exposure to experimentally infected pigs. *J Infect Dis* 175:7–15
60. Gray GC, McCarthy T, Capuano AW, Setterquist SF, Olsen CW, Alavanja MC (2007) Swine workers and swine influenza virus infections. *Emerg Infect Dis* 13:1871–1878
61. BBC News (2009) "Canada farm worker 'infects pigs'". 3 May 2009. <http://news.bbc.co.uk/1/hi/world/americas/8031309.stm>. Retrieved 2009–05–03
62. Ito T, Couceiro N, Kelm S, Baum LG, Krauss, S, Castrucci MR, Donatelli I, Kida H, Paulson JC, Webster RG and Kawaoka Y (1998) Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J Virol* 72:7367–73
63. Landolt GA, Karasin AI, Phillips, L and Olsen, CW (2003) Comparison of the pathogenesis of two genetically different H3N2 influenza A viruses in pigs. *J. Clin. Microbiol* 141: 1936–1941
64. Olsen CW (2002). Influenza in pigs and their role as the intermediate host. In: Nicholson K, Webster R, Hay A and Cox N (Eds). *Textbook of influenza*. 2nd ed. Oxford: Blackwell Science (Cited by Olsen et al. 2002)
65. Olsen CW, Brammer L, Easterday BC, ArdenN, BelayE, Baker I and Cox NJ (2002) Serologic Evidence of H1 Swine Influenza Virus Infection in Swine Farm Residents and Employees *Emerging Infectious Diseases*. Vol. 8:814–819
66. Yua, Zhoua H, Jun Y, Li GX, Zhang GH, Li LH, Li PY, Ming L, Guang ZT (2009) Further evidence for infection of pigs with human-like H1N1 influenza viruses in China. *Virus Res* 140:85–90
67. Kluska V, Macku M and Mensik J (1961) Evidence for swine influenza antibodies in human. *Cesk Pediat* 116: 408–14 cited by Myers KP, Olsen C.W and Gray GC 2007. Cases of swine influenza in humans. A review of the literature. *Clin. Inf. Dis*. 44:1084–88
68. Larsen DL, Karasin A, Zuckermann F and Olsen, CW (2000) Systemic and mucosal immune responses to H1N1 influenza virus infection in pigs. *Vet Microbiol* 74:117–131
69. Johnson NP, and Mueller J (2002) Updating the accounts: global mortality of the 1918–1920 “Spanish” influenza pandemic. *Bull Hist Med*. 76: 105–115
70. Adolfo GS (2009) The 2009 H1N1 virus causing the current “swine” flu. Available at:<http://www.isv-online.org/articles/list.html>. Accessed 23 Oct 2009