



# Emergence of a novel swine-origin influenza A virus (S-OIV) H1N1 virus in humans

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## ABSTRACT

A recently emerged novel influenza A H1N1 virus continues to spread globally. The virus contains a novel constellation of gene segments, the nearest known precursors being viruses found in swine and it likely arose through reassortment of two or more viruses of swine origin. H1N1, H1N2 and H3N2 subtype swine influenza viruses have occasionally infected humans before but such zoonotic transmission events did not lead to sustained human-to-human transmission in the manner this swine-origin influenza virus (S-OIV) has done. Its transmission among humans appears to be higher than that observed with seasonal influenza. Children and young adults appear to those most affected and also those who appear to maintain transmission. Clinical disease generally appears mild but complications leading to hospitalization can occur, especially in those with underlying lung or cardiac disease, diabetes or those on immunosuppressive therapies. There are concerns that the virus may reassort with existing human influenza virus giving rise to more transmissible or more pathogenic viruses. The virus appears to retain the potential to transmit back to swine and thus continued reassortment with swine viruses is a cause for concern.

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## 1. Introduction

The one predictable aspect of influenza is its unpredictability. While attention was focused on the threat of an avian influenza H5N1 pandemic emerging from Asia, a novel influenza virus of swine origin emerged in North America, and is now spreading worldwide. The virus appears to confound us even in its nomenclature<sup>1</sup> and the semantics of what constitutes a pandemic.<sup>2</sup>

During April 2009, a novel H1N1 virus was detected in epidemiologically unrelated cases of influenza-like illness in California and was subsequently recognized to be the cause of a major outbreak of respiratory disease in Mexico that had been ongoing for some weeks previously. The virus was found to be an H1N1 virus that was antigenically and genetically unrelated to human seasonal influenza viruses and genetically related to viruses known to circulate in swine. In the ensuing weeks (as of 1st June 2009) this swine-origin influenza virus (S-OIV) H1N1 virus caused 17,410 virologically confirmed human cases and 115 deaths in 62 countries in the Americas, Europe, Asia and Australasia. The majority of the cases so far have been in Mexico (5029 with 97 deaths), USA (8975 with 15 deaths) and Canada (1336 with 2 deaths).

Influenza A viruses are single stranded RNA viruses of negative sense with an eight segmented genome that belongs to the family Orthomyxoviridae. The viral haemagglutinin (HA) and neuraminidase (NA) proteins are envelope glycoproteins and are the key antigens against which humoral immune responses are directed. They are used for the subtyping of influenza A viruses into 16 HA and 9 NA subtypes. Aquatic birds are the natural reservoir of all influenza virus subtypes but some subtypes have become established in other species; H1 and H3 in pigs, H3 and H7 in horses and recently equine H3 subtype viruses have become established in dogs in North America. Pandemics are believed to arise when a novel avian influenza HA and/or NA (together with the PB1 gene segment in the pandemics of 1957 and 1968) is picked up through reassortment by pre-existing human influenza viruses (reviewed in Ref. [3]), or by a purely avian virus adapting to efficient human transmission.<sup>4</sup> Pigs have been proposed to serve as intermediate hosts where such adaptation and reassortment of avian precursors may occur (reviewed in Ref. [5]).

Highly pathogenic avian influenza (H5N1) is entrenched in poultry flocks worldwide and continues to transmit zoonotically to humans, causing a disease with overall mortality of over 60%. The argument for the preparations for a possible H5N1 pandemic was not its inevitability or even that it was the most likely pandemic candidate, but the severity of such a rare outcome.<sup>6</sup> This threat spurred global influenza pandemic preparedness in relation to options for containment, logistics of health care, non-pharmaceutical inter-

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ventions, antivirals and vaccines. All of these, with the possible exception of vaccines, are equally applicable in the current global efforts to confront the novel S-OIV H1N1 virus.

## 2. Influenza viruses in swine

The H1N1 pandemic of 1918 is believed to have also affected swine in 1918.<sup>7</sup> Its descendants have remained endemic in swine up to now. The first influenza viruses to be isolated in culture were swine H1N1 influenza viruses<sup>8</sup> and they are antigenically very similar to the recently reconstructed 1918 pandemic virus.<sup>9</sup> These swine H1N1 viruses, called the “classical swine” H1N1 viruses, continued to circulate in pigs in Asia, the America’s and until the 1980s also in Europe. Until relatively recently, they remained antigenically stable.<sup>10</sup> The emergence in 1979 of a novel H1N1 virus of avian origin in pigs in Europe (avian-like swine H1N1 viruses) led to the replacement of classical swine H1N1 viruses from pigs in Europe but the classical swine viruses continue to circulate in Asia and America up to now. Human H3N2 viruses have repeatedly established themselves in swine at different times in Europe and in Asia, and more recently in USA. In Europe, the avian-like swine H1N1 viruses reassorted in swine with human origin H3N2 viruses, to give rise to the European swine H3N2 lineage which have avian-like swine H1N1 internal genes and human origin H3 and N2. Separately, the European avian-like swine H1N1 viruses picked up human N2 genes to generate H1N2 viruses (reviewed in Refs. [11,12]).

In Asia, classical swine H1N1 continues to be endemic in pigs. In addition, different lineages of human H3N2 viruses have repeatedly become established in swine.<sup>13–15</sup> Interestingly, each H3N2 lineage undergoes less antigenic drift in swine than in humans, leading Shortridge et al. to presciently note that “persistence of such (human) influenza viruses in pigs could serve as a reservoir for subsequent infection of man”.<sup>14</sup> European swine H3N2 viruses,<sup>16</sup> other avian origin H1N1 viruses<sup>17</sup> and other reassortants have also been reported in Asia (reviewed in Refs. [11]).

Classical swine H1N1 remained the dominant swine virus in North America until the 1998 when “triple reassortant H3N2 viruses began to be isolated in the USA.<sup>18</sup> These viruses had HA, NA and PB1 of human origin, NP, M and NS genes of classical swine and PB2 and PA genes of North American avian virus origin.<sup>19,20</sup> Unlike the classical swine viruses which at most caused mild respiratory disease in pigs, these triple reassortant viruses had unusual pathogenicity being associated with spontaneous abortion and even death of pigs. Furthermore, they continued to acquire H3 HA genes of diverse origin, very likely through repeated acquisition of different human HA genes. The triple reassortant H3N2 viruses also continued to acquire other virus genes via reassortment to generate triple reassortant H1N2 viruses (acquiring a classical swine H1) or H1N1 viruses (acquiring both H1 and N1 from classical swine viruses). These triple reassortant H1N1 and H1N2 viruses have become antigenically very diverse and increasingly more distant from the classical swine H1N1 viruses.<sup>10</sup>

During this time there was also a range of other reassortant swine viruses detected in Europe (e.g. H1N2; H1N7) and Asia (H3N1; H3N2; H1N1; H1N2).<sup>11,15,21–24</sup> Furthermore, avian viruses have been transiently detected in swine in many parts of the world, including H1N1, H9N2, H4N6 and H5N2<sup>13,17,22,25–27</sup> but it is not clear whether these viruses will establish long term transmission in pigs. While highly pathogenic H5N1 viruses have been occasionally detected in pigs,<sup>22,28</sup> experimental studies indicate that pig-to-pig transmission is limited<sup>29,30</sup> and serological studies do not reveal widespread transmission of H5N1 viruses in pigs in areas where this virus remains endemic in poultry.<sup>29,31</sup>

## 3. Zoonotic transmission of swine viruses to humans and swine as intermediate hosts for inter-species transmission of avian viruses to humans

Human and avian viruses can infect pigs<sup>32</sup> and the respiratory tract of pigs is believed to express both sialic acid (SA)  $\alpha$ 2,3Gal (bind avian influenza) and SA $\alpha$ 2,6Gal (bind human influenza) receptors that will permit infection with both avian and human influenza viruses.<sup>33</sup> There is also ample evidence that avian and human viruses establish long term lineages in pigs and that these viruses reassort in pigs (see above). Reassortment between avian and human viruses implies infection of both viruses in the same host and same cell. Humans are poorly permissive to avian viruses<sup>34</sup> and avian species are poorly permissive to human viruses. Since swine are known to be permissive to both avian and human influenza viruses, they have been proposed as a “mixing vessel” for the generation of pandemic viruses through reassortment.<sup>35</sup> It should be noted however, that there is no direct evidence that pigs played a role in the genesis of any of the three pandemics of the 20th century.

In common with human influenza viruses, swine influenza viruses generally bind to SA $\alpha$ 2,6Gal receptors.<sup>36</sup> Swine viruses of subtypes H1N1, H3N2 and H1N2 viruses of diverse lineages have been reported to zoonotically transmit and cause disease in humans causing flu-like illness but was fatal in 17% of cases.<sup>12,37,38</sup> Such transmission has been detected in North America, Europe and Asia, but may be more common than hitherto recognized. In 1976, 13 cases of classical swine H1N1 with one death was reported at Fort Dix, New Jersey and serological testing revealed the evidence of infection in many others at the same military facility.<sup>39</sup> This led to the USA-wide swine-flu vaccine campaign which was later aborted because of occasional side effects (Guillain–Barre syndrome), and even more importantly, because the anticipated swine-flu pandemic failed to materialize.

Since December 2005, the triple reassortant H1 viruses have transmitted to 11 humans in the USA, 10 caused by the triple reassortant H1N1 and one by the triple reassortant H1N2 virus.<sup>40</sup> Another transmission of an European swine H1N1 virus was reported in Europe.<sup>41</sup> Some of these patients had close exposure to pigs. While some of these zoonotic infections led to occasional transmission to other patients, none of them led to sustained human-to-human transmission.

Antibodies to swine influenza viruses (H1N1; H1N2) have been detected in humans with exposure to pigs.<sup>42,43</sup> The true incidence of natural human infection with swine viruses remains unclear.

## 4. Genetic and antigenic characterization of swine-origin influenza virus H1N1

Phylogenetic analyses of the current novel S-OIV H1N1 revealed that the HA, NP and NS genes arise from the classical swine H1N1 lineage, the NA and M genes from the avian-like Eurasian swine H1N1 lineage while the PB2, PB1 and PA are from the North America H3N2 triple reassortant lineage. While it is true that the S-OIV H1N1 virus has virus gene segments of swine, human and avian origin, these genes were already established in the triple reassortant swine in North America and in the Eurasian swine H3N2 or H1N1 viruses for many years. Thus the immediate reassortment event that led to the generation of S-OIV was very probably reassortment between two or more swine viruses, viz. the triple reassortant H1N2 (or H1N1) and the Eurasian H3N2 or H1N1 swine viruses.<sup>43b</sup> The currently available genetic sequence information does not allow identification of the immediate precursor of the S-OIV H1N1 virus or where such a reassortment may have taken place. This reflects the paucity of surveillance of swine influenza viruses worldwide,

and in Mexico and other Central and South American countries in particular.

Analysis of the PB2, PB1-F1 and NS genes of the Mexican and US virus isolates sequenced to date has not identified molecular signatures that have been previously known to confer increased virulence observed with the 1918 pandemic virus or in HPAI H5N1 viruses.<sup>44</sup> The HA retains the receptor binding specificity to “human” SA $\alpha$ 2,6 receptors, similar to that of classical swine H1N1 viruses. In common with other viruses of the European swine lineage from which the S-OIV M gene segment is derived, the M2 protein contains a marker (S31N) for amantadine resistance. The viruses do not have evidence of resistance to the neuraminidase inhibitor class of antivirals.<sup>45</sup>

At present, there is high (99.9%) sequence identity within each gene segment of the S-OIV strains that have been genetically sequenced.<sup>44</sup> This suggests that the inter-species transmission event occurred relatively recently and that it was a single event or multiple inter-species transmission events from a homogeneous gene pool.

Antigenically, the S-OIV viruses are similar to classical swine viruses and to triple reassortant H1N1 viruses that have circulated in USA over the last decade or so. However, there is little antigenic cross-reactivity with contemporary human seasonal H1N1 viruses.<sup>44</sup> This antigenic gap between S-OIV and contemporary seasonal human H1N1 viruses is due to the fact that human H1N1 viruses have been under consistent immune selection pressure from the “herd immunity” being built up in humans while similar immune selection pressure was apparently less intense in swine. Interestingly, age stratified human sero-prevalence studies show that while children and young adults have little or no cross-reacting antibodies to S-OIV, a substantial proportion of humans over 60 years of age (i.e. those born before 1949) have cross-reacting antibodies by HI and neutralization tests.<sup>44</sup> This may reflect the fact that human H1N1 viruses have continued to diverge from swine influenza viruses over time. H1N1 viruses circulating in humans before the 1950s are likely to be more closely related antigenically to classical swine H1N1 and thus to S-OIV than contemporary human H1N1 viruses. Thus those infected with seasonal human H1N1 in this period may be expected to have more cross-reactive antibody to S-OIV.

## 5. Diagnosis of swine-like human H1N1

Upper respiratory specimens such as nasopharyngeal aspirates or nasopharyngeal swabs, throat or nose swabs are suitable for the detection of S-OIV. World Health Organization recommends that suspected clinical cases of swine-like H1N1 influenza A infection are confirmed by (1) specific RT-PCR assays that differentiate S-OIV from seasonal influenza viruses, (2) the isolation and identification of swine-like H1N1 influenza, or (3) the detection of a fourfold rise of neutralization or HAI antibodies to S-OIV.<sup>47</sup>

It is recommended that patient samples are tested by RT-PCR for an influenza A virus target (e.g. M gene) and for the S-OIV H1N1 HA in parallel. Specimens that are positive in both the M gene and S-OIV H1 and negative for seasonal influenza A H1 and H3 can be confirmed as S-OIV infection. A number of sensitive and specific RT-PCR and real time PCR methods for detecting S-OIV and differentiating it from seasonal H1N1 are now available (Refs. [47,48] and papers in this issue of the journal).

The S-OIV H1N1 can be isolated in MDCK cells in the presence of trypsin (as for other seasonal influenza viruses) or in embryonated hens egg. Turkey, chicken, guinea pig and human red blood cells will agglutinate S-OIV. Virus culture is recommended to be carried out in BSL-3<sup>49</sup> or BSL-2 with BSL-3 practice.<sup>50</sup>

Because they are generally targeted at conserved virus antigens (e.g. nucleoprotein), existing point-of-care rapid antigen detection ELISA tests have comparable analytical and clinical sensitivity for S-OIV and for seasonal influenza A viruses.<sup>51,52</sup> But their sensitivity is lower than that of RT-PCR or culture and these tests do not differentiate seasonal influenza A from the novel swine-like H1N1 infections.

## 6. Clinical features and epidemiology

The majority of cases of S-OIV have been mild influenza-like illness. Fever is not invariably present. Some patients have had gastrointestinal symptoms including diarrhea. Approximately 2–5% of virologically confirmed cases in USA and Canada and 6% of cases in Mexico have been hospitalized. One-fifth of hospitalized cases in California required management in intensive care units. However, as the denominator of S-OIV infections in the community is being under-estimated and thus the true hospitalization rate is likely to be lower. About half of hospitalized patients have had underlying conditions such as asthma and other lung diseases, diabetes, morbid obesity, auto-immune disorders, immunosuppressive therapies, neurological or cardiovascular disorders or pregnancy. In Mexico, where the largest numbers of fatalities have been seen, severe pneumonia with multifocal infiltrates and rapid progression to acute respiratory distress syndrome (ARDS) and multi-organ failure has been reported. Severe disease was not invariably associated with secondary bacterial infections. Case fatality rates in Mexico are estimated to be around 0.4%<sup>53</sup> while countries other than Mexico have to date reported a crude case fatality of 18 deaths in 12,381 virologically confirmed cases.<sup>54</sup> It should be noted that many of the case reports outside of Mexico so far have been from the Northern hemisphere with the spread of the virus occurring outside of the typical influenza virus season. Information from the Southern hemisphere will be important in assessing the true burden of disease severity. Initial guidance of clinical management has been provided by WHO<sup>55</sup> and US CDC.<sup>56</sup>

The majority of virologically confirmed cases of S-OIV infection in Mexico ( $\geq 98\%$ ) were in those  $\leq 60$  years of age.<sup>57</sup> This is concordant with the age stratified sero-epidemiology data which suggests that persons  $\geq 60$  years of age are more likely to have neutralizing antibodies to the virus.<sup>46</sup> The infection seems at least as transmissible as seasonal influenza. The basic reproduction number ( $R_0$ ) has been estimated to be 1.4–1.6,<sup>53</sup> 2.2–3.1<sup>58</sup> in different analysis of the Mexican data and 2.3 (95% confidence interval 2.0–2.6).<sup>59</sup> Secondary attack rates in a well defined outbreak in a Mexican Village (La Gloria) was 61% in those under 15 years and 29% in people above that age.<sup>53</sup> In the USA, there is evidence of higher levels of influenza-like illness than is normal for this time of year with most of the influenza viruses being detected now are novel H1N1 viruses providing evidence that the virus continues to transmit during a period that is not the conventional influenza season in that country.

Vaccine virus seed has recently been provided to commercial vaccine manufacturers and one remains hopeful that a safe and effective vaccine will be on the horizon. Since S-OIV is so far antigenically homogenous, such vaccines, if sufficiently immunogenic, are likely to afford protection for the foreseeable future.

## 7. Is it a pandemic?

A pandemic is generally defined as a novel infection that spreads globally. On that definition, S-OIV H1N1 is already “pandemic”. However, an influenza pandemic has in the past been defined as: (a) an outbreak that arises in one geographic area that spreads across the world, a high percentage of individuals are infected resulting in increased mortality rates. (b) Caused by a new influenza A virus sub-

type, the haemagglutinin of which is not related to influenza viruses circulating immediately before the outbreak and could not have arisen from those viruses by mutation.<sup>60</sup> By these criteria, it is not clear whether S-OIV will qualify to be termed a pandemic, even if (when) it spreads worldwide. When H1N1 re-emerged in 1977 after its absence in humans for 20 years, it spread worldwide, but was not yet regarded as a pandemic. That virus however, directly arose from the previous human H1N1 viruses. On this occasion, S-OIV is a novel animal virus that has acquired transmissibility in humans. Although the H1 subtype is currently circulating in humans and thus S-OIV H1N1 is not a subtype new to humans, it is clearly a virus that jumped species to humans from a non-human animal host and did not arise from previously circulating human viruses by mutation (i.e. by antigenic drift). There is also an argument being made by some, that to qualify as a pandemic, an influenza virus has to have a minimal severity. Disease severity however, can change with different geographic contexts and under different seasonal conditions and may vary as a virus adapts to its new host. It is generally accepted that the 1918 Spanish flu pandemic virus was relatively mild in its first wave and acquired its virulence when it returned in the winter. It is difficult enough to establish transmissibility of a novel virus in humans; to impose the condition of severity in addition to transmissibility will mean that (as it is clear from this example of S-OIV), a “pandemic” will only be called long after the event. On 11th June 2009, the World Health Organization declared that S-OIV was now pandemic.

## 8. The future?

S-OIV continues to spread globally and it is unlikely that the S-OIV virus will be contained in the manner that SARS was, i.e. by the determined application of public health measures. The transmission and clinical severity of S-OIV in the Northern hemisphere summer appear comparable with that of seasonal influenza but it remains to be seen how transmissibility and virulence manifest themselves during the winter in the Southern hemisphere, i.e. during the typical transmission season for influenza. Children and young adults appear most susceptible to the infection although when persons >60 years get infected, it can cause significant morbidity. The possibility of acquisition of oseltamivir resistance, either through reassortment with seasonal H1N1 viruses which are uniformly resistant to this drug or through a separate mutation event is a reality. The virus may acquire the replication complex (polymerase genes and nucleoprotein) of seasonal human influenza viruses via reassortment and this could potentially lead to changes in virus replication competence and virulence. The S-OIV virus appears to be able to transmit back from humans to pigs, as occurred in a pig farm in Canada.<sup>61</sup> Thus, as S-OIV spreads globally in humans, we need to be alert to the possibility of an S-OIV panzootic in pigs. Such a situation will provide the virus with other options for reassortment within pigs. Some have speculated that this may increase the possibility of reassortment of S-OIV with HPAI H5N1. While such a possibility cannot be completely excluded, it has to be kept in mind that although H5N1 viruses have occasionally infected pigs, it has not got established in this species (see above). Hence the chance of interaction, while real, is not huge. It is also noted that human H3N2 viruses have been established in pigs in Asia for over a decade<sup>13</sup> and thus there has been ample opportunity for H5N1 to reassort with a “human” influenza virus within pigs in Asia, opportunity that it has so far failed to exploit. Thus there is no reason to believe that the outcome will be any different, if S-OIV were to become endemic in pigs in Asia. If S-OIV was to genetically reassort with avian influenza H5N1 however, the implications could be far more threatening than S-OIV itself. The fact that the triple reassortant swine viruses, with whom S-OIV shares the key “replication

cassette” (i.e. polymerase and nucleoprotein gene segments), have a proven track record of repeated reassortment with other swine viruses does provide cause for continued vigilance.

## Conflicts of interest

None.

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