DOI:10.1111/j.1750-2659.2012.00337.x
www.influenzajournal.com

Original Article

Transmission of influenza A(H1N1) 2009 pandemic viruses in Australian swine

Yi-Mo Deng,[a] Pina Iannello,[a] Ina Smith,[b] James Watson,[b] Ian G. Barr,[a,c] Peter Daniels,[b] Naomi Komadina,[a] Bruce Harrower,[d] Frank Y. K. Wong[b]

[a]WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, Melbourne, Victoria, Australia. [b]Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, Victoria, Australia. [c]School of Applied Sciences, Monash University, Churchill, Victoria, Australia. [d]Virology, Queensland Health Scientific Service, Brisbane, Queensland, Australia.
Correspondence: Yi-Mo Deng, WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, North Melbourne, VIC 3051 Australia.
E-mail: yi-mo.deng@influenzacentre.org

Accepted 17 December 2011. Published Online 15 February 2012.

Background Swine have receptors for both human and avian influenza viruses and are a natural host for influenza A viruses. The 2009 influenza A(H1N1) pandemic (H1N1pdm) virus that was derived from avian, human and swine influenza viruses has infected pigs in various countries.

Objectives To investigate the relationship between the H1N1pdm viruses isolated from piggery outbreaks in Australia and human samples associated with one of the outbreaks by phylogenetic analysis, and to determine whether there was any reassortment event occurring during the human-pig interspecies transmission.

Methods Real-time RT-PCR and full genome sequencing were carried out on RNA isolated from nasal swabs and/or virus cultures. Phylogenetic analysis was performed using the Geneious package.

Results The influenza H1N1pdm outbreaks were detected in three pig farms located in three different states in Australia. Further analysis of the Queensland outbreak led to the identification of two distinct virus strains in the pigs. Two staff working in the same piggery were also infected with the same two strains found in the pigs. Full genome sequence analysis on the viruses isolated from pigs and humans did not identify any reassortment of these H1N1pdm viruses with seasonal or avian influenza A viruses.

Conclusions This is the first report of swine infected with influenza in Australia and marked the end of the influenza-free era for the Australian swine industry. Although no reassortment was detected in these cases, the ability of these viruses to cross between pigs and humans highlights the importance of monitoring swine for novel influenza infections.

Keywords 2009 A(H1N1) pandemic, influenza A, swine.

Please cite this paper as: Deng *et al.* (2012). Transmission of influenza A(H1N1) 2009 pandemic viruses in Australian swine. Influenza and Other Respiratory Viruses 6(3), e42–e47.

Introduction

Swine have receptors for both human and avian influenza viruses and are a natural host for influenza A viruses, potentially serving as a mixing vessel for co-infecting influenza A viruses to exchange different gene segments and create novel reassortant viruses. Swine influenza viruses have long circulated in pigs in different regions of the world, including the North American swine H1N1 lineage,[1] the triple-reassortant swine H3N2 lineage[2,3] and the Eurasian avian-like swine H1N1 lineage.[4,5] In Australia, there have been no reports of swine influenza in pigs prior to 2009, and swine influenza is classified as a notifiable disease (Australian Government DAFF, 2008).

In April 2009, a novel A(H1N1) virus was first discovered infecting humans in Mexico and spread rapidly across the world, resulting in the 2009 A(H1N1) pandemic (H1N1pdm).[6] Through phylogenetic analysis, the H1N1pdm virus was found to be a swine–human–avian triple-reassortant virus with gene segments of the polymerase basic protein 2 (PB2) and polymerase acidic protein (PA) from avian, polymerase basic protein 1 (PB1) from human H3N2, hemagglutinin (HA), nucleoprotein (NP) and non-structural protein (NS) from the classical swine lineage, and the neuraminidase (NA) and matrix protein (M) from Eurasian avian-like swine H1N1 lineage.[7] Apart from infecting millions of humans, the H1N1pdm virus has also infected pigs probably via human contact. The first confirmed H1N1pdm influenza outbreak in a commercial swine herd was in Alberta, Canada, in late April 2009.[8] Subsequently, many other countries around the world also reported outbreaks of H1N1pdm influenza in farmed

pigs.[9–11] The majority of those cases implicated humans as the source of infection. There have been cases where novel reassortant viruses were detected in pigs that were derived from H1N1pdm and other swine influenza strains.[11–14] More recently, it has been reported that humans were infected with reassortant viruses between H1N1pdm and swine triple-reassortant H3N2 in the United States.[15] There are concerns that further mutations in these reassortant viruses might occur in pigs to make them more adaptable to humans, potentially leading to another influenza pandemic.[11]

Here we report the characterization of the first influenza outbreaks in commercial pig farms in Australia caused by H1N1pdm influenza. Most importantly, two staff working with sick pigs in the same Queensland piggery also developed influenza-like illness (ILI) after the outbreak of disease in pigs and were infected with two H1N1pdm viruses unique to that piggery outbreak, but genetically distinct from other viruses circulating in the human population. Our results suggest that human–pig–human transmission of H1N1pdm 2009 viruses had most likely occurred in these instances.

Materials and methods

Clinical specimen collection and influenza virus characterization

Nasal swabs and blood samples from infected pigs were submitted to the Australian Animal Health Laboratory, and all the animal samples and subsequent virus isolation were prepared in physical containment level 3 biosecurity facilities. Swabs in viral transport media (VTM) were typically treated with antibiotics (penicillin, streptomycin and gentamycin) and incubated for at least 30 minutes before filtration through a 0·2-μm filter. Embryonated SPF eggs of 3, 9- to 11-day old (SPAFAS, Woodend, Australia) were inoculated into the allantoic sac with 0·2 ml per egg for each sample and incubated at 35°C for up to 5 days for virus propagation based on protocols from the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.[16] Allantoic fluid was tested for haemagglutinating activity with 0·5% chicken red blood cells. The virus was passaged up to five times using 0·2 ml as inoculum for each egg passage. Infected allantoic fluid was confirmed for haemagglutinating agents and subjected to molecular analysis. Human clinical specimens of influenza linked to the Queensland piggery outbreak were collected under the direction of the Darling Downs Public Health Unit and submitted through Queensland Health Pathology Services to Queensland Health Forensic and Scientific Services, Australia, for influenza screening. These samples were submitted to the WHO Collaborating Centre for Reference and Research on Influenza at VIDRL, Melbourne, Australia.

Influenza detection by real-time RT-PCR

RNA extracted from the clinical specimens or allantoic fluid were used for real-time RT-PCR for influenza detection on a ABI 7500Fast instrument, according to the CDC[17] procedure.

Genome sequencing and phylogenetic analysis

Reverse transcription was first carried out using SuperScript III RT system (Invitrogen) according to the manufacture's instruction with a universal primer, and the resulting cDNA (5 μl each) was subjected to PCR amplification of all eight influenza genes using Platinum Taq DNA polymerase high fidelity and the specific primers developed in our laboratory (primer sequences available on request). Sequencing of the PCR products was performed as described previously.[18] Sequence assembly and multiple sequence alignments were performed using the SeqMan Pro module of Lasergene version 8.0 software (DNASTAR, Madison, WI, USA). A maximum likelihood tree was created using PhyML[19] with HKY85 as the substitution model on the Geneious 5.14 software (Biomatters Ltd, Auckland, New Zealand) and FigTree v1.3.1.

Pyrosequencing

cDNA from viral RNA isolated from pigs was used as template for PCR with eight pairs of primers covering full genomes of H1N1pdm we developed for pyrosequencing.[20] The PCR products were used for pyrosequencing analysis as described before.[20] Briefly, biotinylated PCR amplicons were bound to streptavidin-coated beads and were then subjected to denaturation and washing to generate single stranded DNAs, which were then released to a PSQ plate pre-filled with 40 μl annealing buffer containing pyrosequencing primer (100 μm). The sample plate was heated at 80°C for 2 minutes and cooled to room temperature for primer annealing. Pyrosequencing reactions were performed with the PyroMark Gold enzyme, substrate and nucleotides using the PyroMark ID instrument (Qiagen, Valencia, CA, USA). Pyrosequencing data were analysed using Identifier software (Qiagen) with a library constructed containing the signature sequences of each gene from all subtypes to determine the origin of each gene.

Nucleotide sequence accession numbers

GenBank accession numbers of the complete genome sequences of A/swine/Queensland/02865-7/2009 from egg isolates or original specimen were assigned CY080437-449; for HA gene of A/swine/Victoria/0902767/2009 was CY080402; and for HA gene of A/swine/Victoria/0902797/2009 was CY080404. GenBank accession numbers of the complete genome sequences of A/Brisbane/2015/2009 were assigned HQ712177-183, and for HA gene of A/Brisbane/2014/2009 was HQ712184.

Results

Influenza H1N1pdm 2009 outbreaks in Australian pigs

Since July 2009, at least three H1N1pdm outbreaks in pig farms have been detected in three states in Australia. The first case was in western New South Wales at the end of July, followed by an outbreak in northern Victoria on 18 August and the third one in south-east Queensland on 23rd August. In all cases, disease was self-limited in the swine, and almost all of them made an uneventful recovery. Hemagglutinin genes from viruses isolated from representative pigs of each piggery were sequenced in an attempt to identify the origins of these viruses. Full HA sequences were obtained from the viruses from pigs in the Queensland and Victorian piggeries. Only partial HA sequence was obtained from the outbreak in New South Wales, and these data were not included in the phylogenetic analysis. The HA sequences isolated from the two Victorian pigs were identical, and the HA sequences from the four Queensland pigs showed that the HA sequence obtained from one pig (A/swine/Queensland/02865-5/2009) had a two amino acids difference compared to the HA sequence from the other three pigs (A/swine/Queensland/02865-2/2009, A/swine/Queensland/02865-7/2009, and A/swine/Queensland/02865-10/2009). When the HA sequences were further analysed phylogenetically with the known sequences of H1N1pdm viruses isolated from both human and swine in Australia and other countries, it was found that the viruses from different piggeries formed distinct groups, indicating they occurred via independent infections from different people (Figure 1). Despite the overall high homology in the HA genes between the pig and human H1N1pdm viruses, three HA mutations – A17T, D111N and I283V (numbering starting from the first Methionine) – were identified that were unique to the Queensland farm outbreak (Table 1). The two H1N1pdm viruses isolated from the Victorian pig farm (A/swine/Victoria/0902767/2009 and A/swine/Victoria/0902797/2009) showed almost identical HA sequences to a human H1N1pdm virus isolated from Melbourne, Victoria, in June (A/Victoria/2025/2009), with only two silent mutations.

Possible further pig-to-human transmission of H1N1pdm viruses in the Queensland outbreak

The piggery outbreak in Queensland was initially attended by a veterinarian on 24 August 2009 following observation of pigs being off their feed and coughing. Approximately 25% of animals in a shed holding 450 sows showed signs of inappetence, coughing and elevated temperatures of around 40·5°C. The course of disease was observed to be 5–7 days following onset of clinical signs with no long-term health effects. At least one farm worker had reported ILI around the time of investigation, and swine influenza was suspected but unfortunately no sample was taken from the worker. Nasal swabs and blood samples were collected from symptomatic pigs, and multiple tissue samples were also collected by necropsy from a deceased pig that had a long-standing pre-existing pleuropneumonia infection. Duplicate nasal swab samples from three infected pigs and fresh tracheal tissue from the deceased pig were confirmed to carry H1N1pdm virus based on real-time RT-PCR results on M, HA and NP genes. H1N1pdm virus was isolated from the above four positive samples after at least four passages through embryonated chicken eggs.

Soon after the Queensland outbreak, two other staff who attended the sick pigs at the same farm subsequently developed ILI and nasal swabs were taken from them on 27 and 28 August, 2009, respectively. Both samples were found to be H1N1pdm positive by real-time RT-PCR; however, no virus was recovered from these two specimens.

Hemagglutinin sequences from the specimens of these two staff revealed that they had been infected with two distinct viruses separately (Figure 1 and Table 1). The first virus was named A/Brisbane/2014/2009, which had an HA identical to the pig virus A/swine/Queensland/0902865-5/2009 with the A17T mutation; the second was named A/Brisbane/2015/2009, which was identical to the other three pig viruses, except for a mixed N/D at position 111 in the HA of A/Brisbane/2015/2009. The A17T and D111N mutations were not found in any of the approximately 200 H1N1pdm viruses sequenced from the human samples in Australia around the same period in 2009.

No reassortant virus in Australian pigs

The possible reassortment of the pig H1N1pdm virus strains with other influenza A viruses was also investigated. Full influenza genome sequencing was attempted on two original human specimens and four original pig specimens obtained from the Queensland outbreak. Owing to low viral load in the original samples, full genome sequences were only obtained from one human specimen (A/Brisbane/2015/2009), and only partial sequences were acquired from the swine specimen producing the A/swine/Queensland/09022865-7/2009 isolate. However, full genome sequences were achieved for the A/swine/Queensland/09022865-7/2009 virus isolated in embryonated chicken eggs. Their gene sequences were compared with each other and the sequences from other H1N1pdm viruses (Table 2). All the genes except HA and NP were identical between original A/Brisbane/2015/2009 specimen and egg grown A/swine/Queensland/0902865-7/2009 virus. The mutations in the HA (T35S and Q240R) and NP (G102R) were only found in the virus propagated in eggs, but not the original pig swab, and these were likely to be mutations arising from egg adaptation. Amino acid sequences of the M2 and NA proteins indicated that these viruses were

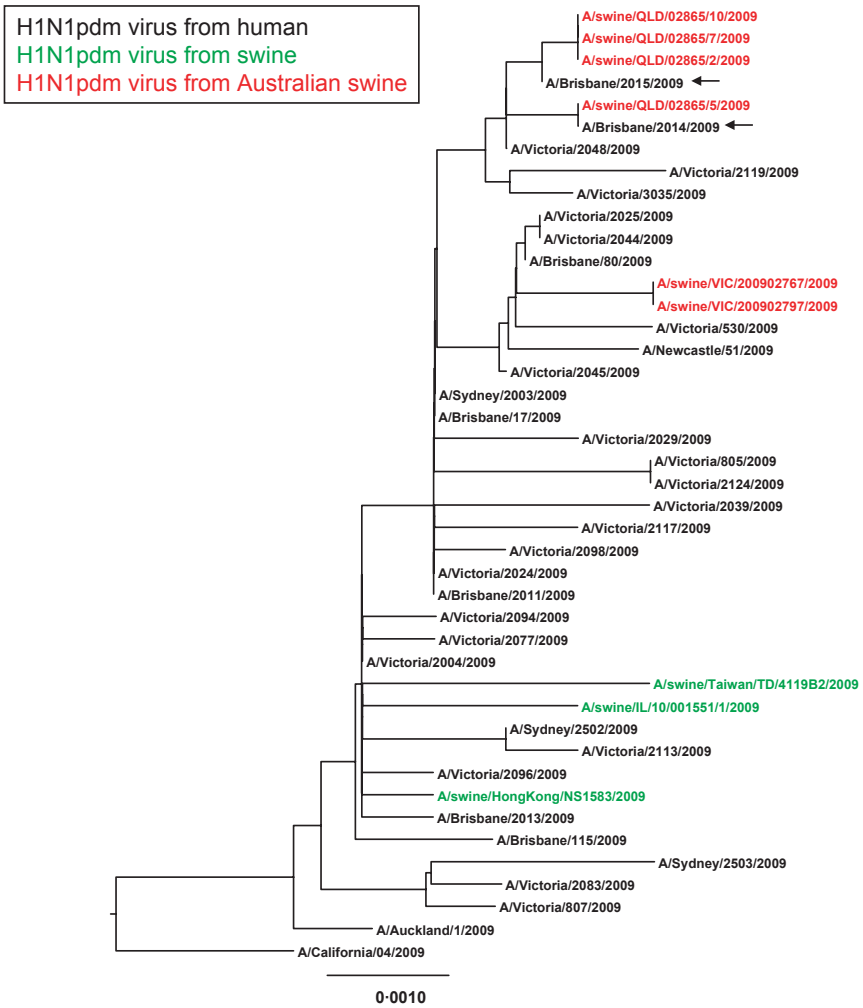

Figure 1. Phylogenetic analysis of HA sequences from influenza H1N1pdm viruses from both swine and humans in Australian. Hemagglutinin sequences of H1N1pdm viruses obtained from pigs in Victoria and Queensland, Australia, during August 2009 were compared to those H1N1pdm isolated from humans in Australia in 2009. Represented HA sequences obtained from pigs and humans in other countries (downloaded from GenBank) were also included in the analysis. A maximum likelihood tree was created using PhyML with HKY85 as the substitution model on the Geneious 5.14 software and viewed by FigTree v.1.3.1. The two HA sequences from staff working at the Queensland piggery are marked by the arrows.

resistant to the adamantanes (31N in M2) but not oseltamivir (275H in NA), which was consistent to most of the circulating H1N1pdm viruses. Sequences from all eight genes confirmed that these viruses belonged to the H1N1pdm lineage. Further, pyrosequencing of all eight genes was performed on RNA isolated from ten original porcine nasal swabs from the Victorian and Queensland pig farms, and again no reassortment between H1N1pdm and other influenza A viruses (human, swine or avian) was identified in these samples.

Discussion

Pigs in Australia have been reported to be free of swine influenza viruses prior to 2009.[21] This study represents the first cases of three independent human-to-pig transmission of H1N1pdm influenza viruses in three geographically well separated piggeries in Australia. In addition, transmission of two highly related but genetically distinct virus strains occurred within the infected pig herd at the same farm in Queensland. The Australian pigs may have been susceptible to H1N1pdm infections because this virus originated recently from swine; and Australian pigs may have also lacked of any pre-existing immunity in as swine influenza viruses have not been detected in Australia prior to 2009. In spite of this, H1N1pdm infected Australian pigs generally recovered from the disease after 5–7 days without any long-term health problems, similar to other studies in countries which have endemic swine influenza.[8–11]

Table 1. Substitutions in hemagglutinin protein of pandemic H1N1 2009 influenza viruses isolated from humans and swine in Australia

Virus strain	Sample date	Substitution at indicated amino acid position*								
		17	100	111	214	220	222	283	338	428
A/California/07/2009	04/2009	A	P	D	T	S	R	I	I	V
A/Auckland/1/2009	04/2009		S		A				V	
A/Brisbane/2011/2009	05/2009		S		A	T			V	I
A/swine/Queensland/02865-2/2009	08/2009		S	N	A	T		V	V	I
A/swine/Queensland/02865-5/2009	08/2009	T	S		A	T		V	V	I
A/swine/Queensland/02865-7/2009	08/2009		S	N	A	T		V	V	I
A/swine/Queensland/02865-10/2009	08/2009		S	N	A	T		V	V	I
A/Brisbane/2014/2009	08/2009	T	S		A	T		V	V	I
A/Brisbane/2015/2009	08/2009		S	D/N	A	T		V	V	I
A/Victoria/2025/2009	06/2009		S		A	T	K		V	I
A/swine/Victoria/0902797/2009	08/2009		S		A	T	K		V	I
A/swine/Victoria/0902767/2009	08/2009		S		A	T	K		V	I

*Amino acid position started at the first Methionine.

Table 2. Sequence similarities between the full genome sequence of A/Brisbane/2015/2009 and other H1N1pdm viruses

Nucleotide identity	A/Brisbane/2015/2009 original specimen							
	Hemagglutinin (HA)* (%)	Neuraminidase (%)	Matrix protein (%)	Polymerase basic protein 2 (%)	Polymerase basic protein 1 (%)	Polymerase acidic protein (%)	Nucleoprotein (%)	Non-structural protein (%)
A/swine/Queensland/02865-7/2009 original specimen	99·90	100 (partial)	n.a	100 (partial)	100 (partial)	100 (partial)	100 (partial)	n.a
A/swine/Queensland/02865-7/2009 egg isolate	99·90	100	100	100	100	100	99·90	100
A/Brisbane/2011/2009	99·90	99·90	99·90	99·90	99·80	100	99·80	100
A/swine/Alberta/HTH-33-8/2009	99·40	99·40	99·50	99·50	99·60	99·70	99·20	99·40
A/California/07/2009	99·50	99·70	99·70	99·70	99·90	99·90	99·60	99·80
A/Auckland/1/2009	98·90	99·50	99·70	99·20	99·90	96·90	99·70	99·80

*The only nucleotide difference between the HA of A/Brisbane/2015/2009 and /swine/Queensland/02865-07/2009 original specimen is a mix of N94 and N94D for A/Brisbane/2015/2009, and pure N94 for A/swine/Queensland/02865-07/2009 original specimen.

In this study, several lines of evidence were obtained, which suggested human-to-pig-to-human transmission of 2009 A(H1N1) pandemic viruses including (i) the two farm staff who developed influenza-like symptoms while working with the sick pigs in the Queensland farm following the initial cases of infected pigs; (ii) the full virus genome sequences from at least one staff were identical to the virus genes isolated from one pig; and (iii) most importantly, the signature HA mutations unique to the H1N1pdm viruses from the pigs but absent from other H1N1pdm viruses circulating in the human population at the same time were also observed in these two human specimens.

Novel swine influenza reassortant viruses between the H1N1pdm and classical swine influenza viruses have been detected in a number of countries, including Hong Kong,[11] Germany,[13] Italy[12] and United States.[14] This is not surprising because swine influenza viruses are endemically present in these pig herds. Transmission of influenza viruses between human and pigs is a public health concern, because reassortment can take place in the pig reservoir and further

cross-species infections may facilitate creation of a new reassortant influenza virus. Indeed, sporadic cases of humans infected with a reassortant virus that included H1N1pdm matrix gene and other seven genes from swine triple-reassortant H3N2 lineages have recently been reported in the United States.[15]. Fortunately, Australian pigs appeared to be free of endemic swine influenza prior to 2009, hence the risk of infected H1N1pdm virus reassorting with other swine influenza in these pigs was not present; however, it is still possible that reassortment may occur from co-infection with other human or avian influenza viruses if the H1N1pdm becomes endemic in Australian pigs. In the cases reported here, strict quarantining of infected properties was applied in these pig farms once the H1N1pdm infection were detected and the virus did not appear to spread further to other pig farms.

In conclusion, these findings drew an end to the era of influenza-free pigs that had been observed in Australia prior to 2009. The extent to which H1N1pdm is currently circulating or not in Australian pig farms is currently unknown because of discontinued surveillance, although the most recent diagnosed outbreak was in June 2011 from a Western Australian piggery (data not shown). Our study highlights the importance of close surveillance of influenza viruses in both humans and pigs, to minimize further cross-species transmission between these populations. Genetic analysis of complete influenza genomes is also important to monitor for the potential emergence of reassortant viruses that might lead to a new human pandemic virus.

Author contributions

Yi-Mo Deng was responsible for designing the experiments, analysing the data and writing the manuscript; Pina Iannello, Ina Smith and Naomi Komadina performed the experiments; Ian Barr, Peter Daniels and Frank Wong participated in experimental design and manuscript editing; Bruce Harrower provided the human samples and erformed the initial test on them.

Acknowledgements

We thank Prof. Anne Kelso for helpful discussion and critical reading of the manuscript. We also thank the state animal health divisions of the New South Wales, Queensland and Victoria Departments of Primary Industries for submissions of diagnostic pig samples; staff of the Darling Downs Public Health Unit for forwarding human clinical samples from the outbreak. The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health and Ageing.

References

1 Lewis PA, Shope RE. Swine influenza: Ii. A Hemophilic bacillus from the respiratory tract of infected swine. J Exp Med 1931; 54:361–371.
2 Brown IH, Harris PA, McCauley JW, Alexander DJ. 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 1998; 79(Pt 12):2947–2955.
3 Webby RJ, Swenson SL, Krauss SL, Gerrish PJ, Goyal SM, Webster RG. Evolution of swine H3N2 influenza viruses in the United States. J Virol 2000; 74:8243–8251.
4 Brown IH, Ludwig S, Olsen CW *et al.* Antigenic and genetic analyses of H1N1 influenza A viruses from European pigs. J Gen Virol 1997; 78(Pt 3):553–562.
5 Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev 1992; 56:152–179.
6 Dawood FS, Jain S, Finelli L *et al.* Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 360:2605–2615.
7 Smith GJ, Vijaykrishna D, Bahl J *et al.* Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 2009; 459:1122–1125.
8 Pasma T, Joseph T. Pandemic (H1N1) 2009 infection in swine herds, Manitoba, Canada. Emerg Infect Dis 2010; 16:706–708.
9 Pereda A, Cappuccio J, Quiroga MA *et al.* Pandemic (H1N1) 2009 outbreak on pig farm, Argentina. Emerg Infect Dis 2010; 16:304–307.
10 Sreta D, Tantawet S, Na Ayudhya SN *et al.* Pandemic (H1N1) 2009 virus on commercial swine farm, Thailand. Emerg Infect Dis 2010; 16:1587–1590.
11 Vijaykrishna D, Poon LL, Zhu HC *et al.* Reassortment of pandemic H1N1/2009 influenza A virus in swine. Science 2010; 328:1529.
12 Moreno A, Di Trani L, Faccini S *et al.* Novel H1N2 swine influenza reassortant strain in pigs derived from the pandemic H1N1/2009 virus. Vet Microbiol 2011; 149:472–477.
13 Starick E, Lange E, Fereidouni S *et al.* Re-assorted pandemic (H1N1) 2009 influenza A virus discovered from pigs in Germany. J Gen Virol 2011; 92:1184–1188.
14 Ducatez MF, Hause B, Stigger-Rosser E *et al.* Multiple reassortment between pandemic (H1N1) 2009 and endemic influenza viruses in pigs, United States. Emerg Infect Dis 2011; 17:1624–1629.
15 CDC. Swine-origin influenza A(H3N2) virus infection in two children – Indiana and Pennsylvania, July–August 2011. MMWR 2011; 60:1213–1215.
16 OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Available at http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ (Accessed 10 November 2008).
17 Centres for Disease Control P. CDC Protocol of Realtime RTPCR for Influenza A (H1N1), Rev 2. Available at http://www.who.int/csr/resources/publications/swineflu/realtimertpcr/en/index.html (Accessed 11 May 2009).
18 Barr IG, Cui L, Komadina N *et al.* A new pandemic influenza A(H1N1) genetic variant predominated in the winter 2010 influenza season in Australia, New Zealand and Singapore. Euro Surveill 2010; 15:pii=19692.
19 Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003; 52:696–704.
20 Deng YM, Caldwell N, Barr IG. Rapid detection and subtyping of human influenza A viruses and reassortants by pyrosequencing. PLoS ONE 2011; 6:(8):e23400.
21 Brockwell-Staats C, Webster RG, Webby RJ. Diversity of influenza viruses in swine and the emergence of a novel human pandemic influenza A (H1N1). Influenza Other Respi Viruses 2009; 3:207–213.