# Pandemic H1N1 2009 influenza in pigs in Australia

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**The novel H1N1 2009 virus that is the cause of the most recent human influenza pandemic is able to infect a number of animal hosts, most notably reported in domesticated swine[1-4]. The first confirmed 2009 pandemic H1N1 (H1N1pdm) influenza infection of a commercial swine herd occurred in Alberta, Canada in late April 2009[5]. The early incidences of H1N1pdm influenza in swine were of great concern to public and animal health agencies alike, and numerous subsequent cases were reported to the World Organization for Animal Health (OIE) by different countries following prevalence of the pandemic virus in the human population, including Australia[5-7]. In almost all cases, outbreak investigations have indicated an epidemiological link with farm in-contact persons reporting recent influenza-like illness (ILI), some diagnostically confirmed as H1N1pdm infections. These have suggested interspecies transmissions from human to swine. This article describes the first reported cases and our investigations of swine influenza due to H1N1pdm virus in Australia.**

Following Canada and Argentina, Australia was the third country to report H1N1pdm influenza in a domesticated pig herd[7]. It was the first confirmed incidence of influenza, of any type, in Australian swine. The index Australian outbreak occurred at the end of July 2009 in north-western New South Wales at a farm running a small 280-sow integrated farrow to finish piggery[7,8]. Indications of illness, which included mild dry coughing by affected pigs of most age groups and reduced feed intake and lethargy in individually housed pregnant sows, were first noticed on 24 July. Prior to this, the herd in this remotely located farm had been free from other significant endemic respiratory pathogens. A veterinarian inspected the herd on 30 July and observed approximately 5% of the pigs expressing a non-productive cough without fever. The absence of several farm staff on sick leave with ILI at the time led to the suspicion of possible influenza in pigs. Duplicate nasal swabs and blood from a representation of symptomatic pigs were collected and sent to the diagnostic laboratories at the Elizabeth Macarthur Agricultural Institute (EMAI) at Menangle, NSW, and the CSIRO Australian Animal Health Laboratory (AAHL), Geelong in Victoria, for independent testing. Respective test results from both serological and PCR assays confirmed a positive diagnosis for H1N1pdm influenza.

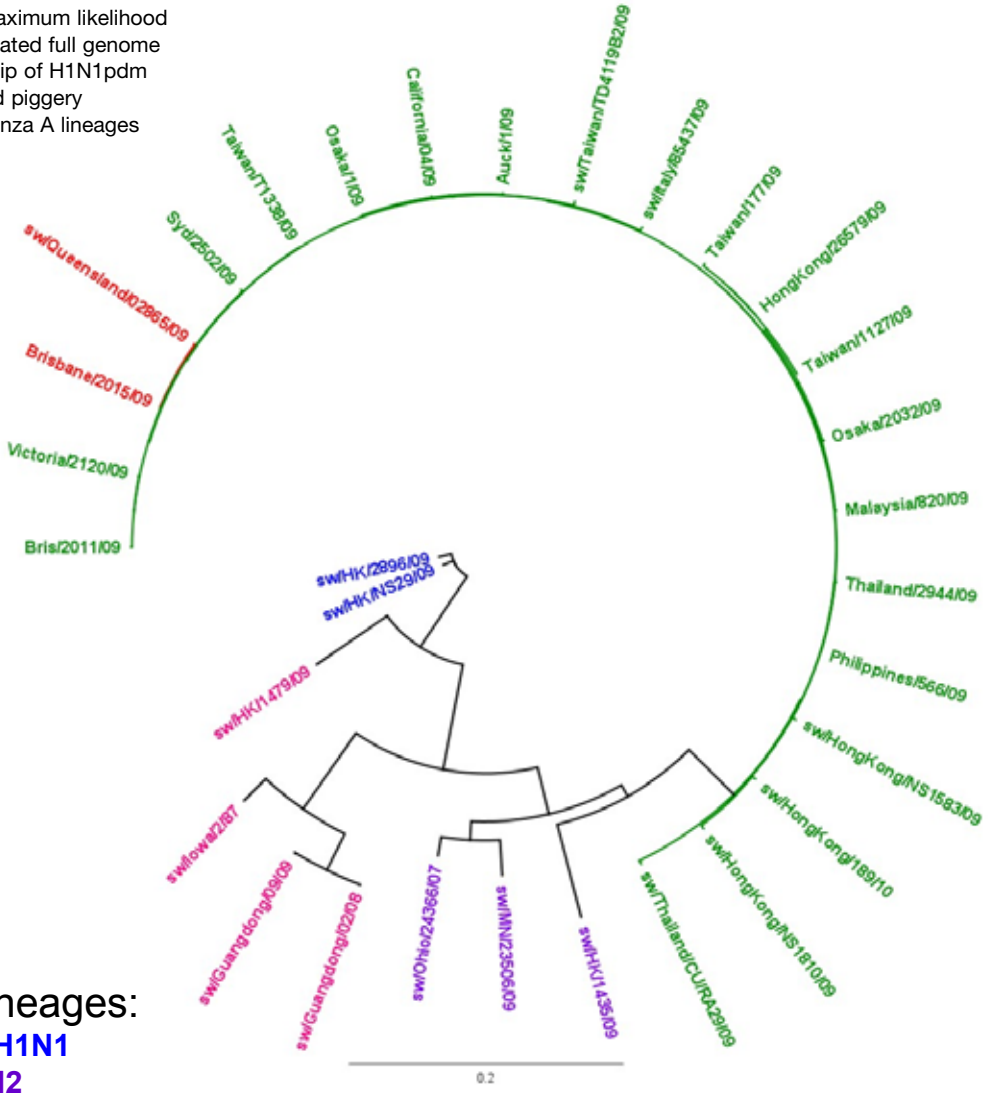
Shortly after the index outbreak in NSW, two further unrelated outbreaks were reported by different states in Australia, in a piggery herd in northern Victoria on 18 August 2009 and at a piggery located in Dalby Shire in south-east Queensland on 23 August[8]. Affected pigs in the Victorian outbreak had minimal clinical disease, although some inappetence and coughing were observed. A farm worker suffering from ILI at the time provided the impetus for diagnostic samples to be collected from representative pigs, which tested positive at AAHL for H1N1pdm influenza. The case in Queensland was attended by a veterinarian on 24 August with pigs being off their feed and coughing. The property held a total of 3000 pigs, with approximately 110 sows in a single shed showing clinical signs, which included elevated temperatures around 40.5°C. Again, farm workers had reported sick with ILI around the time of investigation. Nasal swabs and blood were collected from symptomatic pigs and the veterinarian also performed necropsy on a dead sow with a history of pre-existing pleuropneumonia. Tissue samples were also collected from this sow[8]. All samples were sent to the Queensland Biosecurity Sciences Laboratory, Yeerongpilly, for diagnostic testing, where nasal swabs from three of the symptomatic pigs and swabs from the lung, trachea and nasal mucosa of the sow tested positive for influenza A virus[8]. Subsets of nasal swabs from the infected pigs and tissue from the dead sow were forwarded to AAHL on 25 August and confirmed positive for H1N1pdm influenza virus. Following this case, a second independent outbreak of H1N1pdm influenza was also reported at a piggery in south-west Queensland on 14 October 2009 (http://www.dpi.qld.gov.au/4790_13586.htm). In general, H1N1pdm influenza in pigs was clinically mild and self-limiting with no spread to

animals outside the affected properties[11]. The course of disease was observed to be 5–7 days following onset of clinical signs with no long-term health effects. Infection did not spread beyond the affected properties.

A number of molecular and serologic diagnostic tests were used to investigate the H1N1pdm influenza cases in Australian pigs. TaqMan®-based, real-time, reverse transcription-PCR (rRT-PCR) assays targeting three different virus gene segments were used to confirm for H1N1pdm virus in pig samples. These included a pan-reactive rRT-PCR assay against the influenza A matrix (M1) gene modified from an established USDA protocol[9] to improve detection of the H1N1pdm virus. Additional rRT-PCRs that respectively targeted the haemagglutinin (H1) and neuraminidase (N1) genes of the H1N1pdm virus were further used to support a positive influenza A result. The latest updated versions of these PCRs were recently published by the UK Veterinary Laboratories Agency and the USDA respectively[10-11]. The presence of H1N1pdm virus antibodies in pig sera was confirmed using the haemagglutinin inhibition (HI) test with 0.5% chicken red blood cells against the H1N1pdm virus reference antigen, A/Auckland/1/2009(H1N1). Virus isolation was attempted on PCR positive samples in 9 to 11-day-old embryonated SPF chicken eggs and Madin-Darby Canine Kidney (MDCK) cells. Virus was isolated from the northern Victorian and south-east Queensland samples, but not from the index NSW outbreak. The viruses from pigs typically did not grow well in the laboratory, requiring a minimum of three passages before haemagglutinating agents could be detected.

In common with cases in other countries, H1N1pdm influenza infection of Australian commercial swine herds was suspected to have originated from 'spill-over' transmissions from the human population[1-3,8,12]. One or more primary farm in-contact persons with ILI at the time of onset of illness in pigs had been associated with all of the Australian cases investigated[8]. Unfortunately, except for the Queensland outbreak, specimens from the human cases of ILI linked to the piggery outbreaks

Figure 1. Dendrogram from the maximum likelihood phylogenetic analysis of concatenated full genome sequences showing the relationship of H1N1pdm viruses from the initial Queensland piggery outbreak to the major swine influenza A lineages (modified from reference 13).




Swine influenza lineages:

**European 'avian-like' H1N1**

**Triple reassortant H1N2**

**Classical swine H1N1**

**H1N1pdm 2009**

**Viruses associated with Queensland piggery outbreak**

were not available for characterisation. Phylogenetic analysis of full-length haemagglutinin (HA) gene sequences derived from the Victorian and Queensland swine virus samples together with available sequences from H1N1pdm viruses from humans in Australia showed that independent human-to-pig transmissions had likely occurred in the separate farm outbreaks[13]. Our project team showed that viruses from the differently located farms (in Victoria and Queensland respectively) were clearly distinct from each other but had highest respective relatedness, with nearly identical HA sequence homologies, to the pandemic viruses circulating in the human population at the same location at the time[13].

In addition, HA sequences obtained from specimens collected from two farm staff who had developed ILI after attending sick pigs at the Queensland piggery showed respective infections with two distinct viruses, based on substitutions at different amino acid positions[13]. These same genetic variants were also isolated from the pig-derived samples and were unique to the viruses associated with this outbreak, providing for the first time strong evidence of the zoonotic transmission of H1N1pdm virus from infected pigs back to humans. Furthermore, whole genome sequences obtained from one human and a pig specimen respectively were identical, with phylogenetic analysis confirming that these viruses belonged to the H1N1pdm lineage (Figure 1).

Unlike the situation in other parts of the world, Australian swine are considered to be free from influenza caused by various 'classical' subtypes of swine influenza A viruses[14,15]. Swine influenza is thereby on the list of nationally notifiable animal diseases in Australia as a measure towards protecting the country's swine industry from an exotic disease[16]. The described spill-over transmissions of H1N1pdm influenza to farmed pigs at the human-animal interface provided the first confirmation of swine influenza in Australia. In these cases, state veterinary emergency disease response agencies mobilised biosecurity measures following diagnostic confirmation of H1N1pdm influenza, which in accordance with the national veterinary emergency control strategy (AUSVETPLAN) included strict quarantining of the affected properties[8]. H1N1pdm influenza is now globally endemic and the disease in humans has been reclassified by the World Health Organization to be in the post-pandemic period[17]. The ability of H1N1pdm viruses to circulate in pigs has provided potential opportunities for reassortments with other influenza A viruses that may result in progeny with greater threat to public health[18]. However, despite providing evidence of bi-directional transmissions between humans and swine, our analysis of the genome segments of H1N1pdm viruses from Australian pig farms has shown no evidence of reassortment with non-pandemic viruses. These investigations support OIE recommendations for the continued surveillance of epidemiologic events of significance in animals due to influenza viruses, including H1N1pdm (http://www.oie.int).

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