Locally Acquired Human Infection with Swine-Origin Influenza A(H3N2) Variant Virus, Australia, 2018

Yi-Mo Deng, Frank Y.K. Wong, Natalie Spirason, Matthew Kaye, Rebecca Beazley, Miguel L. Grau, Songhua Shan, Vittoria Stevens, Kanta Subbarao, Sheena Sullivan, Ian G. Barr, Vijaykrishna Dhanasekaran

In 2018, a 15-year-old female adolescent in Australia was infected with swine influenza A(H3N2) variant virus. The virus contained hemagglutinin and neuraminidase genes derived from 1990s-like human seasonal viruses and internal protein genes from influenza A(H1N1)pdm09 virus, highlighting the potential risk that swine influenza A virus poses to human health in Australia.

Long-term circulation of influenza A viruses (IAVs) among swine poses a public health threat. The 2009 pandemic was caused by a reassortant swine influenza A(H1N1) virus with genes that originated from human and avian IAVs that had circulated among swine for several years (*1*,*2*). Since then, globally enhanced influenza surveillance among swine has indicated continuous introduction of human seasonal influenza viruses into swine, followed by reassortment with influenza A viruses endemic in swine (IAV-S) and persistence of many lineages in swine for several decades (*3*). Although IAV-S are normally limited to transmission among swine, since 2010, a total of 430 cases of human infection with swine-origin influenza A(H3N2) variant viruses (H3N2v) have been detected in the United States (*4*), primarily in young persons exposed to swine at agricultural fairs. Most patients had self-limited influenza-like illness (*5*). Recent data also suggest that IAV-S have been endemic to Australia for many decades, including viruses that were originally derived from human H3N2 viruses as early as 1968, pre-2009 seasonal H1N1 viruses, and influenza A(H1N1)pdm09 (pH1N1) viruses (*6*).

Affiliations: World Health Organization Collaborating Centre for Reference and Research on Influenza, Melbourne, Victoria, Australia (Y.-M. Deng, N. Spirason, M. Kaye, K. Subbarao, S. Sullivan, I.G. Barr, V. Dhanasekaran); CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia (F.Y.K. Wong, S. Shan, V. Stevens); South Australian Department of Health and Wellbeing, Adelaide, South Australia, Australia (R. Beazley); Monash University, Melbourne (M.L. Grau, V. Dhanasekaran); University of Melbourne, Melbourne (S. Sullivan, I.G. Barr)

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The Study

In September 2018, a case of human infection with a swine-origin influenza virus was detected in Australia through routine human influenza virus surveillance by the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza (Melbourne, Victoria, Australia), which is part of the WHO Global Influenza Surveillance and Response System. The sample was from a 15-year-old female adolescent living in a semirural area in South Australia, ≈100 km from Adelaide. The patient sought outpatient care for a mild respiratory illness ≈8 days after illness onset. The attending physician collected a nasal swab sample and sent it for testing to a laboratory in Adelaide, where influenza A was detected by real-time reverse transcription PCR (RT-PCR) but not subtyped. The sample was subsequently forwarded to the WHO Collaborating Centre for further characterization. It was later determined that the patient had not been vaccinated against influenza in 2018 and had had contact with animals at an agricultural show in South Australia the day before illness onset.

We isolated an influenza A virus in SIAT-1 MDCK cells (*7*) and designated it as A/South Australia/85/2018 (Appendix, https://wwwnc.cdc.gov/EID/article/26/1/19-1144-App1.pdf). Testing of this isolate by real-time RT-PCR with an influenza diagnostic kit from the US Centers for Disease Control and Prevention confirmed that the virus was an

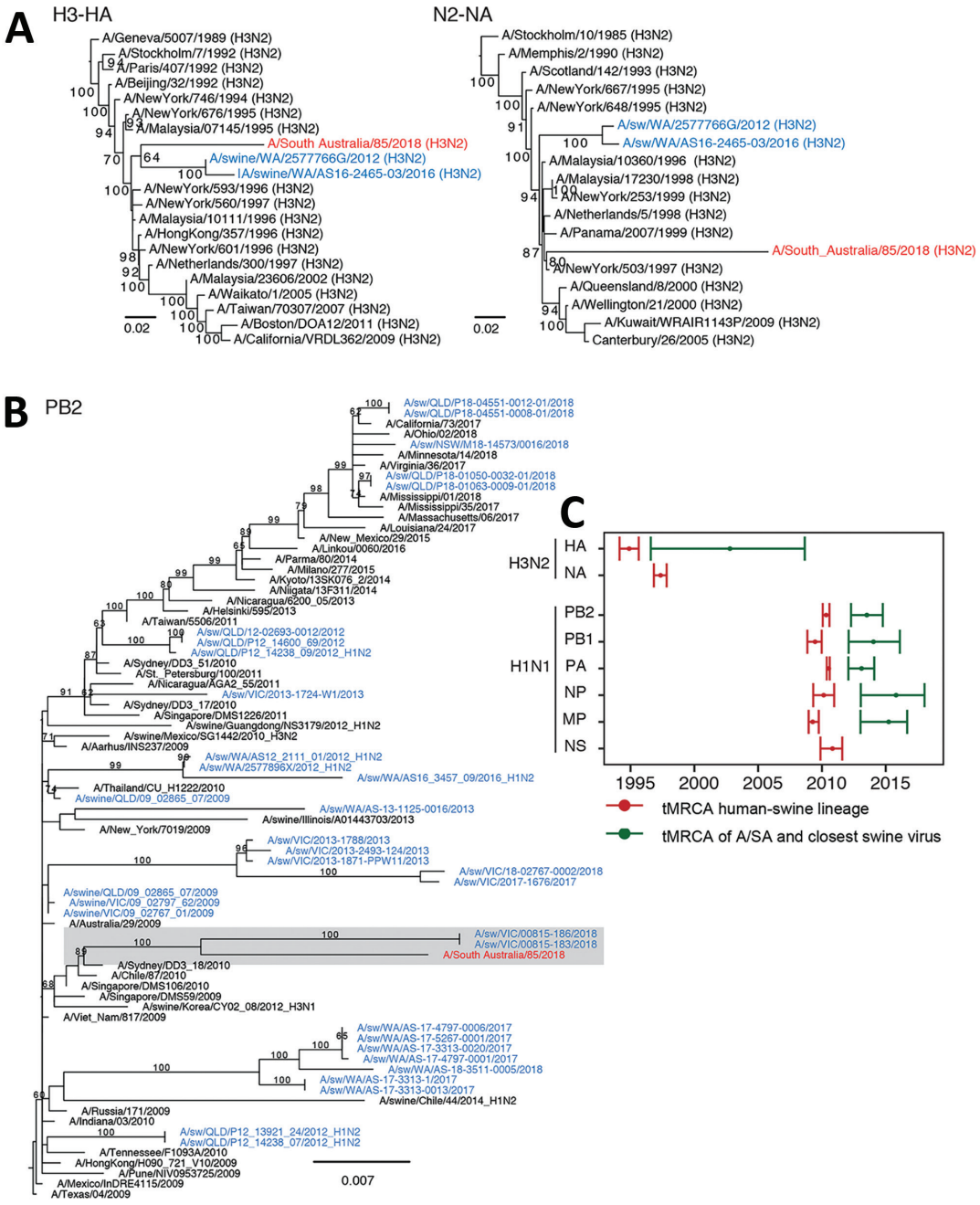

Figure 1. Genetic origin of influenza A/South Australia/85/2018 virus isolated from a human patient in Australia (red) from swine influenza A(H3N2) and H1N1pdm09 viruses. Blue indicates influenza A viruses from swine in Australia. A, B) Maximum-likelihood phylogenies estimated by using RAxML version 8 (*10*) of the HA and NA genes (A) and PB2 gene (B) showing bootstrap values at branch nodes (Appendix, https://wwwnc.cdc.gov/EID/article/26/1/19-1144-App1.pdf). The origins of the remaining 5 internal proteins genes (PB1, PA, NP, MP, and NS) are provided in Appendix Figure 2, and the GenBank accession numbers and dates of sampling are provided in Appendix Table 4. Scale bars indicate nucleotide substitutions per site. C) Calculation of tMRCA. Red indicates means and 95% CIs of the time of origin of each of the Australia swine influenza A virus lineages from human seasonal influenza viruses. Numbers denote viruses that shared the same tMRCA and that formed a similar lineage. Green indicates the time of divergence of A/South Australia/85/2018 from A/swine/WA/2577766G/2012 (H3N2) (for the H3 HA gene) and A/swine/Victoria/18-04095-0003/2018 (H1N1) (for 5 internal protein genes: PB2, PB1, PA, NP, and MP). N2 and NS proteins of A/South Australia/85/2018 are directly derived from human viruses. Divergence times were estimated by using the uncorrelated log-normal relaxed clock model (*11*) in a Bayesian Markov chain Monte Carlo framework in BEAST version 1.10 (https://beast.community). A/SA, A/South Australia/85/2018 virus; HA, hemagglutinin; MP, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic; A(H1N1) pdm09 virus, 2009 pandemic influenza H1N1 virus; tMRCA, time to most recent common ancestor.

influenza A(H3N2) virus with a pH1N1-like nucleoprotein (NP) gene. Hemagglutination inhibition (HI) assays of the isolate showed that this virus had >5-fold antigenic divergence to ferret antiserum raised against human H3N2 viruses collected during 1993–2016 and an H3N2v from the United States (A/Minnesota/11/2010) (Appendix Table 1) (*6*). The A/South Australia/85/2018 virus showed good cross-reactivity

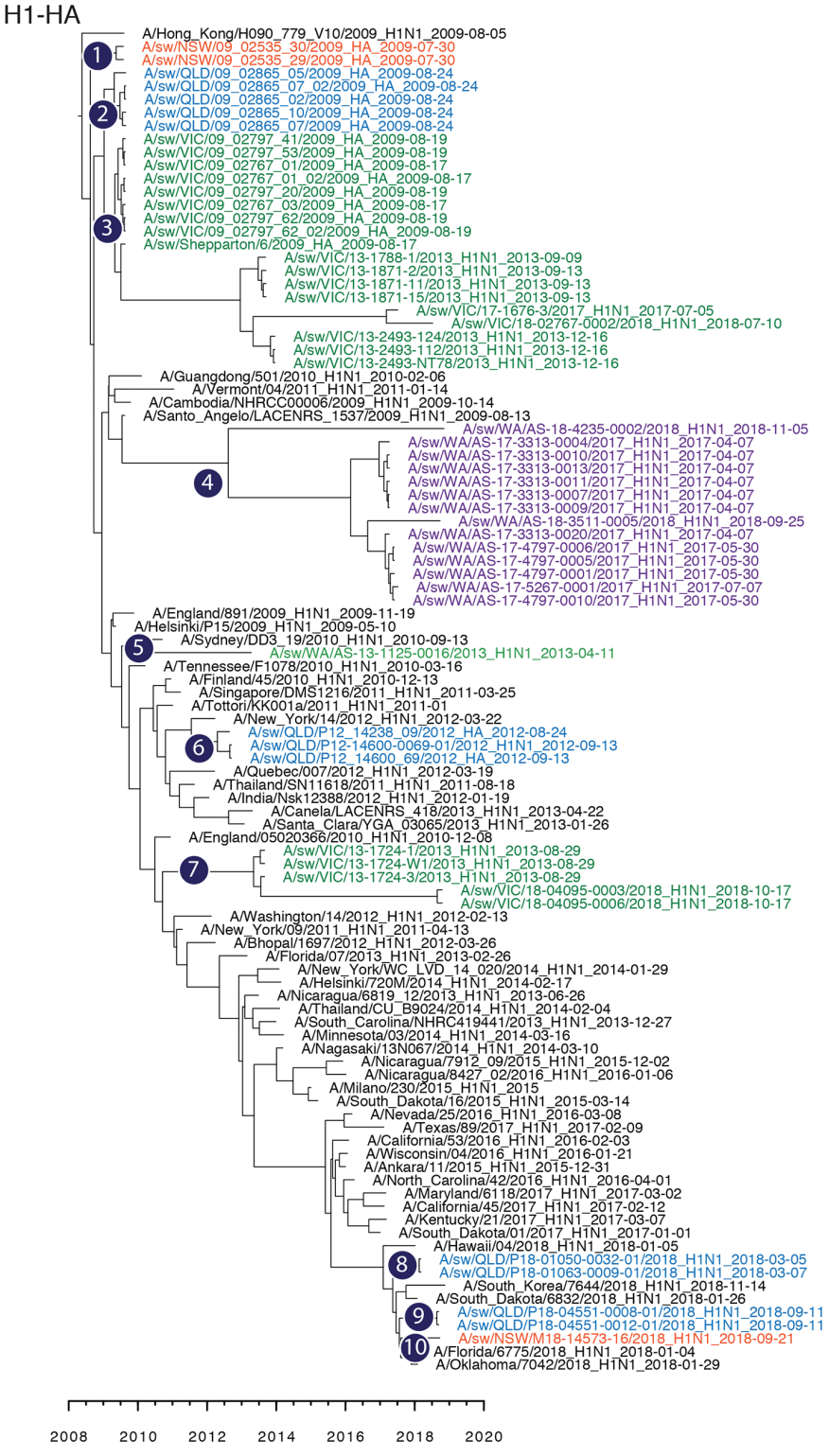

Figure 2. Maximum-clade credibility tree showing the time of emergence from humans and divergence of swine influenza A and influenza A(H1N1)pdm09 virus among swine populations in Australia. Numbers 1–10 denote inferences of individual introductions from humans to swine, and virus names are colored by state of collection: red, New South Wales; blue, Queensland; green, Victoria; purple, Western Australia. Time-scaled phylogenies were estimated by using the uncorrelated log-normal relaxed clock model (*11*) in a Bayesian Markov chain Monte Carlo framework in BEAST version 1.10 (https://beast.community). HA, hemagglutinin.

(within 2-fold of the homologous HI titer) with pig antiserum to a US swine A(H3N2) virus, A/swine/New York/A01104005/2011 (Appendix Table 2). Although both viruses showed high genetic divergence, they contained a common hemagglutinin (HA) Y155H substitution within antigenic site B (Appendix Table 3); its role in antigenicity remains unknown.

Whole-genome sequencing of A/South Australia/85/2018 indicated that the virus was a 2:6 reassortant of 2 human influenza virus lineages introduced to swine in Australia; the H3 HA and N2 NA genes originated independently from seasonal human influenza viruses that circulated during 1995–1999, and the genes for internal proteins originated from pH1N1 viruses from late 2009 through early 2010. Using a fluorescence-based NA enzyme inhibition assay, we found that A/South Australia/85/2018 was sensitive to 1 class of influenza drugs, the NA inhibitors (oseltamivir and zanamivir); however, it would be expected to be resistant to the adamantane class of drugs (amantadine/rimantadine) because it had an S31N substitution in the matrix (M) 2 gene, which is known to confer high-level resistance to adamantane (*8*).

To identify the evolutionary origins and the epidemiologic links of A/South Australia/85/2018, we further generated the whole and partial genomes of an additional 44 available IAV-S collected in piggeries across several states in Australia during 2013–2018; all were pH1N1-like viruses (Appendix Table 4). These viruses were derived from swine nasal, tracheal, or pooled lung tissue samples submitted on an ad hoc basis for diagnostic investigations during 2012–2018 to the Australian Animal Health Laboratories (Geelong, Victoria, Australia) by commercial piggeries in New South Wales, Queensland, Victoria, and Western Australia.

Phylogenies inferred with all sequences of swine influenza viruses from Australia, as well as with a representative set of those collected from humans and swine globally (*6*,*9*) (Appendix), showed that the HA gene of A/South Australia/85/2018 was most closely related to swine H3N2 viruses collected from a commercial piggery in Western Australia during 2012–2016 (*6*) (Figure 1, panel A). This H3 swine lineage from Australia was poorly supported (64% bootstrap) and originated from the phylogenetic backbone of human seasonal H3N2 virus, clustering with viruses collected during 1995–1996, whereas their NA was most closely related to that of human H3N2 viruses circulating during 1997–1999 (Appendix Figure 1) and not most closely related to that of swine samples from Australia.

Of note, 5 of 6 internal segments of A/South Australia/85/2018 were most closely related to a swine pH1N1 virus collected in Victoria in 2018 (A/swine/Victoria/00815–183/2018) (Figure 1, panel B). Although there were no IAV-S data from South Australia to trace the immediate origins of A/South Australia/85/2018, these phylogenetic relationships confirm that A/South Australia/85/2018 was acquired locally from swine herds endemically infected with influenza A viruses that had circulated since the mid-1990s and the 2009 H1N1 pandemic.

The genomic relationship of A/South Australia/85/2018 to IAV-S collected across at least 2 geographically distinct states, Western Australia for the HA gene and Victoria for 5 internal segments, suggests the possibility of IAV-S movement between states in Australia, although IAV-S data for Australia are missing for at least 2–10 years (Figure 1, panel C). This suggestion is, however, contradictory to the data from IAV-S HA collected across 4 states in this study: 10 distinct monophyletic lineages (Figure 2) derived independently from the human pH1N1 lineage, with each group exclusive to 1 state, suggesting that there are spatial restrictions for farms in Australia.

Conclusions

A comparison of divergence times between the IAV-S segments from Australia showed that reassortment of endemic viruses with introduced human lineages had been continual (Appendix Figure 3), thereby potentially maintaining sustained transmission on individual swine farms. The risk for emergence of A/South Australia/85/2018-like viruses in humans is potentially high because all 6 internal protein genes are derived from human-adapted pH1N1 virus. The human-origin HA and NA genes of A/South Australia/85/2018 were widely circulating in the human population 20–25 years ago. Hence, children probably have little or no immunity to the HA/NA of this virus, making them more susceptible to infection with this virus subtype, as in the case reported here and in children infected with swine H3N2v virus in the United States (*12*–*14*).

The genomic and antigenic properties and epidemiologic characteristics of zoonotic IAV-S are useful for identifying the potential risk for emergence and spread into the human population. These data also enable better identification of potential nationally relevant mitigation strategies, including measures such as public awareness programs and influenza vaccination of swine herds to eliminate sustained transmission of influenza virus in swine populations (*15*). Our study highlights the risk to the general human population in Australia for infection with IAV-S and the need for more vigilant surveillance of swine and persons who are in close contact with swine to enable early detection and characterization of zoonotic influenza infections.

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About the Author

Dr. Deng leads the molecular biology group at the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia. Her primary research interest is the use of novel sequencing and analysis methods to infer the epidemiology and mechanisms of evolution of zoonotic and human influenza viruses.

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Address for correspondence: Yi-Mo Deng, WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty Institute for Infection and Immunity, 792 Elizabeth St, Melbourne, VIC 3000, Australia; email: yi-mo.deng@influenzacentre.org; Vijaykrishna Dhanasekaran, Department of Microbiology, Biomedicine Discovery Institute, Monash University, 19 Innovation Walk, Clayton, VIC 3800, Australia; email: vijay.dhanasekaran@monash.edu

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Appendix

Methods

Collection of human and swine viruses

The human nasal swab sample was forwarded for further characterization to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne (WHO CC). Virus isolation was attempted in SIAT-1-MDCK cells (*1*), using standard procedures (*2*). Swine nasal, tracheal, or pooled lung tissue samples were submitted between 2012 and 2018 on an ad hoc basis to the Australian Animal Health Laboratories (AAHL) in Geelong for diagnostic investigations by commercial piggeries in New South Wales (NSW), Queensland (QLD), Victoria (VIC) and Western Australia (WA). Swine IAV (swIAV) isolates were obtained by propagation in MDCK cells (*3*). Haemagglutination titers were obtained on cultures showing cytopathic effects using 0.5% (vol/vol) chicken or 1% (v/v) turkey red blood cells.

Hemagglutinin inhibition Assay

The antigenic relationships of the human and swine IAV were investigated by hemagglutination inhibition (HI) assays using panels of post-infection ferret and swine antisera raised against representative human and swine IAV (see Appendix Table 1 and S2). HI assays were performed with either turkey, guinea pig or chicken red blood cells as previously described using standard techniques (*2*).

Viral genome sequencing and assembly

Viral RNA was extracted using either QIAcube with QIAamp 96 Virus QIAcube HT kit (QIAGEN, https://www.qiagen.com) for human nasal swabs or MagMax Express magnetic particle processor with MagMax-96 viral RNA isolation kit (Thermo Fisher, https://www.thermofisher.com) for swine samples according to the manufacturer's instructions. Virus subtyping was performed using the CDC Influenza Virus Real-Time RT-

PCR kits provided by the International Reagent Resources (https://www.internationalreagentresource.org) at the WHO CC with SensiFAST Probe Lo-ROX One-Step Kit (Bioline, https://www.bioline.com).

Virus genome segments were amplified by one-step reverse transcription-PCR (RT-PCR) using SuperScript III one-step RT-PCR system with Platinum *Taq* DNA polymerase (Thermo Fisher) and the IAV gene primers with the cycling conditions previously described (*4*). Amplicons were analyzed on TapeStation 4200 (Agilent, https://www.agilent.com) to determine quality and quantity. IAV genomes were sequenced on an IonTorrent PGM (Thermo Fisher) for human IAV, and assembled using FluLINE as previously described (*5*). Genome sequences of swIAV samples were obtained using the Illumina MiSeq (Illumina, https://www.illumina.com) as previously described (*6*).

Phylogenetic analysis

The complete swIAV genome coding sequences determined in this study were compared with sequences obtained from the Influenza Research Database (IRD) and Global Initiative on Sharing All Influenza Data (GISAID) database. BLAST searches of each gene segment of A/SA was used to identify the influenza lineage to which each segment belonged to. Preliminary analysis was conducted using all available H3N2 HA and NA sequences collected during the 1990s and representative sequences from other years, while for the H1N1pdm09 datasets we included 30 sequences from each year from 2009–2018. Following data curation, phylogenetic relationships were estimated for each gene segment independently using the maximum likelihood (ML) method in RAxML v8 (*7*) using the General Time Reversible (GTR) nucleotide substitution model with a gamma (Γ) distribution of among-site rate. Branch support was estimated using a ML bootstrap analysis with replicates ranging from 10 to >1000 replicates for the different datasets analyzed. Time-scaled phylogenies were estimated using the uncorrelated log-normal relaxed clock model (*8*) in a Bayesian Markov Chain Monte Carlo (MCMC) framework in BEAST v1.10 (*9*). Trees were visualized and annotated using FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Accession number(s)

The influenza nucleotide sequences generated from the human case in this study can be obtained using GISAID isolate ID EPI_ISL_341299; and influenza sequences from swine can be obtained from NCBI GenBank using accession numbers MN200947 to MN201152.

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Appendix Table 1. Hemagglutinin inhibition assay using ferret antisera against human and swine H3N2 viruses isolated during 1993–2018.

	Antisera[1]											
Antigen	A/ Shangdong/ 9/93	A/ Johannesburg/ 33/94	A/ Wuhan/ 359/95	A/ Tasmania/ 1/97	A/ Sydney/ 5/97	A/ Moscow/ 10/99	A/ Minnesota/ 11/2010 (H3N2v)	A/ swine/WA/ 2577766G /2012	A/ Texas/ 50/ 2012	A/Hong Kong/ 4801/ 2014	A/ Singapore/ INFIMH-16–0019/2016	A/South Australia/ 85/2018
A/Shangdong/9/93	**1280**	160	40	<20	<20	<20	20	320	<20	<20	<40	<40
A/Johannesburg/33/94	160	**640**	40	<20	20	<20	<20	160	<20	<20	<40	<40
A/Wuhan/359/95	40	20	**640**	80	40	20	<20	80	<20	<20	<40	<40
A/Tasmania/1/97	20	<20	640	**>2560**	20	40	<20	40	<20	<20	<40	<40
A/Sydney/5/97	<20	<20	80	<20	**>2560**	320	20	40	<20	<20	<40	<40
A/Moscow/10/99	<20	<20	<20	<20	>2560	**>2560**	<20	40	<20	<20	<40	40
A/Minnesota/11/2010 (H3N2v)	20	<20	<20	<20	<20	<20	**320**	<20	<20	<20	<40	<40
A/swine/WA/2577766G/2012	40	20	<20	<20	<20	<20	<20	**1280**	<20	<20	<40	80
A/Texas/50/2012	<20	<20	<20	<20	<20	<20	<20	<20	**80**	40	<40	<40
A/Hong Kong/4801/2014	<20	<20	<20	<20	<20	20	<20	<20	80	**320**	40	80
A/Singapore/INFIMH-16–0019/2016	<20	<20	<20	<20	<20	<20	<20	<20	<20	80	**80**	<40
A/South Australia/85/2018	40	<20	<20	<20	<20	<20	<20	40	<20	<20	<40	**1280**

Appendix Table 2. Hemagglutinin inhibition assay using representative swine antisera

	Antisera[2]							
Antigen	A/sw/Ts/ 4199–1/98	A/sw/Co/ 23619/99	A/sw/Mn/ 01146/06	A/sw/NY/ A01104005/11	A/sw/Iowa/ A01480656/14	A/Sydney/ 5/97	A/Wuhan/ 359/95	A/Moscow/ 10/99
A/swine/Texas/4199–1/1998	**1280**	20	40	<20	20	<20	160	<20
A/swine/Colorado/23619/1999	160	**5120**	1280	40	80	160	160	160
A/swine/Minnesota/01146/2006	80	80	**>5120**	640	80	<20	40	20
A/swine/NewYork/A01104005/2011	40	20	160	**640**	40	<20	<20	<20
A/swine/Iowa/A01480656/2014	160	80	1280	640	**640**	80	320	<20
A/Sydney/5/97	80	640	160	<20	80	**320**	320	40
A/Wuhan/359/95	640	80	320	80	160	80	**1280**	<20
A/Moscow/10/99	80	320	640	<20	<20	160	20	**640**
A/Shangdong/9/93	640	20	160	80	20	<20	320	<20
A/swine/WA/2577766G/2012	320	<20	320	20	20	<20	320	<20
A/swine/WA/2465–2/2016	80	<20	640	20	160	80	80	<20
A/swine/Vic/17–03926/2017	<20	<20	<20	<20	<20	<20	<20	<20
A/South Australia/85/2018	160	20	1280	320	40	40	160	20

Appendix Table 3. Amino acid variation in the H3-HA antigenic epitopes

	H3-HA amino acid position (in the antigenic epitopes)						Sequence similarity to A/South Australia /85/2019
Virus strain	145	155	156	158	189	193	
Consensus	**N**	**H**	**X**	**N**	**X**	**N**	
A/South Australia/85/2018		Y	K		S	G	100%
A/swine/WA/2577766G/2012			K	D	S	S	92%
A/swine/WA/2465–2/2016	K		E		R		91%
A/swine/Minnesota/01146/2006			N		R		90%
A/swine/NY/A01104005/2011		Y	N		K		89%

Appendix Table 4. Meta-data of swine IAV sequenced in this study

Virus strain	**No. segments sequenced**	**Collection date**	**Sample type**	**Passage details**	**Genbank accession no.**
A/swine/New South Wales/M18–14573–16/2018(H1N1)	8	21/09/2018	Nasal swab	Original	MN200985-MN200992
A/swine/New South Wales/M18–14573–18/2018(H1N1)	1	21/09/2018	Nasal swab	Original	MN200947
A/swine/Queensland/P12–14600–0069–01/2012(H1N1)	8	13/09/2012	Nasal swab	E2	MN200993-MN201000
A/swine/Queensland/P18–01050–0032–01/2018(H1N1)	8	5/03/2018	lung	Original	MN201001-MN201008
A/swine/Queensland/P18–01063–0009–01/2018(H1N1)	8	7/03/2018	Nasal swab	Original	MN201009-MN201016
A/swine/Queensland/P18–04551–0008–01/2018(H1N1)	8	11/09/2018	Nasal swab	MDCK1	MN201017-MN201024
A/swine/Queensland/P18–04551–0011–01/2018(H1N1)	1	11/09/2018	Nasal swab	MDCK1	MN200948
A/swine/Queensland/P18–04551–0012–01/2018(H1N1)	8	11/09/2018	Nasal swab	MDCK1	MN201025-MN201032
A/swine/Queensland/P18–04551–0017–01/2018(H1N1)	1	11/09/2018	Nasal swab	MDCK1	MN200949
A/swine/Victoria/18–04095–0003/2018(H1N1)	8	17/10/2018	Nasal swab	MDCK2	MN201081-MN201088
A/swine/Victoria/18–04095–0006/2018(H1N1)	8	17/10/2018	Nasal swab	MDCK1	MN201089-MN201096
A/swine/Victoria/18–02767–0001/2018(H1N1)	1	10/07/2018	lung	Original	MN200950
A/swine/Victoria/18–02767–0002/2018(H1N1)	8	10/07/2018	lung	Original	MN201073-MN201080
A/swine/Victoria/18–02767–0003/2018(H1N1)	1	10/07/2018	lung	Original	MN200951
A/swine/Victoria/13–1724–1/2013(H1N1)	2	29/08/2013	Nasal swab	Original	MN200952-MN200953
A/swine/Victoria/13–1724–3/2013(H1N1)	2	29/08/2013	Nasal swab	Original	MN200954-MN200955
A/swine/Victoria/13–1724-W1/2013(H1N1)	8	29/08/2013	Nasal swab	Original	MN201033-MN201040
A/swine/Victoria/13–1788–1/2013(H1N1)	8	9/09/2013	Bronchial swab	Original	MN201041-MN201048
A/swine/Victoria/13–1871–2/2013(H1N1)	1	13/09/2013	Nasal swab	Original	MN200956
A/swine/Victoria/13–1871–11/2013(H1N1)	8	13/09/2013	Nasal swab	Original	MN201049-MN201056
A/swine/Victoria/13–1871–15/2013(H1N1)	1	13/09/2013	Nasal swab	Original	MN200957
A/swine/Victoria/13–2493–112/2013(H1N1)	2	16/12/2013	Nasal swab	E1	MN200958-MN200959
A/swine/Victoria/13–2493–124/2013(H1N1)	8	16/12/2013	Nasal swab	Original	MN201057-MN201064
A/swine/Victoria/13–2493-NT78/2013(H1N1)	3	16/12/2013	Nasal swab	E1	MN200960-MN200962
A/swine/Victoria/17–1676–3/2017(H1N1)	8	5/07/2017	lung	E1	MN201065-MN201072
A/swine/Western Australia/AS-13–1125–0016/2013(H1N1)	8	11/04/2013	Pooled tissue	Original	MN201097-MN201104
A/swine/Western Australia/AS-17–3313–0004/2017(H1N1)	1	7/04/2017	Nasal swab	Original	MN200963
A/swine/Western Australia/AS-17–3313–0007/2017(H1N1)	1	7/04/2017	Nasal swab	Original	MN200964
A/swine/Western Australia/AS-17–3313–0009/2017(H1N1)	1	7/04/2017	Nasal swab	Original	MN200965
A/swine/Western Australia/AS-17–3313–0010/2017(H1N1)	1	7/04/2017	Nasal swab	Original	MN200966

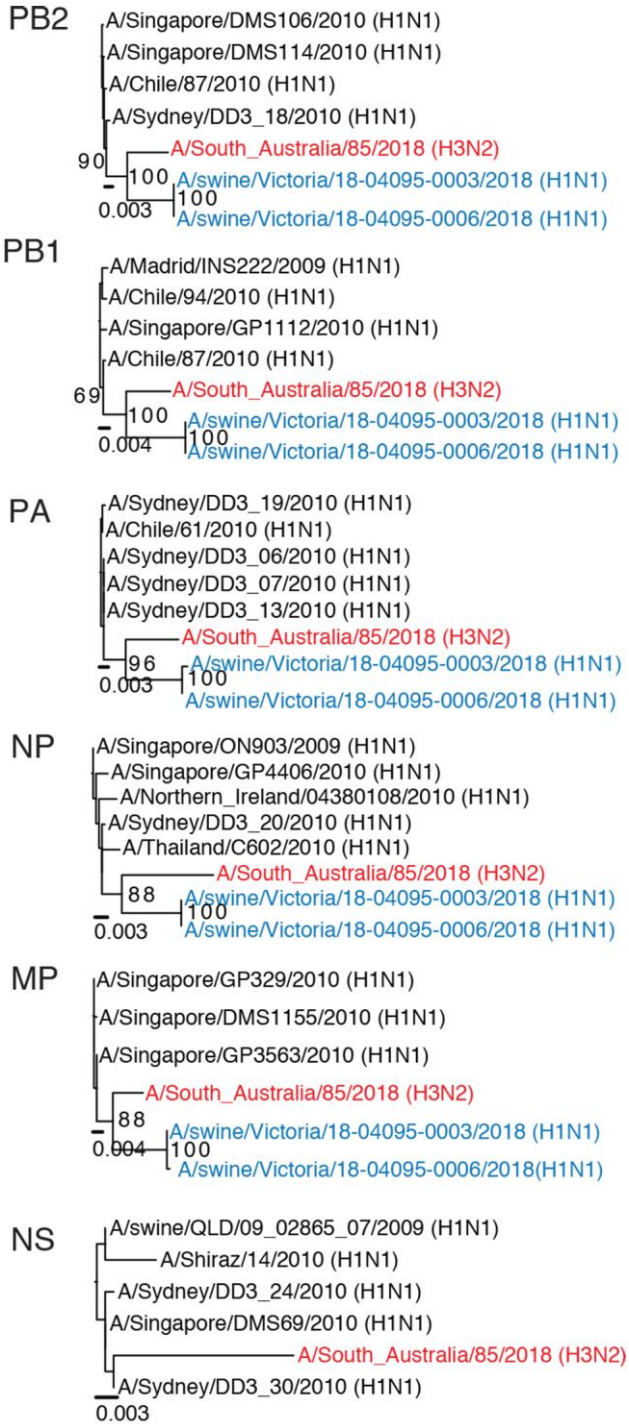
Virus strain	No. segments sequenced	Collection date	Sample type	Passage details	Genbank accession no.
A/swine/Western Australia/AS-17–3313–0011/2017(H1N1)	1	7/04/2017	Nasal swab	Original	MN200967
A/swine/Western Australia/AS-17–3313–0013/2017(H1N1)	8	7/04/2017	Nasal swab	Original	MN201105-MN201112
A/swine/Western Australia/AS-17–3313–0020/2017(H1N1)	8	7/04/2017	Nasal swab	MDCK1	MN201113-MN201120
A/swine/Western Australia/AS-17–3313–0001/2017(H1N1)	3	7/04/2017	Nasal swab	Original	MN200968-MN200970
A/swine/Western Australia/AS-17–4797–0001/2017(H1N1)	8	30/05/2017	Nasal swab	MDCK1	MN201121-MN201128
A/swine/Western Australia/AS-17–4797–0005/2017(H1N1)	3	30/05/2017	Nasal swab	Original	MN200971-MN200973
A/swine/Western Australia/AS-17–4797–0006/2017(H1N1)	8	30/05/2017	Nasal swab	MDCK1	MN201129-MN201136
A/swine/Western Australia/AS-17–4797–0010/2017(H1N1)	3	30/05/2017	Nasal swab	Original	MN200974-MN200976
A/swine/Western Australia/AS-17–5267–0001/2017(H1N1)	8	7/07/2017	Lung	MDCK1	MN201137-MN201144
A/swine/Western Australia/AS-18–3511–0001/2018(H1N1)	1	25/09/2018	Nasal swab	Original	MN200977
A/swine/Western Australia/AS-18–3511–0002/2018(H1N1)	1	25/09/2018	Nasal swab	Original	MN200978
A/swine/Western Australia/AS-18–3511–0004/2018(H1N1)	1	25/09/2018	Nasal swab	Original	MN200979
A/swine/Western Australia/AS-18–3511–0005/2018(H1N1)	8	25/09/2018	Nasal swab	Original	MN201145-MN201152
A/swine/Western Australia/AS-18–4235–0002/2018(H1N1)	5	5/11/2018	Lung	Original	MN200980-MN200984

Appendix Table 5. Sequences used in phylogenetic analysis obtained from GISAID*

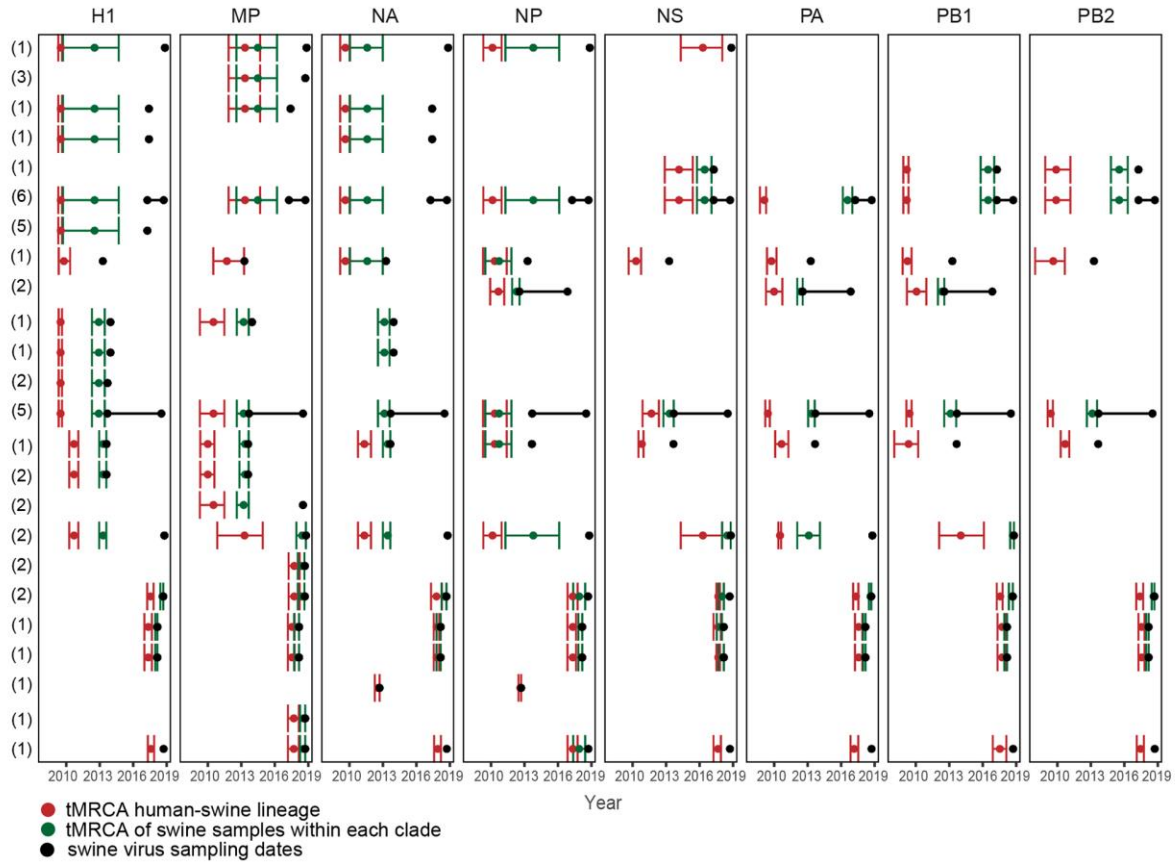
Isolate ID	Segment	Country	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI398040	HA	United States	2012-Aug-05	A/Ohio/57/2012	Ohio Department of Health Laboratories	Centers for Disease Control and Prevention
EPI393661	HA	United States	2012-Aug-13	A/Ohio/62/2012	Ohio Department of Health Laboratories	Centers for Disease Control and Prevention
EPI575533	HA	Congo	2014-Dec-08	A/Congo/2461/2014	INRB Service de Virologie	Centers for Disease Control and Prevention
EPI651720	HA	United States	2015-Sep-02	A/New Hampshire/38/2015	New Hampshire Public Health Laboratories	Centers for Disease Control and Prevention
EPI346424	HA	United States	2011-Nov-14	A/Iowa/09/2011	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention
EPI694865	NA	Italy	2014-Jan-08	A/swine/Italy/50064-2/2014	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie
EPI506844	NA	China	2013-Nov-15	A/Hangzhou/A773/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention
EPI566710	NA	Myanmar	2013-Jul-22	A/Myanmar/13M089/2013		Niigata University (DPH)
EPI649441	NA	Guatemala	2015-May-03	A/Guatemala/15/2015	Laboratorio Nacional De Salud Guatemala	Centers for Disease Control and Prevention
EPI588529	NA	United States	2015-Mar-08	A/Texas/36/2015	Baylor College of Medicine	Centers for Disease Control and Prevention
EPI397439	NA	United States	2012-Aug-20	A/Wisconsin/30/2012	Wisconsin State Laboratory of Hygiene	Centers for Disease Control and Prevention
EPI579059	NA	Kazakhstan	2015-Jan-08	A/Kazakhstan/39/2015	CSEE	Centers for Disease Control and Prevention

*https://www.gisaid.org.

Appendix Figure 1. Evolutionary relationships of the HA and NA genes of A/South Australia/85/2018. Maximum likelihood phylogenies estimated using RAxML v8 [7] showing bootstrap values at branch nodes. Scale-bar represents nucleotide substitutions per site. The human case is colored red, while swIAV collected in Australia are shown in blue.



Appendix Figure 2. Origins of the PB1, PA, NP, MP and NS genes of A/South Australia/85/2018. MP, matrix protein; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1, polymerase basic 1.



Appendix Figure 3. Origins of Australian swine H1N1pdm09 genes from humans. The mean and 95% confidence intervals of the time of origin of each of the Australian swIAV lineages from human seasonal influenza viruses is shown in red, while the time of divergence of each sub-lineage is shown in green. Numbers denote viruses sharing the same tMRCAs and that formed a similar lineage Divergence times were estimated using the uncorrelated log-normal relaxed clock model in a Bayesian Markov Chain Monte Carlo (MCMC) framework in BEAST v1.10. Trees were visualized and annotated using FigTree v1.4 (See Appendix methods).