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Global transmission of influenza viruses from humans to swine

Martha I. Nelson,[1] Marie R. Gramer,[2] Amy L. Vincent[3] and Edward C. Holmes[1,4]

Correspondence
Martha I. Nelson
nelsonma@mail.nih.gov

[1]Fogarty International Center, National Institutes of Health, Bethesda, MD 20892, USA

[2]University of Minnesota Veterinary Diagnostic Laboratory, St Paul, MN 55108, USA

[3]Virus and Prion Diseases Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA 50010, USA

[4]Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA

To determine the extent to which influenza viruses jump between human and swine hosts, we undertook a large-scale phylogenetic analysis of pandemic A/H1N1/09 (H1N1pdm09) influenza virus genome sequence data. From this, we identified at least 49 human-to-swine transmission events that occurred globally during 2009–2011, thereby highlighting the ability of the H1N1pdm09 virus to transmit repeatedly from humans to swine, even following adaptive evolution in humans. Similarly, we identified at least 23 separate introductions of human seasonal (non-pandemic) H1 and H3 influenza viruses into swine globally since 1990. Overall, these results reveal the frequency with which swine are exposed to human influenza viruses, indicate that humans make a substantial contribution to the genetic diversity of influenza viruses in swine, and emphasize the need to improve biosecurity measures at the human–swine interface, including influenza vaccination of swine workers.

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INTRODUCTION

The 2009 pandemic H1N1 virus (H1N1pdm09) represents the best-documented emergence of a swine pathogen in humans, particularly as the virus was associated with widespread global morbidity, mortality and years of life lost in humans (Viboud *et al.*, 2010). The H1N1pdm09 virus was generated by a reassortment event between Eurasian swine H1N1 influenza viruses and North American triple-reassortant H1 viruses, with the former contributing the N1 and M segments and the latter donating the PB2, PB1, PA, H1, NP and NS segments (Garten *et al.*, 2009). Although similar influenza virus reassortants containing segments of both Eurasian swine virus and triple-reassortant swine virus origins have been detected in Asia (Lam *et al.*, 2011; Smith *et al.*, 2009), no 'smoking gun' viruses that are closely related progenitors have been detected in swine in any locality.

Following the identification of H1N1pdm09 in humans in April 2009, the virus was transmitted rapidly from humans back into swine. The first isolation of a H1N1pdm09 virus in swine was from a pig farm in Alberta, Canada, in May 2009 (Howden *et al.*, 2009), and the H1N1pdm09 virus was subsequently isolated from outbreaks in swine in all other major continents: Africa (Njabo *et al.*, 2012), Asia (Kim *et al.*, 2011; Sreta *et al.*, 2010), Australia (Holyoake *et al.*, 2011), Europe (Hofshagen *et al.*, 2009; Howard *et al.*, 2011) and South America (Pereda *et al.*, 2010). Upon introduction into the swine population, H1N1pdm09 viruses co-circulated with endemic swine influenza viruses and exchanged genome segments via reassortment (Ducatez *et al.*, 2011; Lam *et al.*, 2011; Vijaykrishna *et al.*, 2010). In 2011, 12 reassortant H3N2 (H3N2v) swine influenza viruses with matrix (M) segments of pandemic H1N1pdm09 origin were isolated from humans in the USA (CDC, 2012).

Seasonal human influenza viruses have also been isolated periodically from swine, with a few major lineages becoming endemic in swine: most notably the human-origin H3N2 viruses that proliferated in European swine in the 1970s (Ottis *et al.*, 1982), the H1δ lineage of human origin that emerged in North American swine in 2003 (Karasin *et al.*, 2006), and the human H3, N2 and PB1 segments that were associated with the triple-reassortant viruses that were identified in 1998 in North American swine (Zhou *et al.*, 1999). In addition, singletons and smaller clusters of swine influenza viruses that contain one or more segments of human seasonal influenza virus origin have been identified in several countries, including Argentina (Cappuccio *et al.*, 2011; Pereda *et al.*, 2011) and Italy (Moreno *et al.*, 2012).

Seven supplementary figures and two supplementary tables are available with the online version of this paper.

Table 1. Country and state/province of virus collection, month and year of collection, number of swine isolates, and bootstrap support ⩾70 % for 49 introductions of H1N1pdm09 influenza virus from humans into swine during 2009–2011

Introduction no.	Country of collection*	State or province of collection (if available)*	Month/year of collection	No. of isolates (swine)	Bootstrap support (%) for viral introductions†		
					Internal gene phylogeny	HA phylogeny	NA phylogeny
1	Canada	ALB	May 2009	2	85‡	95	
2	Canada	ALB, MB	Aug 2009	2	94		
3	Australia	VIC	Aug 2009	6	95‡	86	
4	Singapore		Aug 2009	1	72		
5	USA	MN	Aug–Sep 2009	4	100		
6	Canada	MB	Sep–Oct 2009	3		92	73
7	HK		Oct 2009	2	100		
8	Canada	QC	Nov 2009	1	85		
9	Canada	SK	Nov 2009	1	95		
10	Canada		Nov–Dec 2009	3		84	72
11	Canada	QC	Nov–Dec 2009	4		97	
12	Italy		Nov 2009	1	92		
13	S. Korea		Nov–Dec 2009	3	100‡	98	70‡
14	Canada	QC	Nov–Dec 2009	5		90	84‡
15	USA	IL	Nov 2009	1		71	
16	Taiwan		Nov 2009–Sep 2010	4	98		82‡
17	Thailand		Nov 2009–Jan 2010	6	100		
18	China	Guangdong	Dec 2009	3	100	89	
19	China	Guangdong	Dec 2009	3	100	85	
20	HK		Dec 2009	2	100	94	
21	HK		Dec 2009	5	100		96
22	Italy		Dec 2009	2	100	98	
23	S. Korea		Dec 2009	8	100	84	93
24	S. Korea		Dec 2009–Jan 2010	9	100	72	90
25	USA	IL, MN	Dec 2009–Feb 2010	8		87	72‡
26	USA	NC	Dec 2009–Mar 2010	2		90	
27	USA	IL	Dec 2009–Jan 2010	3		94‡	76
28	China	Nanchang	Jan 2010	4	100	81	
29	China	Guangdong	Jan 2010	5	100		
30	Thailand		Feb–Jun 2010	4	97		
31	USA	IL, MO	Mar 2010	3		98	
32	China		May 2010	10	100		
33	USA	IL	May 2010	3		92‡	97
34	Thailand		Sep 2010	2	100	80	
35	Costa Rica		Nov 2010	6		100	100
36	Cuba		Nov 2010	3		98‡	100
37	Thailand		Nov 2010	2			99
38	USA	IA, IL	Nov–Dec 2010	3		98	97‡

Table 1. cont.

Introduction no.	Country of collection*	State or province of collection (if available)*	Month/year of collection	No. of isolates (swine)	Bootstrap support (%) for viral introductions†		
					Internal gene phylogeny	HA phylogeny	NA phylogeny
39	USA	IA	Nov 2010	2		100	100
40	USA	IL	Nov 2010–Mar 2011	3			100
41	USA	MN, NE	Nov 2010–May 2011	3		99	92
42	USA	NC, PA	Nov 2010–Feb 2011	2		100	100
43	USA	IL	Nov 2010–Feb 2011	3			94
44	USA	MN	Dec 2010–Jan 2011	2			100
45	USA	IL	Dec 2010–Mar 2011	2		100	
46	USA	MN	Dec 2010–Jan 2011	2		100	98
47	USA	IL, KY, OH	Jan–Mar 2011	9		95	92
48	USA	MN	Feb–Apr 2011	2		100	99
49	USA	TX	Jul 2011	2	100	100	100

*HK, Hong Kong. For Canadian provinces: ALB, Alberta; MB, Manitoba; QC, Quebec; SK, Saskatchewan; for Australia: VIC, Victoria; for the USA: IA, Iowa; IL, Illinois; KY, Kentucky; MN, Minnesota; MO, Missouri; NE, Nebraska; OH, Ohio; NC, North Carolina; PA, Pennsylvania; TX, Texas.

†Boxes that are empty indicate that no sequences were available for these genome segments or that bootstrap support was <70 %.

‡Bootstrap support was ⩾70 %, but only for a subset of the isolates in a given cluster.

The increased global surveillance of influenza in pigs provides the first opportunity to estimate the extent of human-to-swine transmission of the H1N1pdm09 virus. The main aim of our study was therefore to estimate, using a simple phylogenetic approach, the total number of introductions of the H1N1pdm09 virus from humans into swine using a dataset of H1N1pdm09 viruses collected globally in swine during 2009–2011. We then compared this estimate with the equivalent transmission frequency of human seasonal (non-pandemic) H1 and H3 into swine since 1990.

RESULTS

Frequent transmission of H1N1pdm09 virus from humans into swine

Across the haemagglutinin (HA), neuraminidase (NA) and concatenated internal gene phylogenies, we identified a total of 49 discrete introductions of H1N1pdm09 influenza virus from humans into swine during 2009–2011 [Table 1; Figs 1 and S1–S3 (available in JGV Online)]. These introductions were observed in 12 countries and semi-autonomous regions: Australia, Canada, China, Costa Rica, Cuba, Hong Kong (SAR), Italy, Singapore, South Korea, Taiwan, Thailand and the USA. The majority of viral introductions into swine were identified in the USA and Canada (19 and eight introductions, respectively; Table 1), reflecting the higher number of H1N1pdm09 influenza virus sequences available from North American swine. Given our strict criteria for defining viral introductions (bootstrap values ⩾70 %) and limited global sampling, these numbers are likely to underestimate the extent of global spillover from humans to swine of the H1N1pdm09 virus significantly. Our conservative methods are particularly prone to miss introductions involving single isolates. For example, single H1N1pdm09 isolates from Mexico, Japan, England, Cameroon, Brazil and Colombia (A/sw/4/Mexico/2009, A/sw/Osaka/1/2009, A/sw/England/73690/2010, A/sw/Cameroon/11rs149-198/2010, A/sw/Brazil/12A/2010, A/sw/Colombia/1403/2010) are likely to represent additional introductions. These additional probable introductions are identified by # on the detailed HA, NA and concatenated phylogenies presented in Figs S1–S3.

Frequency of human-to-swine transmission of seasonal influenza viruses

Across the H1, H3, N1 and N2 phylogenies, at least 23 separate introductions of seasonal influenza virus from humans to swine were observed during the period 1990–2011 (Figs S4–S7). The vast majority of these introductions were identified after 1996, when surveillance of influenza virus in swine increased. Six human-to-swine transmission events involved the human seasonal H1 segment, eight involved the human H3 segment, six involved the human N1 segment and 16 involved the human N2 segment (Table 2). The higher number of introductions of the human N2 segment is

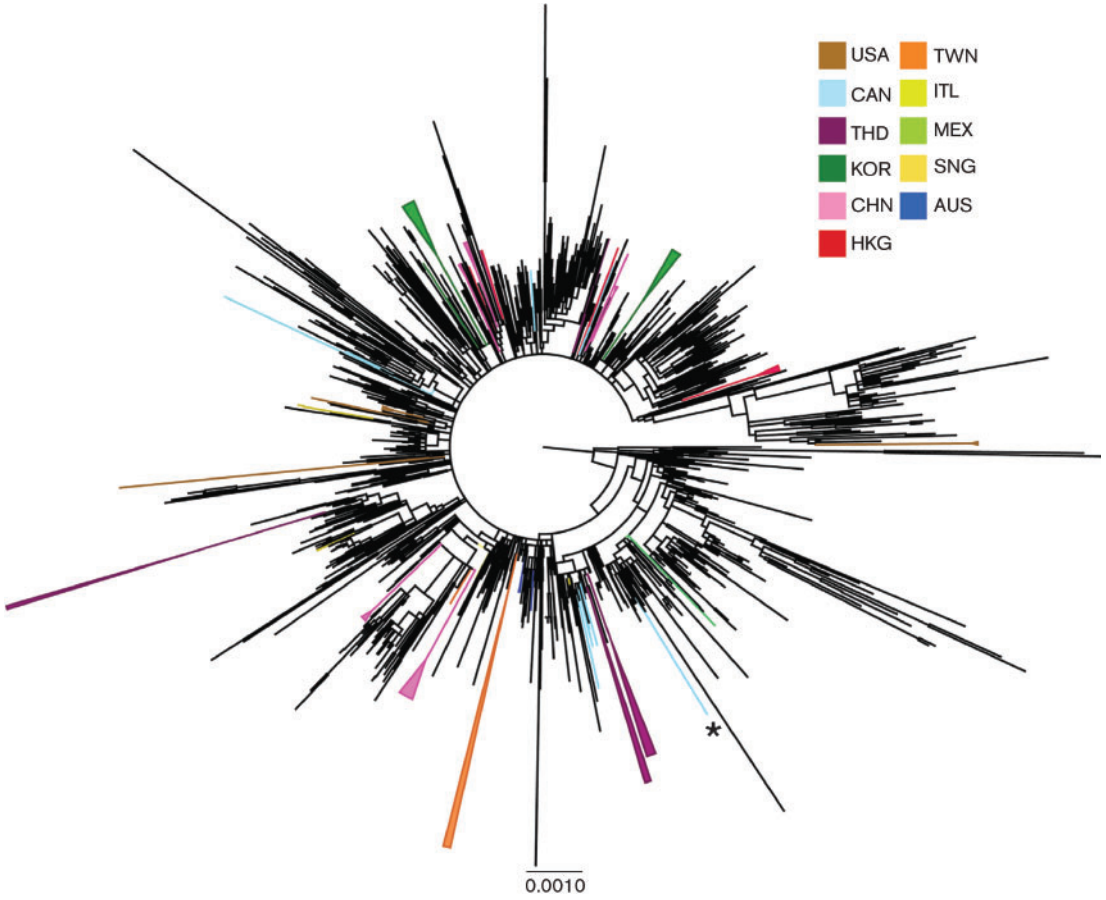

Fig. 1. Phylogenetic relationships of the six concatenated internal gene segment (PB2, PB1, PA, NP, M, NS) sequences from 100 H1N1pdm09 influenza virus isolates collected in swine globally during 2009–2011, and 1008 H1N1pdm09 influenza viruses collected globally in humans during the same time period (total dataset, $n=1108$ sequences; colour-coded black). Each swine H1N1pdm09 isolate is colour-coded by the country of origin: USA, brown; Canada, light blue; Thailand, purple; South Korea, dark green; China, pink; Hong Kong, red; Taiwan, orange; Italy, olive; Mexico, light green; Singapore, yellow; Australia, dark blue. The first introduction of H1N1pdm09 identified in swine, in May 2009 in Canada, is denoted by an asterisk. An identical phylogeny with all viral introductions labelled in detail is provided in Fig. S1.

probably related to several factors, including (a) the overall higher number of H3N2 virus introductions ($n=12$) due to the dominance of the H3N2 subtype in humans during the period of this study, (b) the frequency of introductions ($n=3$) of the human H1N2 variant that circulated globally in humans from 2001 to 2003, and (c) the frequency of reassortment involving N2 segments of human origin and HA segments of swine origin. For example, the 20 H1N2 reassortant viruses from Italy that are associated with introduction #11 have N2 sequences that are related most closely to the N2 of human seasonal H3N2 isolates collected in 1997–1998, but H1 sequences related to European swine influenza viruses.

Twelve of the introductions of seasonal influenza virus were first identified in Asia, six were first identified in the USA, three in Canada and two in Europe (Table 2). As with the H1N1pdm09 viruses, the methods that we used to define a human-to-swine transmission event are highly conservative, and numerous probable introductions were excluded from our final estimate, particularly those involving singletons. Six singleton swine isolates, including three from Argentina, which were identified to be of human origin on both the HA and NA trees but were not supported by high bootstrap values, are highlighted (letters a–g, Table 2; Figs S4–S7). Additionally, eight swine H3N2 isolates collected in Hong Kong during 2000–2002 are interspersed with human isolates collected from that same time period on both the H3 and N2 phylogenies, suggesting additional introductions of human H3N2 viruses into swine in Hong Kong, although again lacking the phylogenetic resolution required to be defined as discrete introductions.

Estimated time periods of human-to-swine transmission

The long branch lengths associated with several introductions of human-origin influenza viruses into swine indicate

Table 2. Country and year of collection, viral subtype, year of collection of the most phylogenetically related human influenza viruses, number of swine isolates, and bootstrap support ⩾70 % for 23 introductions of seasonal influenza viruses (H1, H3, N1 and N2 segments) from humans into swine during 1990–2011

Introduction no.	Country and year of collection	Subtype	Related human viruses (year)	No. of isolates (swine)	Bootstrap support (%) for viral introductions			
					H1	H3	N1	N2
1	Japan 1992	H1N1	1991	1			79	
2	Canada 1997	H3N2	1997	1		§		98
3	Japan 1997	H3N2	1997	4		§		84
4	USA 1998–1999 (triple-reassortant I), S. Korea 2005–2006, China 2007	H3N2	1995–1996	10		100		97
5	HK 1999–2000, China 2003–2008	H3N2	1999	26		93		94*
6	France 1999	H3N2	1998	1		§		99
7	HK 1999–2004, China 2004–2010	H1N2‖	1996	18				100
8	USA 1999, S. Korea 2004	H3N2	1999	2		§		95
9	Taiwan 2002, Indonesia 2004	H3N1 H3N2	1990	2		96		
10	USA 2003–2011, Canada 2005–2011 (triple-reassortant II)	H3N2	1998	126†		100		97, 70‡
11	Italy 2003–2010	H1N2‖	1997–1998	20				100
12	Canada 2004	H1N2	2002–2003	2				92
13	Thailand 2004–2009	H3N2	1995–1996	8		100		100
14	China 2004–2006	H1N1	1997	2	93		100	
15	USA 2005–2011 (δ-2)	H1N1 H1N2	2002–2003	43†	97		100*	97, 70‡
16	HK 2006	H1N1	2006–2007	1	100		88	
17	S. Korea 2007	H3N2	1995–1996	3		100		97
18	USA 2007–2011 (δ-1)	H1N2	2002–2003	97	97			97, 70‡
19	China 2008	H1N1	2000–2001	1	§		80	
20	USA 2009	H1N1	2007	1	90			
21	Canada 2009	H1N1	2008–2009	2	81		90	
22	China 2010, HK 2011	H3N2	2005	3		100		100
23	Vietnam 2010	H3N2	2005	6		100		100
a	HK 1999	H3N2	1999–2000	1		–		–
b	HK 2000–2002	H3N2	2000–2002	8		–		–
c	Canada 2003	H1N2	2002–2003	1	–			–
d	USA 2003	H3N2	2001–2002	1		–		–
e	Argentina 2008	H3N2	2001–2002	1		–		–
f	Argentina 2009	H1N1	2005–2007	1	–		–	
g	Argentina 2010	H1N2	2002–2003	1	–			–

*Bootstrap support was ⩾70 %, but only for a subset of the isolates in a given cluster.

†In cases of reassortment, the number of isolates on HA phylogeny is provided.

‡In cases of reassortment, bootstrap values for both NA clades that are associated with an introduction on the HA tree are provided.

§The HA associated with this introduction was identified to be of human seasonal influenza virus origin on the HA phylogeny (or the HA1 phylogeny; introduction no. 3), but was not associated with a significant bootstrap value.

‖These H1N2 viruses are not related to the seasonal H1N2 viruses that circulated globally in humans in 2001–2003, but rather are human–swine reassortants.

that numerous viral introductions circulated in swine for many years before being detected. Given the high intensity of sampling of influenza virus in humans, the duration of unsampled circulation in swine can be estimated simply and directly by the differences in time between when the isolates were collected in swine and when the most closely related human isolates were collected. For example, the eight H3N2 viruses associated with introduction #13 were collected in swine in Thailand in 2004–2009, but are related most closely to A/Wuhan/359/1995(H3N2)-like viruses from 1995–1996 on the H3 and N2 phylogenies (Figs S4 and S6, respectively), representing 8 years of unsampled circulation in swine. The time difference between the 2008 H3N2 and 2010 H1N2 isolates collected in swine in Argentina and the most closely related human seasonal isolates is approximately 6–8 years. Likewise, the isolate A/sw/Argentina/CIP051-StaFeN2/2010(H1N2) is related most closely to the reassortant H1N2 viruses that circulated in humans during 2002–2003 (Figs S5 and S6), suggesting that this virus has circulated undetected in swine in Argentina or elsewhere since at least 2003, when the H1N2 viruses disappeared globally in humans. Incorporating more viral sequence data from swine by also including partial HA (HA1) sequences filled in some of these gaps in surveillance (H1–HA1 and H3–HA1 trees available from the authors upon request). For example, the USA 2007–2011 (δ-1) introduction (#18) is detected 2 years earlier in 2005 when HA1 data are included. Finally, 11 years separate the three H3N2 swine isolates collected in South Korea in 2007 (introduction #17) from the most phylogenetically related human seasonal H3N2 viruses collected in 1995–1996. However, the relatively short branch lengths associated with these 2007 South Korean isolates raises the question of whether the evolutionary rate was particularly low on this branch, or whether these sequences could be erroneous. The short branch length that separates A/sw/Fujian/0325/2008(H1N1) (introduction #19) from the most closely related human seasonal H1N1 viruses collected in 2000–2001, relative to the approximately 7–8-year difference in collection date, also raises the question of possible sequencing error.

Spatial patterns of human-origin influenza viruses in swine

The number of swine H1N1pdm09 isolates associated with each introduction (ranging from one to ten isolates; Table 1) represents the extent of onward swine-to-swine transmission that can be inferred from our phylogenetic analysis. Although the vast majority of introductions of human H1N1pdm09 viruses into swine exhibit local onward transmission in pigs, reflected in introductions that are associated with more than one swine isolate, we infer that global viral movement has not yet been important in the dissemination of the H1N1pdm09 virus in swine. Specifically, each cluster of swine H1N1pdm09 influenza viruses that are associated with a human-to-swine transmission event is highly spatially structured, with no swine introduction including viruses collected in more than one country. However, within-country spread of H1N1pdm09 viruses has occurred in the USA, as six viral US introductions include multiple states, representing viral dissemination within the Midwestern USA (Table 1). Limited spread within Canada between Alberta and Manitoba was also detected (introduction #2; Table 1). Within-country spread was not observed in other countries where information on the province or region of viral collection was also available, including China and Australia.

Similarly, 18 of the 23 introductions of human seasonal H1 and H3 influenza viruses into swine involve a single country (Table 2). Six of these single-country introductions involve singleton swine influenza viruses, with no evidence of onward transmission. In contrast, four viral introductions include isolates collected in different countries, indicating viral migration within North America and into and within Asia. Aside from the major introduction of triple-reassortant H3N2 viruses that spread from North America to Asia (introduction #4) and from the USA to Canada (introduction #10), possible limited viral migration occurred between Taiwan and Indonesia (introduction #9) and the USA and South Korea (introduction #8).

DISCUSSION

Despite evolving the capability to transmit efficiently in humans, our results indicate that the H1N1pdm09 influenza virus has retained its ability to transmit back into swine and to co-circulate with other swine influenza viruses, increasing genetic diversity and providing the opportunity for reassortment. The large number of H1N1pdm09 transmission events observed in this study, even using conservative methods, highlights the frequency of human–swine contact rates globally and the continual exposure of swine to human influenza viruses. Indeed, this transmission rate is considerably higher than would be inferred from the relatively lower number ($n=23$) of human-to-swine transmission events of seasonal H1 and H3 influenza viruses that were identified during the past two decades. Importantly, detection of seasonal human influenza viruses in swine did not increase during 2009–2011, despite widespread global circulation of seasonal H3N2 viruses in humans during 2010–2011. Hence, the different transmission frequencies observed are not explained by the increase in swine influenza surveillance since 2009 and are unlikely to be an artefact of sampling. Furthermore, the low phylogenetic resolution of the H1N1pdm09 phylogeny, due to the virus's recent emergence and relatively low genetic diversity, impeded the identification of what are likely to be many more introductions of this virus into swine than could be defined here.

Although it is tempting to compare the frequencies of human-to-swine transmission with the 27 swine-origin influenza viruses that were identified in humans in the USA over a time period similar to that of our study (1990–2010) (Shu *et al.*, 2012), such a comparison is biased greatly by the substantially higher levels of global surveillance of

influenza in humans than consistently occurs in swine. However, the intensity of influenza surveillance in humans does facilitate estimation of the time of emergence of the 23 introductions of human seasonal influenza viruses into swine. For example, the introductions of H1N1pdm09 viruses were generally detected in swine within 1 year, whereas several of the human seasonal influenza viruses that were introduced into swine were not detected for 5–10 years. Whilst the time to detection in swine may relate to intrinsic viral characteristics (e.g. the virus must first adapt, then replicate competently in the new host, then spread to many hosts before being detected), it is also possible that the difference in time between when a human-to-swine transmission event is detected in swine and the year of collection of the most closely related human influenza viruses provides an indication of intensity of swine surveillance in a given region. The smallest gaps in detection are in Canada, the USA and Hong Kong, where ongoing viral surveillance is conducted in swine, while larger time gaps occurred in countries such as South Korea, Italy, Thailand and Argentina, where sampling appears to be more sporadic, based on publicly available sequence data.

The global frequency of introductions of H1N1pdm09 viruses into swine also provides an opportunity to track, in detail, the onward transmission and spatial dissemination of these new viral clades and how they relate to swine movements (Nelson *et al.*, 2011). It will be particularly important to determine which of these H1N1pdm09 lineages sustains transmission in swine over the long term, to observe their population dynamics in a new swine host and to identify which lineages spread globally. The multiple introductions of human influenza viruses into swine provide a natural experiment for observing adaptive evolution of influenza from human to swine hosts, in identifying instances of parallel adaptive evolution, and in the comparison of rates of evolutionary change and selection pressures along the branches that define human-to-swine transmission events. Clearly, the ability to trace such dynamics relies upon the continued surveillance of influenza in swine globally, even after the initial interest in the 2009 H1N1 pandemic has subsided.

METHODS

Sequence data used in this study. To estimate the number of human-to-swine transmission events of the H1N1pdm09 virus, we compiled gene sequence data from 263 H1N1pdm09 influenza viruses that were collected in swine during 2009–2011 in 18 countries: Argentina, Australia, Brazil, Cameroon, Canada, China, Colombia, Costa Rica, Cuba, Hong Kong, Japan, Mexico, Singapore, South Korea, Taiwan, Thailand, the UK and the USA (Table S1). Due to the frequency of reassortment between H1N1pdm09 viruses and other swine influenza viruses involving the HA and NA segments, concatenated internal gene segments (PB2, PB1, PA, NP, M and NS) and the HA and NA segments were studied separately. Separate phylogenies were also inferred for each of the internal gene segments to identify and remove any viruses with reassorted internal genes. As background data, 1008 complete genome sequences from H1N1pdm09 influenza viruses that were collected in humans during 2009–2011 were downloaded from the Influenza Virus Resource on GenBank; due to the size of the original dataset (n=2718 genomes), 50 genome sequences were selected randomly from highly sampled countries (i.e. those with >50 genome sequences) (Bao *et al.*, 2008).

To identify swine influenza viruses related to human seasonal H1 and H3 influenza viruses, four phylogenetic trees were inferred using all full-length H1 (n=991, excluding pandemic viruses), H3 (n=326), N1 (n=858) and N2 (n=559) sequences collected from swine since 1990 that are available in GenBank (accession numbers are available from the authors). Due to the frequency of reassortment and low availability of whole-genome sequence data for these swine viral isolates, alignments of the concatenated internal genome segments were not included for this part of the study. These data came from Argentina, Belgium, Canada, China, Denmark, France, Germany, Hong Kong, Hungary, Italy, Japan, Spain, South Korea, Sweden, Taiwan, Thailand, the UK and the USA. From these phylogenies, 152 human-origin swine H1 sequences, 221 human-origin swine H3 sequences, 18 human-origin swine N1 sequences and 430 human-origin swine N2 sequences were identified globally and used in the final analysis. This analysis also included, as background data, 1000 randomly selected human seasonal H1, H3, N1 and N2 segments collected from 1990 to 2011. A large clade of European sequences (e.g. A/sw/Scotland/410440/1994) that were of human seasonal H1 virus origin were not included because they clearly were introduced from humans into swine at least a decade prior to 1990, the beginning of our study period.

To identify any additional human-to-swine transmission events that could not be detected from trees inferred with full-length HA sequence data, two additional phylogenies were inferred using all partial HA1 sequence data (918 nt) from the swine H1 (n=1200 sequences) and H3 (n=1290 sequences) subtypes available since 1990, including HA1 sequence data from the same 1000 human influenza viruses that were selected as background in the full-length HA phylogenies. Using HA1 data, we identified 50 additional swine H1 sequences of human origin and 70 additional swine H3 sequences of human origin. However, all but two isolates [A/sw/Obihiro/3/1993(H3N2) and A/sw/Obihiro/1/1994(H3N2)] were associated with introductions that are already identified in the analysis of full-length HA and NA sequences.

Phylogenetic analysis of H1N1pdm09. Alignments were constructed manually using the Se-Al program (Rambaut, 2002) for three sets of swine H1N1pdm09 virus sequence data, with the 1008 human H1N1pdm09 isolates included as background: (i) the HA segment [n=232 swine H1N1pdm09 isolates, 199 of which were downloaded from GenBank, and 33 provided by the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL) from samples collected from their routine veterinary diagnostic laboratory submission and/or tested via the US Department of Agriculture (USDA) National Animal Health Laboratory Network (NAHLN) Swine Influenza Surveillance System]; (ii) the NA segment (n=229 swine H1N1pdm09 isolates, 197 from GenBank and 32 from UMVDL); and (iii) concatenated internal gene segments (n=100 swine H1N1pdm09 isolates, all downloaded from GenBank, with all reassortants identified from individual gene trees and excluded). For each alignment, a maximum-likelihood (ML) tree was inferred using the PhyML v3.0 program (Guindon *et al.*, 2010), employing SPR (subtree pruning and regrafting) branch-swapping and a general time-reversible model (GTR) model of nucleotide substitution with gamma-distributed rate variation among sites. Statistical support for individual nodes was estimated using 1000 replicate neighbour-joining trees inferred using PAUP* and assuming the GTR+gamma model of nucleotide substitution (Swofford, 2003). Well-supported nodes (≥70% bootstrap support) defining human-to-swine viral introductions were identified visually on each phylogeny. To mitigate possible effects of sequencing

error, the several isolates that were separated by extremely long branch lengths were only categorized as discrete introductions when multiple isolates from the same country exhibited the same phylogenetic pattern.

Phylogenetic analysis of seasonal H1 and H3. Alignments were manually constructed using Se-Al for four sets of swine influenza virus sequences that were identified as of human seasonal (non-pandemic) influenza virus origin: (i) 152 H1 sequences, (ii) 221 H3 sequences, (iii) 18 N1 sequences and (iv) 430 N2 sequences. As background, 1000 human influenza viruses were selected randomly for each segment and downloaded from GenBank. For each alignment, an ML tree was inferred as described above to identify well-supported nodes ($\geqslant$70 % bootstrap support) defining introductions of influenza viruses from humans to swine. The estimated time periods of human-to-swine transmission were inferred directly from the phylogeny by identifying the most closely related human viruses (the sampling date of which is known). This simple phylogenetic method produced results (i.e. times to common ancestry) similar to those obtained using the Bayesian Markov chain Monte Carlo approach available in the BEAST program (Drummond & Rambaut, 2007) and performed during a previous analysis of human influenza viruses that were transmitted to swine (Nelson *et al.*, 2011).

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REFERENCES

Bao, Y., Bolotov, P., Dernovoy, D., Kiryutin, B., Zaslavsky, L., Tatusova, T., Ostell, J. & Lipman, D. (2008). The influenza virus resource at the National Center for Biotechnology Information. *J Virol* **82**, 596–601.

Cappuccio, J. A., Pena, L., Dibárbora, M., Rimondi, A., Piñeyro, P., Insarralde, L., Quiroga, M. A., Machuca, M., Craig, M. I. & other authors (2011). Outbreak of swine influenza in Argentina reveals a non-contemporary human H3N2 virus highly transmissible among pigs. *J Gen Virol* **92**, 2871–2878.

CDC (2012). Update: influenza A (H3N2)v transmission and guidelines – five states, 2011. *MMWR Morb Mortal Wkly Rep* **60**, 1741–1744.

Drummond, A. J. & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* **7**, 214.

Ducatez, M. F., Hause, B., Stigger-Rosser, E., Darnell, D., Corzo, C., Juleen, K., Simonson, R., Brockwell-Staats, C., Rubrum, A. & other authors (2011). Multiple reassortment between pandemic (H1N1) 2009 and endemic influenza viruses in pigs, United States. *Emerg Infect Dis* **17**, 1624–1629.

Garten, R. J., Davis, C. T., Russell, C. A., Shu, B., Lindstrom, S., Balish, A., Sessions, W. M., Xu, X., Skepner, E. & other authors (2009). Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* **325**, 197–201.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* **59**, 307–321.

Hofshagen, M., Gjerset, B., Er, C., Tarpai, A., Brun, E., Dannevig, B., Bruheim, T., Fostad, I. G., Iversen, B. & other authors (2009). Pandemic influenza A(H1N1)v: human to pig transmission in Norway? *Euro Surveill* **14**, 19406.

Holyoake, P. K., Kirkland, P. D., Davis, R. J., Arzey, K. E., Watson, J., Lunt, R. A., Wang, J., Wong, F., Moloney, B. J. & Dunn, S. E. (2011). The first identified case of pandemic H1N1 influenza in pigs in Australia. *Aust Vet J* **89**, 427–431.

Howard, W. A., Essen, S. C., Strugnell, B. W., Russell, C., Barass, L., Reid, S. M. & Brown, I. H. (2011). Reassortant pandemic (H1N1) 2009 virus in pigs, United Kingdom. *Emerg Infect Dis* **17**, 1049–1052.

Howden, K. J., Brockhoff, E. J., Caya, F. D., McLeod, L. J., Lavoie, M., Ing, J. D., Bystrom, J. M., Alexandersen, S., Pasick, J. M. & other authors (2009). An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. *Can Vet J* **50**, 1153–1161.

Karasin, A. I., Carman, S. & Olsen, C. W. (2006). Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada (2003 to 2005). *J Clin Microbiol* **44**, 1123–1126.

Kim, S. H., Moon, O. K., Lee, K. K., Song, Y. K., Yeo, C. I., Bae, C. W., Yoon, H., Lee, O. S., Lee, J. H. & Park, C. K. (2011). Outbreak of pandemic influenza (H1N1) 2009 in pigs in Korea. *Vet Rec* **169**, 155.

Lam, T. T., Zhu, H., Wang, J., Smith, D. K., Holmes, E. C., Webster, R. G., Webby, R., Peiris, J. M. & Guan, Y. (2011). Reassortment events among swine influenza A viruses in China: implications for the origin of the 2009 influenza pandemic. *J Virol* **85**, 10279–10285.

Moreno, A., Chiapponi, C., Boniotti, M. B., Sozzi, E., Foni, E., Barbieri, I., Zanoni, M. G., Faccini, S., Lelli, D. & Cordioli, P. (2012). Genomic characterization of H1N2 swine influenza viruses in Italy. *Vet Microbiol* **156**, 265–276.

Nelson, M. I., Lemey, P., Tan, Y., Vincent, A., Lam, T. T., Detmer, S., Viboud, C., Suchard, M. A., Rambaut, A. & other authors (2011). Spatial dynamics of human-origin H1 influenza A virus in North American swine. *PLoS Pathog* **7**, e1002077.

Njabo, K. Y., Fuller, T. L., Chasar, A., Pollinger, J. P., Cattoli, G., Terregino, C., Monne, I., Reynes, J. M., Njouom, R. & Smith, T. B. (2012). Pandemic A/H1N1/2009 influenza virus in Swine, Cameroon, 2010. *Vet Microbiol* **156**, 189–192.

Ottis, K., Sidoli, L., Bachmann, P. A., Webster, R. G. & Kaplan, M. M. (1982). Human influenza A viruses in pigs: isolation of a H3N2 strain antigenically related to A/England/42/72 and evidence for continuous circulation of human viruses in the pig population. *Arch Virol* **73**, 103–108.

Pereda, A., Cappuccio, J., Quiroga, M. A., Baumeister, E., Insarralde, L., Ibar, M., Sanguinetti, R., Cannilla, M. L., Franzese, D. & other authors (2010). Pandemic (H1N1) 2009 outbreak on pig farm, Argentina. *Emerg Infect Dis* **16**, 304–307.

Pereda, A., Rimondi, A., Cappuccio, J., Sanguinetti, R., Angel, M., Ye, J., Sutton, T., Dibárbora, M., Olivera, V. & other authors (2011). Evidence of reassortment of pandemic H1N1 influenza virus in swine in Argentina: are we facing the expansion of potential epicenters of influenza emergence? *Influenza Other Respir Viruses* **5**, 409–412.

Rambaut, A. (2002). Se-Al: sequence alignment editor, version 2.0. Accessed 11 December 2011. http://tree.bio.ed.ac.uk/software/seal/

Shu, B., Garten, R., Emery, S., Balish, A., Cooper, L., Sessions, W., Deyde, V., Smith, C., Berman, L. & other authors (2012). Genetic analysis and antigenic characterization of swine origin influenza viruses isolated from humans in the United States, 1990–2010. *Virology* **422**, 151–160.

Smith, G. J., Vijaykrishna, D., Bahl, J., Lycett, S. J., Worobey, M., Pybus, O. G., Ma, S. K., Cheung, C. L., Raghwani, J. & other authors

(2009). Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* **459**, 1122–1125.

Sreta, D., Tantawet, S., Na Ayudhya, S. N., Thontiravong, A., Wongphatcharachai, M., Lapkuntod, J., Bunpapong, N., Tuanudom, R., Suradhat, S. & other authors (2010). Pandemic (H1N1) 2009 virus on commercial swine farm, Thailand. *Emerg Infect Dis* **16**, 1587–1590.

Swofford, D. L. (2003). PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, MA: Sinauer Associates.

Viboud, C., Miller, M., Olson, D., Osterholm, M. & Simonsen, L. (2010). Preliminary estimates of mortality and years of life lost associated with the 2009 A/H1N1 pandemic in the US and comparison with past influenza seasons. *PLoS Curr* **2**, RRN1153.

Vijaykrishna, D., Poon, L. L. M., Zhu, H. C., Ma, S. K., Li, O. T. W., Cheung, C. L., Smith, G. J. D., Peiris, J. S. M. & Guan, Y. (2010). Reassortment of pandemic H1N1/2009 influenza A virus in swine. *Science* **328**, 1529.

Zhou, N. N., Senne, D. A., Landgraf, J. S., Swenson, S. L., Erickson, G., Rossow, K., Liu, L., Yoon, K., Krauss, S. & Webster, R. G. (1999). Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J Virol* **73**, 8851–8856.