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Global transmission of influenza viruses from humans to swine

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To determine the extent to which influenza viruses jump between humans 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 repeatedly transmit 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.

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 life-years 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*., 2010; 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 rapidly transmitted 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*., 2011), 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*., 2010; Vijaykrishma *et al.,* 2010). In 2011 twelve reassortant H3N2 (H3N2v) swine influenza viruses with matrix (M) segments of pandemic H1N1pdm09 origin were isolated from humans in the United States (Anonymous 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 America swine (Zhou *et al*., 1999). In addition, singletons and smaller clusters of swine influenza viruses have been identified that contain one or more segments of human seasonal influenza virus origin in several countries, including Argentina (Cappuccio *et al*., 2011; Pereda *et al*., 2011) and Italy (Moreno *et al*., 2012).

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 data set 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 HA, 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, S1-S3). 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 United States. The majority of viral introductions into swine were identified in the United States and Canada (19 and 8 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 significantly underestimate the extent of global spillover from humans to swine of the H1N1pdm09 virus. 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 # symbols 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 likely 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 2001-2003, 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 most closely related 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 United States, three in Canada, and two in Europe (Table 2). As with the H1N1pdm09 viruses, the methods 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, are highlighted that were identified to be of human origin on both the HA and NA trees but which were not supported by high bootstrap values (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 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 most closely related to A/Wuhan/359/1995(H3N2)-like viruses from 1995-1996 on the H3 and N2 phylogenies (Figs. S4 and S6, respectively), representing eight 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 most closely related 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 two years earlier in 2005 when HA1 data is included. Finally, eleven 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 2000-01, relative to the ~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 1-10 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 >1 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 United States, as six viral US introductions include multiple states, representing viral dissemination within the Midwestern US (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 American 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 United States to Canada (introduction #10), possible limited viral migration occurred between Taiwan and Indonesia (introduction #9) and the US 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 artifact 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 United States over a similar time period as our study (1990-2010) (Shu *et al.,* 2012), such a comparison is greatly biased 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 a year, whereas several of the human seasonal influenza viruses that were introduced into swine were not detected for 5-10 years. While 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 US, 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, England, Hong Kong, Japan, Mexico, Singapore, South Korea, Taiwan, Thailand, and the United States (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, 1,008 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 data set (n = 2,718 genomes), 50 genome sequences were randomly selected 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 sequences (n = 559) collected from swine since 1990 that are available on GenBank (accession numbers available from the

242 authors). Due to the frequency of reassortment and low availability of whole-genome sequence
243 data for these swine viral isolates, alignments of the concatenated internal genome segments
244 were not included for this part of the study. These data came from Argentina, Belgium, Canada,
245 China, Denmark, France, Germany, Hong Kong, Hungary, Italy, Japan, Spain, South Korea,
246 Sweden, Taiwan, Thailand, the United Kingdom, and the United States of America. From these
247 phylogenies, 152 human-origin swine H1 sequences, 221 human-origin swine H3 sequences,
248 18 human-origin swine N1 sequences, and 430 human-origin swine N2 sequences were
249 identified globally and used in the final analysis. This analysis also included, as background
250 data, 1,000 randomly selected human seasonal H1, H3, N1, and N2 segments collected from
251 1990-2011. A large clade of European sequences (e.g. A/sw/Scotland/410440/1994) that were
252 of human seasonal H1 virus origin were not included because they clearly were introduced from
253 humans into swine at least a decade prior to 1990, the beginning of our study period.
254 To identify any additional human-to-swine transmission events that could not be
255 detected from trees inferred with full-length HA sequence data, two additional phylogenies were
256 inferred using all partial HA1 sequence data (918 nt) from the swine H1 (n = 1,200 sequences)
257 and H3 (n = 1,290 sequences) subtypes available since 1990, including HA1 sequence data
258 from the same 1,000 human influenza viruses that were selected as background in the full-
259 length HA phylogenies. Using HA1 data, we identified 50 additional swine H1 sequences of
260 human origin and 70 additional swine H3 sequences of human origin. However, all but two
261 isolates (A/sw/Obihiro/3/1993(H3N2) and A/sw/Obihiro/1/1994(H3N2)) were associated with
262 introductions that are already identified in the analysis of full-length HA and NA sequences.
263
264 **Phylogenetic analysis of H1N1pdm09**. Alignments were manually constructed using the SeAl
265 program (Rambaut *et al.,* 2002) for three sets of swine H1N1pdm09 virus sequence data, with
266 the 1,008 human H1N1pdm09 isolates included as background: (i) the HA segment (n = 232
267 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 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 neighbor-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 SeAl 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, 1,000 human influenza viruses were randomly selected for each segment and downloaded from GenBank. For each alignment a maximum likelihood (ML) tree was inferred as described above to identify well-supported nodes (≥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

294 known). This simple phylogenetic method produced similar results (i.e. times to common
295 ancestry) to those obtained using the Bayesian Markov chain Monte Carlo approach available in
296 the BEAST program (Drummond & Rambaut 2007) and performed during a previous analysis of
297 human influenza viruses that were transmitted to swine (Nelson *et al.*, 2011).
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FIGURE LEGENDS

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 1,008 H1N1pdm09 influenza viruses collected globally in humans during the same time period (total data set n = 1,108 sequences; color-coded black). Each swine H1N1pdm09 isolate is color-coded by the country of origin: United States (US) = brown, Canada (CAN) = light blue, Thailand (THD) = purple, South Korea (KOR) = dark green, China (CHN) = pink, Hong Kong (HKG) = red, Taiwan (TWN) = orange, Italy (ITL) = olive, Mexico (MEX) = light green, Singapore (SNG) = yellow, and Australia (AUS) = dark blue. The first introduction of H1N1pdm09 identified in swine, in May 2009 in Canada, is denoted with an asterisk. An identical phylogeny with all viral introductions labeled in detail is provided in Fig. S1.

Table 1. Country and state/province of viral collection, month/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	Country of collection	State or province of collection (if available)[#]	Month, Year of collection	Number 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 09	4	100		
6	Canada	MB	Sep-Oct 09	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 09	3		84	72
11	Canada	QC	Nov-Dec 09	4		97	
12	Italy		Nov 2009	1	92		
13	S Korea		Nov-Dec 09	3	100*	98	70*
14	Canada	QC	Nov-Dec 09	5		90	84*
15	USA	IL	Nov 2009	1		71	
16	Taiwan		Nov 09 - Sep 10	4	98		82*
17	Thailand		Nov 09 - Jan 10	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 09 - Jan 10	9	100	72	90
25	USA	IL, MN	Dec 09 - Feb 10	8		87	72*
26	USA	NC	Dec 09 - Mar 10	2		90	

27	USA	IL	Dec 09 - Jan 10	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*
39	USA	IA	Nov 2010	2		100	100
40	USA	IL	Nov 10 - Mar 11	3			100
41	USA	MN, NE	Nov 10 - May 11	3		99	92
42	USA	NC, PA	Nov 10 - Feb 11	2		100	100
43	USA	IL	Nov 10 - Feb 11	3			94
44	USA	MN	Dec 10 - Jan 11	2			100
45	USA	IL	Dec 10 - Mar 11	2		100	
46	USA	MN	Dec 10 - Jan 11	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

[#]For Canadian provinces, ALB = Alberta, MB = Manitoba, QC = Quebec, and SK = Saskatchewan; for Australia, VIC=Victoria; for the United States, IA = Iowa, IL = Illinois, KY = Kentucky, MN = Minnesota, MO = Missouri, OH = Ohio, NC = North Carolina, PA = Pennsylvania, and 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.

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	Country & year of collection	Subtype	Related human viruses (year)	Number 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	1995-96	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##	1997-98	20				100
12	Canada 2004	H1N2	2002-03	2				92
13	Thailand 2004-2009	H3N2	1995-96	8		100		100
14	China 2004-2006	H1N1	1997	2	93		100	
15	USA 2005-2011 (δ-2)	H1N1 H1N2	2002-03	43#	97		100*	97,70&
16	HK 2006	H1N1	2006-07	1	100		88	
17	S Korea 2007	H3N2	1995-96	3		100		97
18	USA 2007-2011 (δ-1)	H1N2	2002-03	97	97			97,70&
19	China 2008	H1N1	2000-01	1	$		80	
20	USA 2009	H1N1	2007	1	90			
21	Canada 2009	H1N1	2008-09	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	1999-00	1		-		-
b	HK 2000-2002	H3N2	2000-02	8		-		-
c	Canada 2003	H1N2	2002-03	1	-			-
d	USA 2003	H3N2	2001-02	1		-		-
e	Argentina 2008	H3N2	2001-02	1		-		-
f	Argentina 2009	H1N1	2005-07	1	-		-	
g	Argentina 2010	H1N2	2002-03	1	-			-

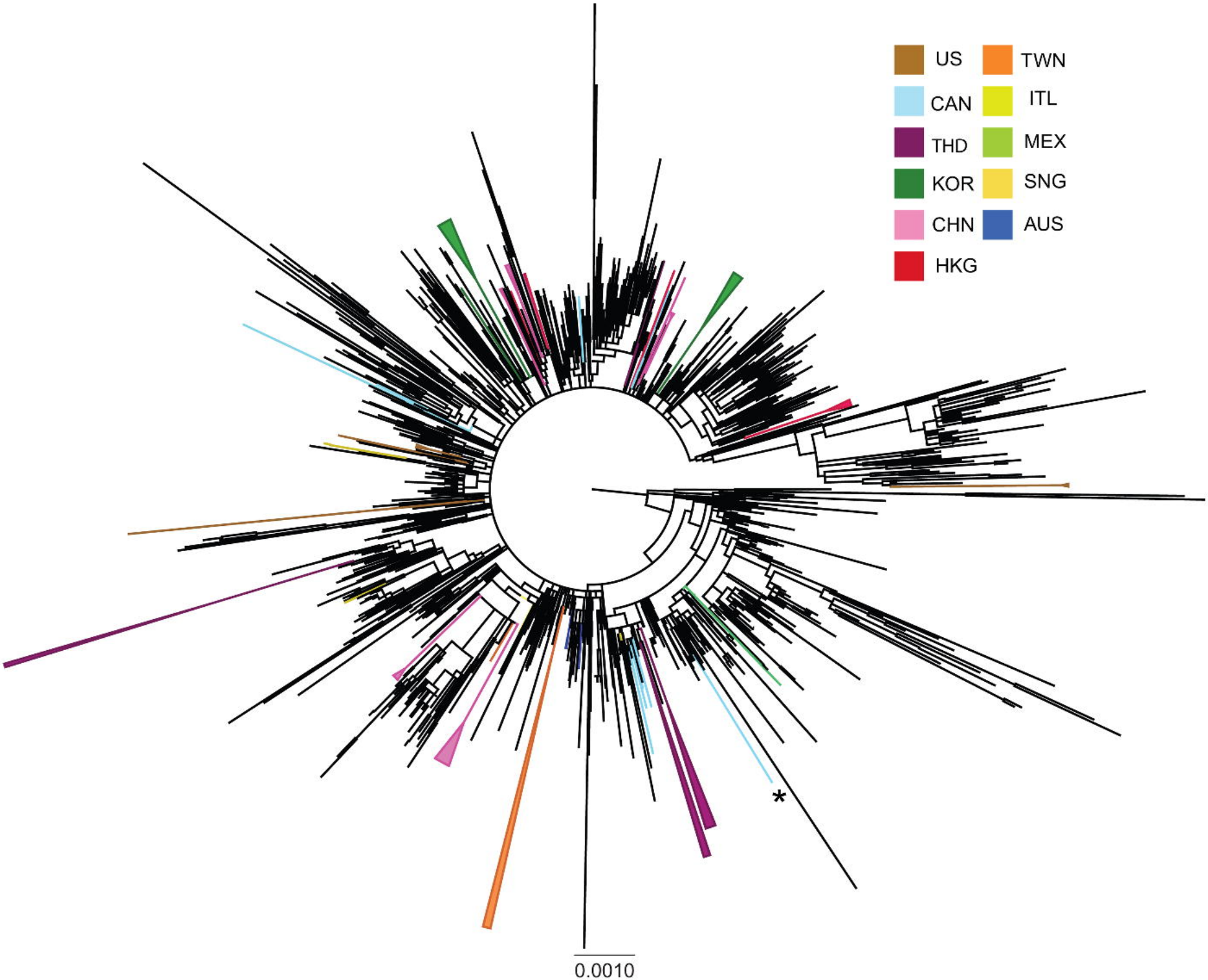
*Bootstrap support was ≥70 but only for a subset of the isolates in a given cluster.

[#]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[$$]), 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 2001-2003 but rather are human-swine reassortants.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