Population susceptibility to North American and Eurasian swine influenza viruses in England, at three time points between 2004 and 2011

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Age-stratified sera collected in 2004, 2008 and 2010 in England were evaluated for antibody to swine influenza A(H3N2) and A(H1N1) viruses from the United States or Europe as a measure of population susceptibility to the emergence of novel viruses. Children under 11 years of age had little or no measurable antibody to recent swine H3N2 viruses despite their high levels of antibody to recent H3N2 seasonal human strains. Adolescents and young adults (born 1968–1999) had higher antibody levels to swine H3N2 viruses. Antibody levels to swine H3N2 influenza show little correlation with exposure to recent seasonal H3N2 (A/Perth/16/2009) strains, but with antibody to older H3N2 strains represented by A/Wuhan/359/1995. Children had the highest seropositivity to influenza A(H1N1)pdm09 virus, and young adults had the lowest antibody levels to A/Perth/16/2009. No age group showed substantial antibody levels to A/Aragon/RR3218/2008, a European swine H1N1 virus belonging to the Eurasian lineage. After vaccination with contemporary trivalent vaccine we observed evidence of boosted reactivity to swine H3N2 viruses in children and adults, while only a limited boosting effect on antibody levels to A/Aragon/RR3218/2008 was observed in both groups. Overall, our results suggest that different vaccination strategies may be necessary according to age if swine viruses emerge as a significant pandemic threat.

Introduction

Pigs are considered a mixing vessel for the reassortment of avian, swine and human influenza viruses. Recent events confirm their important role in the emergence of novel influenza viruses capable of causing a human pandemic [1]. Until the 1990s, classic swine influenza A(H1N1), the most commonly circulating swine influenza virus among pigs, remained genetically fairly constant [2]. However, by the late 1990s, different subtypes (H1N1, H3N2 and H1N2) had emerged and became predominant among North American pig herds [3]. These swine influenza A viruses acquired avian, human, and swine virus gene segments through reassortment [3,4] and various genetic lineages can be distinguished within each subtype [4]. In Europe, swine influenza is primarily caused by the aforementioned subtypes. However, their antigenic and genetic characteristics differ significantly from those found in North America and Asia [5,6]. Genetic diversity has been expanded through multiple introductions of influenza viruses from other animal hosts into pig herds, including from humans [7], most recently demonstrated with A(H1N1)pdm09 virus in Europe, Asia, and the Americas [6,8,9].

For this study of population susceptibility we chose two swine virus subtypes which have most recently caused outbreaks or sporadic cases in humans. These include representatives of swine influenza A(H3N2) viruses (swH3N2) recently isolated from human cases in the United States [10,11] and a swine influenza A(H1N1) viruses (swH1N1) isolated from a zoonotic infection in Europe [12].

The primary objective of this analysis was the improvement of the risk assessment of population susceptibility to currently circulating swine influenza viruses, with the proven ability to cause zoonotic infections.

Methods

We measured haemagglutination inhibition (HI) antibody prevalence to representative current and previous seasonal H3N2 and H1N1 strains, to which the population of the United Kingdom (UK) has been exposed, and compared it with HI antibody reactivity to influenza H3 and H1 strains of swine origin to which the UK population is very unlikely to have been exposed. We also determined vaccine-induced cross-reactive antibodies in pre- and post-immunisation sera.

Serum samples

We used a random selection of anonymised age-stratified residual serum aliquots collected in England

[14] from 1,982 individuals over three time periods as detailed in Table 1. Sera were collected from an age range of 0 to 89 years and stratified by birth cohorts. The 1,982 sera were grouped into panels according to time of serum sample collection (Table 1).

A small additional panel of anonymised children and adult sera before and after vaccination with 2010/11 trivalent inactivated influenza vaccine (TIV) was used to asses levels of vaccine-induced cross-reactive antibodies in children (3–14 years-old; 24 pairs) and adults (20–77 years-old; 24 pairs).

Viruses

Antigenic characterisation of virus isolates was performed using HI assays [13]. Virus strains used for H3N2 analysis were: A/Perth/16/2009 (human H3N2 virus, circulating from 2009 onwards); A/Wuhan/359/1995 (human H3N2 virus, circulating from the mid-1990s); A/Swine/Minnesota/593/1999 (A/sw/Minnesota/593/1999; genetic predecessor of swine H3N2 viruses, which have recently caused limited human infection in North America, kindly provided by Prof I. Brown at the Veterinary Laboratory Agency, UK); and A/Pennsylvania/14/2010 and A/Indiana/08/2011 (swine H3N2 viruses isolated from sporadic cases of human infection in the United States; both kindly provided by the World Health Organization Collaborating Centre (WHO CC) at the National Institute for Medical Research (NIMR), London, UK, who received the samples as part of the WHO Global Influenza Surveillance and Response System (GISRS) Pandemic Influenza Preparedness (PIP) Framework from the WHO CC at CDC, Atlanta), see also Table 2.

Viruses used for H1N1 analysis were: NIBRG122 (reverse genetics virus of A/England/195/2009, the influenza A(H1N1)pdm09 UK prototype strain, provided by the National Institute for Biological Standards and Control (NIBSC)) and A/Aragon/RR3218/2008 (swine H1N1 virus isolated from a sporadic human case in Spain in 2008 [12], kindly provided by the National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain).

The NIBRG122, A/Perth/16/2009, A/Wuhan/359/1995, A/Aragon/RR3218/2008, A/sw/Minnesota/593/1999 and A/Pennsylvania/14/2010 viruses were grown in embryonated hens' eggs. A/Indiana/08/2011 was cultured in MDCK cells.

Serological methods and analysis

Antibody titres were measured by HI [14,15]. All assays were performed using turkey red blood cells (0.5%), with the exception of the analysis with A/Perth/16/2009 virus, for which we used guinea pig red blood cells (0.5%) according to WHO recommendation [16]. Undetectable titres (<8) were assigned a value of 4. Age-related geometric mean titres (GMTs) with 95% confidence intervals (CI) as well as proportion of participants with HI titre ≥32 (defined as seropositive) were calculated. Data were analysed by birth cohorts according to primary influenza exposure (before 1957, exposed to H1N1; 1957–68, exposed to H2N2; 1968–99 exposed to H3N2; from 2000 onwards, representing the very young). Pearson's correlation coefficient (*r*) was used to compare responses between log10 assay titres.

Table 1

Characteristics of serum panels for influenza serosusceptibility analysis, England, 2004–11 (n=1,982)

Panel name	Time of collection	Number of samples		Age Ranges			Analysed with	
		Total	By birth cohort	Birth cohorts	Age at collection (years)	Year of birth	H1N1 subtype	H3N2 subtype
2004 panel	June 2004	687	176	Pre-1957	1–80	1924–2003	A/England/195/2009[a], A/Aragon/RR3218/2008	Not analysed
			87	1957–1967				
			304	1968–1999				
			48	After 2000				
2008 panel	Jan 2008 to April 2009	1,179	588	Pre-1957	0–87	1921–2009	A/England/195/2009[a]	Not analysed
			67	1957–1967				
			314	1968–1999				
			209	After 2000				
2010 panel	Autumn 2010 and spring 2011	116	33	Pre-1957	0–89	1922–2011	A/England/195/2009[a], A/Aragon/RR3218/2008	A/Wuhan/359/95, A/sw/Minnesota/593/99, A/Pensylvania/14/10, A/Indiana/08/2011, A/Perth/16/09
			13	1957–1967				
			49	1968–1999				
			22	After 2000				

[a] The reverse genetics derivative, NIBRG122, was used.

Table 2

Source of viruses and sequence information used in this study (n=25)

Virus	Virus provided by	Sequence Source		Originating laboratory	Submitter
		GenBank	GISAID EpiFlu		
A/Wuhan/359/95	WHO CC National Institute for Medical Research, London	JX518888	NA	NA	NA
A/Moscow/10/99	WHO CC National Institute for Medical Research, London	DQ487341	NA	NA	NA
A/Johannesburg/33/94	WHO CC National Institute for Medical Research, London	CY121349	NA	NA	NA
A/Panama/2007/99	WHO CC National Institute for Medical Research, London	DQ487340	NA	NA	NA
A/Perth/16/2009	WHO CC National Institute for Medical Research, London	GQ293081	NA	NA	NA
A/England/215/2011	NA	JX518887	EPI393290	NA	Centre for Infections, HPA, London, United Kingdom
A/Swine/Minnesota/593/99	Veterinary Laboratories Agency, Weybridge, UK				
	AF251427	NA	NA	NA	
A/Kansas/13/2009	WHO CC for Reference and Research on Influenza, CDC, Atlanta	NA	EPI244297	Kansas Department of Health and Enviroment	WHO CC for Reference and Research on Influenza, CDC, Atlanta
A/Wisconsin/12/2010	WHO CC for Reference and Research on Influenza, CDC, Atlanta	NA	EPI291898	Evanston Hospital and NorthShore University	WHO CC for Reference and Research on Influenza, CDC, Atlanta
A/Pennsylvania/14/2010	WHO CC for Reference and Research on Influenza, CDC, Atlanta	NA	EPI291865	Pennsylvania Department of Health	WHO CC for Reference and Research on Influenza, CDC, Atlanta
A/Indiana/08/2011	WHO CC for Reference and Research on Influenza, CDC, Atlanta	NA	EPI344405	Indiana State Department of Health Laboratories	WHO CC for Reference and Research on Influenza, CDC, Atlanta
A/California/07/2009	National Institute Biological Standards and Control, HPA	ACP44189	NA	NA	NA
A/Swine/Iowa/00239/2004	NA	ABV25643	NA	NA	NA
A/New Jersey/11/1976	NA	ACU80014	NA	NA	NA
A/swine/Wisconsin/1/1961	NA	AAD25302	NA	NA	NA
A/swine/Iowa/15/1930	NA	ABV25634	NA	NA	NA
A/South Carolina/1/1918	NA	AAD17229	NA	NA	NA
A/Puerto Rico/8/1934	NA	ABO21709	NA	NA	NA
A/Roma/1949	NA	ABN59434	NA	NA	NA
A/New Caledonia/20/1999	NA	CAC86622	NA	NA	NA
A/Brisbane/59/2007	NA	ACA28846	NA	NA	NA
A/duck/Italy/69238/2007	NA	ACJ14445	NA	NA	NA
A/mallard/Alberta/35/1976	NA	AAD25304	NA	NA	NA
A/Aragon/RR3218/2008	Instituto de Salud Carlos III, Madrid-Majadahonda, Spain	NA	EPI393289	Instituto de Salud Carlos III, Madrid-Majadahonda, Spain	Centre for Infections, HPA, London, United Kingdom
A/swine/England/WVL7/1992	NA	ACO25133	NA	NA	NA

CDC: Centres for Disease Control and Prevention; HPA: Health Protection Agency; NA: not applicable; UK: United Kingdom; WHO CC: World Health Organization Collaborating Centre.

For analysis of vaccine sera, immunogenicity end points included group GMTs and geometric mean fold changes (GMTR) from pre- to post-vaccination with 95% CI, the proportion of participants with HI titre ≥32 ('seroprotection rate' when evaluating vaccine antigens), and the proportion of seroconverting individuals ('seroconversion rate'; SCR); showing four-fold increase in post- compared with pre-immunisation titres or from HI titre <8 before immunisation to at least 32 after immunisation.

Sequencing of full-length haemagglutinin and phylogenetic analysis

Virus RNA was extracted, underwent RT-PCR, and amplified products were sequenced [13,17]. Accession numbers for GenBank and the Global Initiative on Sharing All Influenza Data (GISAID) are listed in Table 2. Phylogenetic trees were constructed using deduced amino acid sequences with a neighbour-joining algorithm, available in the MEGA 4.0.1 software (http://www.megasoftware.net).

Results

Cross-reactivity of H3N2 viruses

The classical swine lineage virus A/sw/Minnesota/593/1999 showed some reactivity with ferret post-infection antiserum raised to human seasonal viruses from the mid-1990's, suggesting some antigenic similarity between swine and human viruses co-circulating during this period (Table 3).

Figure 1 shows the genetic relationships between haemagglutinin (HA) protein sequences of representative human H3N2 and swH3N2 lineages, including some from human infections with North American swine H3N2 viruses detected since 2009. A/sw/Minnesota/593/99 clusters with human viruses from the mid-1990s, since this virus is a representative from the swine triple reassortant lineage that arose in 1998 and includes an HA gene from human origin. The human lineage further separates into two branches of viruses isolated before or after 1998.

Of 59 residues located at antigenic sites, current human and swine North American H3N2 viruses differ at ca. 16 positions (73% identity at antigenic sites, 89% for the entire HA protein (data not shown). The highest pairwise identity between current North American swine viruses and human H3N2 viruses included in this analysis is shown with A/Wuhan/359/95 (78–83% identity at antigenic sites, 94% for the entire HA), which is consistent with this virus being an ancestor for the HA segment of recent and classic North American swH3N2 viruses.

Age stratified reactivity of human sera to seasonal H3N2 viruses shows a profile consistent with exposure

Table 3

Antigenic analysis of influenza A(H3N2) viruses (seasonal, swH3N2 and swH3N2 variant influenza strains) (n=11)

		A/Perth/16/2009	A/England/215/2011	A/Panama/2007/99	A/Moscow/10/99	A/Wuhan/359/95	A/Johannesburg/34/94	A/Pennsylvania/14/2010	A/Wisconsin/12/2010
		H3N2	H3N2	H3N2	H3N2	H3N2	H3N2	swH3N2	swH3N2
A/Perth/16/2009	H3N2	**2,560**	5,120	<	<	<	<	<	<
A/England/215/2011	H3N2	640	**2,560**	<	<	<	<	<	<
A/Panama/2007/99	H3N2	<	<	**2,560**	5,120	20	<	<	<
A/Moscow/10/99	H3N2	<	<	1,280	**10,240**	<	<	<	<
A/Wuhan/359/95	H3N2	<	<	<	<	**2,560**	160	<	<
A/Johannesburg/33/94	H3N2	<	<	<	<	<	**2,560**	<	<
A/Pennsylvania/14/2010	swH3N2	<	<	<	<	<	<	**5,120**	2,560
A/Wisconsin/12/2010	swH3N2	<	<	<	<	<	<	640	**2,560**
A/Kansas/13/2009	swH3N2	<	<	<	<	<	<	2,560	320
A/Indiana/8/2011	sw(H3N2)v	<	<	<	<	<	<	2,560	5,120
A/sw/Minnesota/593/99	swH3N2	<	<	<	<	160	160	<	<

sw(H3N2)v: variant of recent swH3N2 viruses, which acquired the M gene of the A(H1N1)pdm09 virus.

Haemagglutination inhibition titres for seasonal H3N2 viruses, novel swH3N2 viruses causing sporadic human infections, and swH3N2 viruses with post-infection ferret antiserum. < denotes a titre <40.

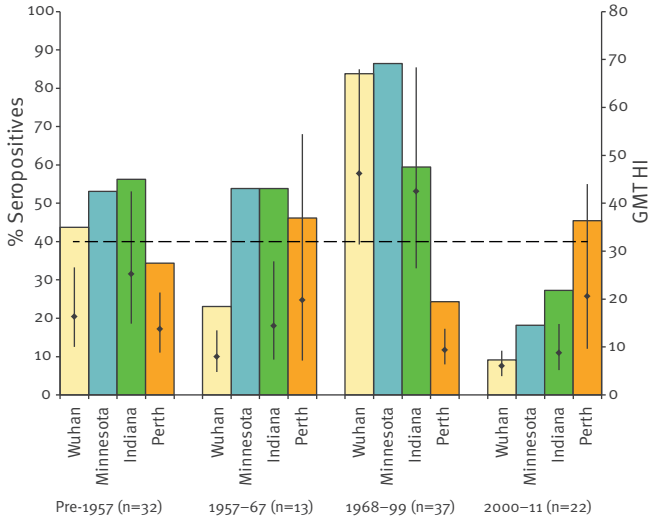
to different circulating strains according to birth cohort (Figure 2). Seropositivity with A/Perth/16/2009, the virus included in the TIV and the most recently circulating H3N2 virus in England, shows the least variation across different ages. The youngest age cohort (born after 2000) and those born between 1957 and 1967 had the highest number of seropositive individuals to this strain, while younger adults born between 1968 and 1999 showed the highest number of seropositives to the previously circulating H3N2 A/Wuhan/359/1995 virus.

Cross-reactive antibody levels to swH3N2 viruses were lowest in children (born after 2000) and older adults (born before 1968) for the two viruses used in the analysis (Figure 2), with the lowest GMTs for the recent swine virus isolate A/Indiana/08/2011 (GMT=9; 95% CI: 5–15) found in the youngest age cohort. However, the two groups with lowest overall GMT seem to differ in susceptibility. We found significantly (p=0.04, Fisher's exact test) fewer seropositives in those 12 years-old and younger (6/22=27% with A/Indiana/08/2011) compared to adults born before 1968 (25/45=56%). Highest levels of cross-reactive antibodies to swH3N2 strains were found in individuals born between 1968 and 1999. The susceptibility profile for the A/Wuhan/359/95 virus was very similar to that of an ancestor strain for swH3N2, A/sw/Minnesota/593/1999.

We observed the strongest correlation between A/Wuhan/359/1995 and A/sw/Minnesota/593/1999 (r=0.80) and weaker correlation between A/Wuhan/359/1995 and A/Indiana/08/2011 (r=0.69) as well as between A/sw/Minnesota/593/1999 and A/Indiana/08/2011 viruses (r=0.5). By contrast, we found no evidence for the pairwise correlations of antibody titres between A/Perth/16/2009 and any of the other H3N2 strains used.

Figure 2

Reactivity in age-stratified sera to different influenza A(H3N2) viruses, England, 2010/11

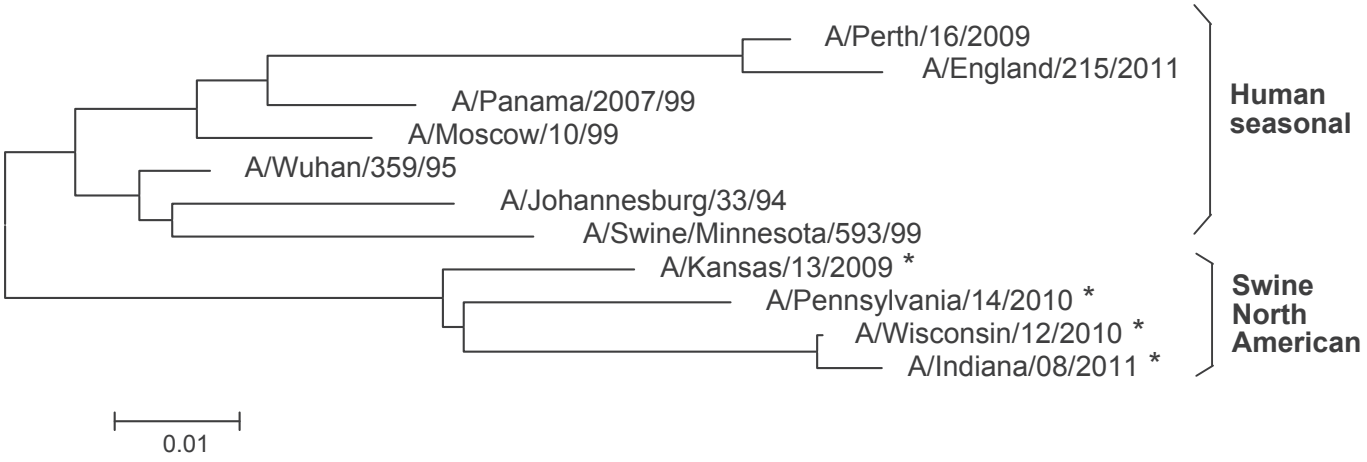


CI: confidence interval; GMT: geometric mean titre; HI: haemagglutination inhibition.

Proportion with HI titre ≥32 (% seropositives) by exposure-related age group for influenza A(H3N2) influenza viruses. The figure shows the results of the analysis of the 2010 panel (Table 1) with four influenza A(H3N2) viruses. The percentage of seropositives for the viruses are depicted in yellow for A/Wuhan/395/1995, blue for A/sw/Minnesota/593/1999, green for A/Indiana/08/2011 and orange for A/Perth/16/2009, while GMTs for analysis with A/Indiana/08/2011, A/Wuhan/395/1995 and A/Perth/16/2009 are illustrated as diamonds in each bar with their 95% CI shown as vertical lines. Cut-off for seropositivity is shown as dotted line. Numbers of samples in each age group are given below the bars. Due to low available serum volume, HI with A/sw/Minnesota/593/1999 virus was started at 1:16 dilution point for all samples and we could therefore not determine GMTs for this analysis.

Figure 1

Phylogenetic tree showing the relationship between human, swine and avian full length haemagglutinin sequences from influenza A(H3N2) viruses



Swine viruses isolated from humans are denoted with *. Mid-point rooted trees were constructed with a neighbour-joining algorithm, using MEGA 4 software.

Cross-reactivity of influenza A(H1N1) viruses

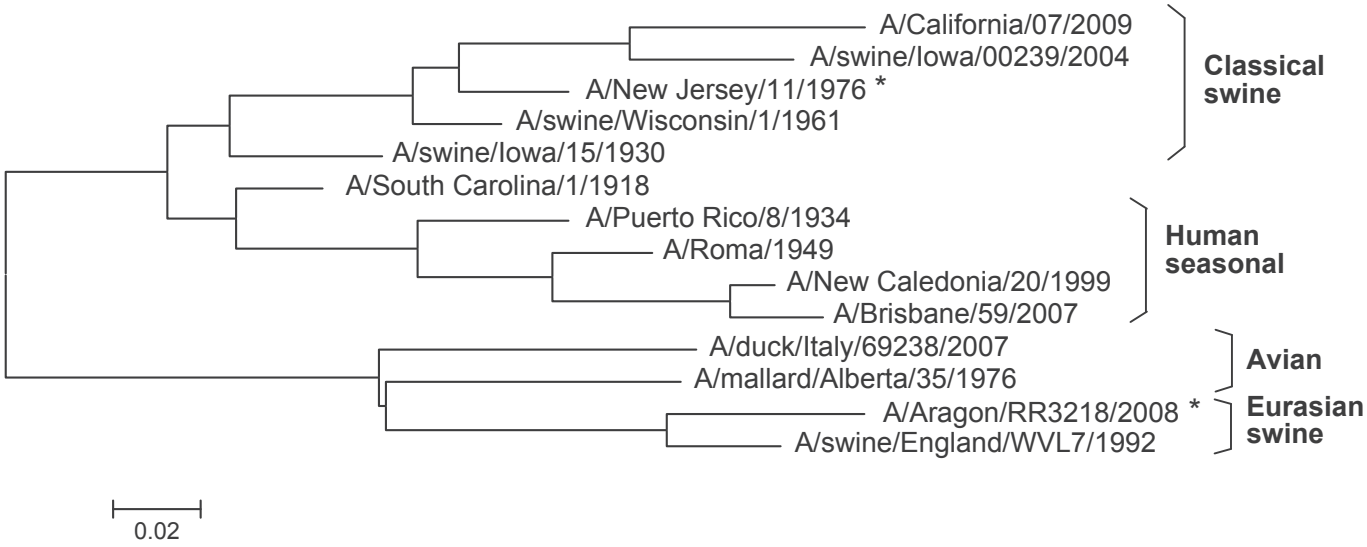
Ferret antiserum raised to human seasonal H1N1 virus strains showed no cross-reactivity with viruses from either the classical or Eurasian swine lineages. The prototype A(H1N1)pdm09 virus A/California/7/2009 from the classical swine lineage showed no reactivity with antiserum raised to either human seasonal H1N1 viruses or Eurasian swine viruses (data not shown and described elsewhere [18]). The recent Eurasian swine virus A/Aragon/RR3218/2008, that caused one sporadic human infection in 2008, had no reactivity with human seasonal virus antiserum.

Figure 3 shows the phylogenetic relationships between the HA sequences of representative human, swine and avian H1N1 viruses isolated since 1918. The pandemic virus A/California/07/2009 has its closest relationship with recent classical swine viruses, which have been circulating in North America and other regions since 1930 [19]. The branch most distal to A/California/7/2009 contains viruses isolated from pigs in Europe including A/Aragon/RR3218/2008, a swine virus isolated from a zoonotic infection in Spain. These viruses are closely grouped with H1N1 viruses of avian origin. These so called Eurasian swine viruses have been circulating in swine since 1979 [20], were entirely derived from avian viruses, and have not yet been detected in North America. These observations clearly show that the HA gene from A/Aragon/RR3218/2008, an Eurasian avian-like swine virus, is genetically distant and has a different ancestor from the A(H1N1)pdm09 virus than their swine counterpart (classical swine lineage) circulating in North America. The observed lack of antigenic relatedness between A/Aragon/RR3218/2008 and the A(H1N1)pdm09 virus is further supported by the fact that, out of 50 residues located at antigenic sites, the two viruses differ at 16 positions (74% identity for the entire HA gene). Only antigenic site Sa is conserved between them. These findings also reveal that, for H1N1 viruses, amino acid differences are present throughout the HA, unlike current swine and human H3N2 viruses, where divergence is located mostly at antigenic sites. Whole-genome analysis showed sequence identities around 80–85% between PB2, PB1, PA, NP and NS genes of A/California/7/2009 and A/Aragon/RR3218/2008.

We compared antibody levels in panels collected at different time points (Figure 4). Cross-reactive antibody levels to H1N1 viruses depended on the collection period. In 2004 (2004 panel), antibody levels to influenza A(H1N1)pdm09 virus were lowest in individuals born after 1999 and highest in individuals aged 37 to 47 at the time (born between 1957 and 1967). After the 2007/08 winter (2008 panel), dominated by influenza A(H1N1) virus circulation, all age groups showed increases in reactive antibody levels to A(H1N1)pdm09 virus. This was most evident in those born before 1957, whilst only moderate increases were observed in those born between 1957–99, and the smallest increase noticed in the youngest age group. After the emergence and wide circulation of the A(H1N1)pdm09 virus (2010 panel), significant increases in antibody levels to this virus were observed in all age groups. The youngest age groups had the highest titres overall (GMT=124, 95% CI: 65–236) against this virus and the highest percentage of seropositive individuals (91%), while the number of seropositives in the older age groups was at least 45% even in the group with the lowest percentage overall, those born before 1957.

Figure 3

Phylogenetic tree showing the relationship between human, swine and avian full length haemagglutinin sequences from influenza A(H1N1) viruses



Swine viruses isolated from humans are denoted with *. Mid-point rooted trees were constructed with a neighbour-joining algorithm, using MEGA 4 software.

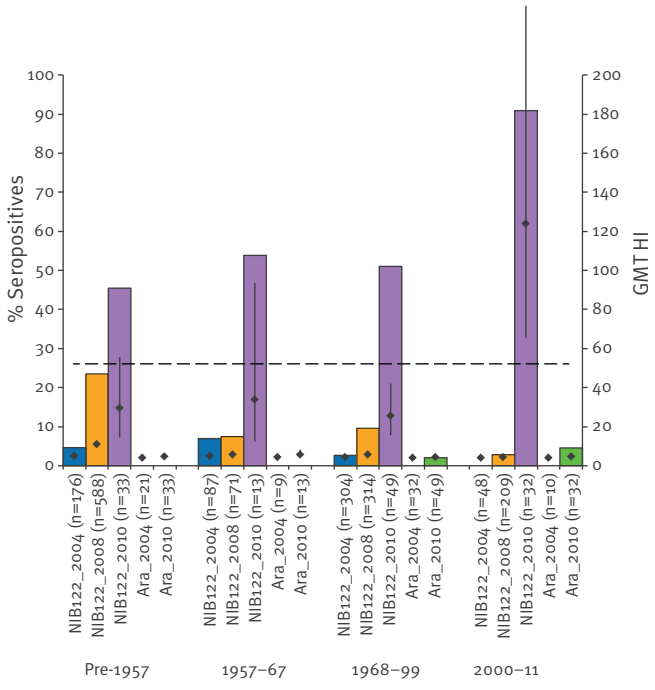
In contrast, we did not identify substantial time-dependent changes of cross-reactive antibody to the A/Aragon/RR3218/2008 strain in 2004 and 2010 panels, and age-related seropositivity suggests high level of susceptibility in all age groups.

Response to trivalent influenza vaccines

Analysis of a small additional panel of anonymised children (3–14 years-old; 24 pairs) and adult sera (20–77 years-old; 24 pairs) before and after vaccination with 2010/11 trivalent inactivated influenza vaccine, showed that children had higher levels of antibody to currently circulating influenza A strains prior to vaccination, which was consistent with our age-stratified cohort analysis, while no significant differences were identified between children and adults for the influenza B component of the TIV (data not shown).

Figure 4

Reactivity in age-stratified sera to influenza A(H1N1) viruses, England, 2004–11



CI: confidence interval; GMT: geometric mean titre; HI: haemagglutination inhibition.

Proportion with HI titre ≥32 (% seropositives) and GMT (95% CI) by exposure-related age group for H1N1 influenza viruses. The figure shows the analysis of three serum panels, collected at different time points (Table 1) with two influenza A(H1N1) viruses. The colouring of the bars indicates, which serum panel and virus were used in an analysis: the percentage of seropositives for the analysis with the NIBRG122 virus (reverse genetics derivative of A/England/195/2009) are indicated in blue (2004 panel), white (2008 panel) and green (2010 panel), while purple bars were used for the analysis with A/Aragon/R3128/2008 of 2010 panel (percentage of seropositives for analysis with A/Aragon/R3128/2008 in the 2004 panel is zero for all four age cohorts). The GMTs for the analysis with the NIBRG122 and A/Aragon/RR3128/2008 viruses are illustrated as diamonds in each bar with their 95% CI shown as vertical lines. Numbers under bars represent the number of samples in each age group. Cut-off for seropositivity is shown as dotted tine.

For influenza A(H3N2) viruses, we observed in children higher pre-vaccine GMTs with the currently circulating seasonal strain A/Perth/16/2009 (GMT=27; 95% CI: 13–55) and four to five times lower titres to recent swH3N2 viruses (A/Pensylvania/14/2010) and A/Wuhan/359/1995, while adults had higher titres to A/Pensylvania/14/2010 and A/Wuhan/359/1995 (GMT=40 and 34; 95% CI: 21–74 and 19–60, respectively), but significantly lower titres to A/Perth/16/2009. Children and adults showed comparable titre increases post vaccination, which were highest for the vaccine virus A/Perth/16/2009 (11.8 and 8.6-fold; 95% CI: 7.3–19.1 and 4.3–17.2, respectively). In both, GMTRs to A/Pensylvania/14/2010 and A/Wuhan/359/1995 viruses were three to four times lower than responses to A/Perth/16/2009. The seroconversion rates were generally higher in children than in adults; in both, rates with the vaccine virus A/Perth/16/2009 were almost twice as high as with the A/Pensylvania/14/2010 and A/Wuhan/359/1995 viruses.

For influenza A(H1N1) viruses, we observed clear differences in pre-vaccine titres for the currently circulating A/California/7/2009 virus, which were highest in children (GMT=76; 95% CI: 45–130) and significantly lower in adults (GMT=9; 95% CI: 5–16). Both age groups had only negligible titres against the A/Aragon/RR3218/2008. Comparing responses to A/California/7/2009 and cross-reactive antibody responses to A/Aragon/RR3218/2008 viruses, similar SCRs and GMTRs for both age groups were observed with the vaccine strain (SCR=96 and 63; GMTR=17.4 and 13.1, for children and adults respectively), while we observed two- to threefold lower SCR and six- to eightfold lower post-vaccine GMTRs with A/Aragon/RR3218/2008 virus.

Discussion

For our serological analysis, we chose three swine influenza isolates from the United States (US) representative of the recent limited human-to-human transmission of swH3N2 viruses in the US, together with historic and recent seasonal H3N2 strains. The swH3N2 viruses included an early isolate, A/sw/Minnesota/593/1999, closely resembling the ancestry of swH3N2 strains, which began circulating in North American pigs in 1998 [21], as well as two strains isolated from recent human cases A/Pennsylvania/14/2010 and A/Indiana/08/2011, the latter of which had acquired one of the eight gene segments (M gene) from the influenza A(H1N1)pdm09 virus [10,22]. We also selected a swine influenza A(H1N1) strain which had caused a sporadic human infection in 2008 in Spain [12] and compared serological responses with those to the A(H1N1)pdm09 virus. The diversity of these swine viruses was shown both in genetic analysis and antigenic characterisation.

Analysis of susceptibility to influenza A(H3N2) swine viruses

We found little evidence for reactive antibodies to North American swH3N2 viruses in children born in

England after 1999, despite moderate levels of antibody to the recent circulating human A/Perth/16/2009 H3N2 strain. This strongly suggests susceptibility of this age group to infection with North American swH3N2 virus. These data predict a high attack rate and greatest impact in young age groups, if these swH3N2 viruses were to emerge as a novel pandemic strain, analogous to the A(H1N1)pdm09 virus. The data are consistent with recently published results from the US [23], Canada [24] and Norway [25], and the observation that the cases identified so far have been mainly in children (ca. 90% in individuals younger than 18 years) [22,26]. They also suggest antibodies induced to the most recently circulating human H3N2 strains lack cross-reactivity with the investigated North American swH3N2 viruses.

Individuals born between 1968 and 1999 (aged 13–44 years in 2012) had the highest level of antibody to swH3N2 viruses, but the lowest level of antibody to the recent H3N2 seasonal A/Perth/16/2009 strain. This also supports the conclusion that antibody reactive with swH3N2 viruses occurs as a result of exposure to older H3N2 strains, either because of antigenic relatedness of older H3N2 strains to swH3N2 viruses or because of an increase in cross-reactive antibodies induced with increasing age. Cross-reactive antibodies in humans seem to correlate with exposure to H3N2 viruses circulating during the 1990s (e.g. A/Wuhan/359/1995 virus). We assume that cross-reactive antibodies in those born between 1968 and 1999 reflect extensive exposure to H3N2 variants circulating in that period and conform to previous observations that the highest attack rates following emergence of antigenic drift variants occur in the youngest age groups. Similar to surveillance data from the US [27] for the last two decades, variants of influenza A(H3N2) were the most commonly circulating strains in Western Europe with multiple drift variants recognised during this period [28-30]. Together, this suggests that the cumulative antibody responses to these H3N2 variants are a consequence of cross-reactivity to swH3N2 viruses, rather than arising from recent exposure to A/Perth/16/2009.

The data also suggest the importance of priming with an antigenically closely matched virus for later protection from a drifted strain – similar to observations in the 2009 pandemic, where individuals which had been exposed to historic H1N1 strains (dating from 1918 to 1956) early in their life seemed to be protected from infection with A(H1N1)pdm09 [14].

The assumption that cross-reactive antibody levels correlate with exposure to H3N2 viruses circulating during the 1990s is supported by the results from phylogenetic analysis (Figure 1) and antigenictiy work in ferrets (Table 3), which together point to similarity of seasonal human viruses of the 1990s and the swH3N2 viruses causing the recent zoonotic cases in the US. One of the influenza strains used in this study (A/Swine/Minnesota/593/1999) dates back to the emergence of influenza A(H3N2) in North American pigs and predates antigenic drift resulting from continuous circulation in pig herds. This isolate shares antigenic epitopes with human H3N2 viruses circulating at the same time, such as A/Wuhan/359/1995. We observed a close match of seroreactivity with A/Wuhan/359/1995 and A/Swine/Minnesota/593/1999 viruses.

In individuals born before 1968 (aged 44 years and older in 2012), antibody titres to A/Perth/16/2009 were of similar level, indicating a similar overall exposure to a recently circulating variant. However, compared to antibody levels in individuals in the 1957–67 birth cohort, we observed lower reactivity with swH3N2 viruses and A/Wuhan/359/1995 in these older adults despite greater likelihood of cumulative exposure to influenza A(H3N2) viruses. We assume that lower levels of cross-reactive antibody to swH3N2 in these individuals could be a result of priming with H3N2 viruses which emerged during the pandemic 1968, or childhood exposure to other, non-H3 influenza subtypes as suggested elsewhere [24]. Nevertheless, the overall GMTs suggest that significant numbers of individuals in England (ca. 50%) may currently be protected from swH3N2 infection.

We also determined the ability of pre- and post-immunisation sera from children and adults immunised with 2010/11 TIV to react with viruses of swine origin as a measure of whether vaccination with seasonal influenza vaccines produces cross-reactive antibodies capable of providing partial protection to emerging zoonotic swine influenza infections. Vaccination with contemporary TIV shows clear evidence of boosting reactivity to swH3N2 viruses after seasonal influenza vaccination. Although boosting was equally efficient in children and adults, vaccination is likely to be most beneficial to the younger age groups because of their generally lower cross-reactive baseline titres.

Analysis of susceptibility to influenza A(H1N1) European swine viruses

We found no evidence of significant pre-existing immunity to a recent Eurasian swH1N1 isolate (A/Aragon/RR3218/2008) in any age group (Figure 4). These findings are consistent with the substantial genetic (Figure 3) and antigenic divergence of this virus from the previous seasonal and current A(H1N1)pdm09 viruses. Baseline immunity analysis in 2009 [14] together with influenza surveillance data [31] point at the importance of priming with historic seasonal H1N1 strains for protection from infection with a newly emerging virus, i.e. A(H1N1)pdm09 [32]. In contrast, the genetic and antigenic divergence of previous and current seasonal H1N1 viruses as compared to the Eurasian swH1N1 points to a lack of priming in the English population.

However, whole genome sequencing data show that this virus has NA and M genes which are similar to those of the A(H1N1)pdm09 virus, with 90% and 94% of sequence identity, respectively, consistent with the

finding that these genes in the 2009 pandemic viruses had originated from the Eurasian lineage of swine viruses [33]. Vaccination with contemporary TIV shows only a limited boosting effect on antibody levels to A/Aragon/RR3218/2008 in both children and adults, and could indicate an inability of current commercial vaccines to protect against swH1N1viruses of the Eurasian lineage.

Our study has several limitations. We used a cut-off value of titres ≥32, while it is unclear whether this titre would indeed confer protection on an individual level, especially for zoonotic infections to which whole populations are immunologically naïve.

This analysis is based on HI data. It has been speculated that neutralisation assays are more likely to detect antibody arising from previous exposure or vaccinations with related strains, which are undetectable by HI [34]. This could have resulted in an underestimation of cross-reactive antibodies. We are also unable to predict the possible contribution of cell-mediated responses to protection. Furthermore, our analysis was opportunistic and intended to be indicative. We used samples available to us, but had only limited numbers of samples with enough remaining volume for this analysis, as the material from the Public Health England serum archive had been used extensively for the UK seasonal seroepidemiology programme. As a result, the described serum panels vary significantly in sample number and the study was underpowered to detect significant differences between adults and children for the analysis of cross-reactive responses post TIV for vaccine trials or by birth cohort in the three population-based serosusceptibility panels (Table 1), especially with the low seroprevalence of antibodies to A/Aragon/RR3218/2008.

The analysis described here has been performed over a period of three years. An identical standard operating procedure was followed throughout; together with use of appropriate and consistent control sera, this should have kept variability of the results to a minimum and allow their comparability.

Although A/Wuhan/359/1995 seems to be an ancestor strain of the investigated swH3N2 viruses, our antigenic characterisation (using ferret sera) indicates that it is not a precise antigenic match. However, seroprevalence data from our human cohort indicate that this virus might be closely related to a shared ancestor. Finally, for the swH1N1 of the Eurasian lineage we selected only one isolate; it is possible that use of other strains might lead to slightly different conclusions regarding cross-protection. However, the phylogenetic data show that viruses in this lineage are significantly distant from previous seasonal H1N1 viruses and the currently circulating A(H1N1)pdm09 viruses (Figure 3), suggesting that the observed lack of cross-reactivity is a universal feature for this group of viruses.

Conclusions

These data and the implied susceptibility to infection in different population subgroups highlight the importance of regular risk assessment of emerging swine origin viruses and virus-specific response planning. Vaccination and control strategies need to target individuals in society who appear to have least protection from infection. The observed differences in seroreactivity when analysing representative swine viruses from different geographical origin and two subtypes, both of which had recently caused infection in humans, emphasise the necessity of regular surveillance activities and interaction between animal and human health agencies.

The data presented here show that swH3N2 and swH1N1 subtypes have a different age-related pattern of potential susceptibility in the human population studied, which is again different from the variant H1N1 subtype that caused the 2009 pandemic. Recommendations for pandemic preparedness need to be adjusted accordingly to take into account virus subtype and source of origin. At a global level, epidemiology of influenza virus in pigs is very complex and diverse. Similarly, recommendations for vaccination with TIV to induce cross-reactive antibody will depend on the nature of the emerging strain and age-dependent priming history in the population.

Globally, very few programmes exist that are based on interconnected animal and human health agencies. It is a clear recommendation from WHO that animal surveillance efforts should be enhanced beyond disease notification, with sharing of viruses between the human and animal sector to improve pandemic risk assessments [35].

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