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Cross-reactive antibody to swine influenza A(H3N2) subtype virus in children and adults before and after immunisation with 2010/11 trivalent inactivated influenza vaccine in Canada, August to November 2010

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In pre- and post-immunisation sera from children (17–120 months-old) and adults (20–59 years-old) immunised with 2010/11 trivalent inactivated influenza vaccine, we assessed age-related patterns of serosusceptibility and vaccine-induced cross-reactive antibodies to a representative swine H3N2 (swH3N2) and a related ancestral human H3N2 (A/Sydney/5/1997) influenza virus. Few children but a greater proportion of adults showed pre-immunisation haemagglutination inhibition titres ≥40 to either virus. Titres increased with age among children but decreased in adults. Fewer than 20% showed a four-fold rise in antibody titres to either virus following immunisation. Further investigation is warranted to guide ongoing risk assessment and response to emerging swine H3N2 viruses.

Introduction

The first documented human infection in North America with an influenza A(H3N2) virus of swine origin (swH3N2) occurred in an Ontario farm worker in 2005 (A/Ontario/RV1273/2005). It involved a swH3N2 lineage that had entered swine from humans in the mid-1990s [1,2]. Sporadic human cases occurred thereafter in the United States (US) and Canada [3,4]. During the latter half of 2011, 12 cases of human infection with a variant of swH3N2 (designated A(H3N2)v by the World Health Organization) [5] were identified in the US, primarily among children including some without recognised swine exposure [6]. Recent analysis has shown that swH3N2 viruses and zoonotic transmissions to humans, including A(H3N2)v, are descendants from a common human influenza virus ancestor, the A/Wuhan/359/1995(H3N2)-like virus [7]. Influenza A/Wuhan/359/1995 has not circulated in humans nor been a component of the trivalent inactivated influenza vaccine (TIV) since 1998, when it was replaced by influenza A/Sydney/5/1997(H3N2) [8-10].

Herein we assess cross-reactive antibody titres to a representative swH3N2 virus in sera collected from Canadian children and adults before and after immunisation with the 2010/11 TIV, containing the same vaccine components as the 2011/12 formulation [10].

Methods

We used a convenience sample of previously collected pre- and post-immunisation sera from children and adults enrolled in 2010/11 TIV immunogenicity trials. Sera had been collected at baseline and 21 to 28 days after the last age-appropriate dose of TIV (Fluviral, GSK, Laval, Quebec, Canada) from Quebec adults in August and September 2010 and from Quebec children in October and November 2010. All had been vaccinated in 2009 with the monovalent AS03-adjuvanted influenza A(H1N1)pdm09 vaccine (Arepanrix; GSK, Laval, Quebec). The study protocols have been described previously [11,12] and had been approved by the ethics board of the Centre Hospitalier Universitaire de Québec.

Sera were tested for antibodies (i) to influenza A/Wisconsin/15/2009(H3N2), considered antigenically equivalent to the influenza A/Perth/16/2009(H3N2)-like component of the 2010/11 (and also 2011/12) northern hemisphere TIV (referred to in this paper as A/Wisconsin), (ii) to a swH3N2 virus (A/ferret/QC/844/2011; F844) isolated from a ferret infected in February 2011 while temporarily housed with swine at the same Quebec animal research facility, and (iii) to influenza A/Sydney/5/97(H3N2) as a human influenza ancestor of swH3N2 (A/Sydney). Because influenza A/

Wuhan/359/1995 virus was not readily accessible, A/Sydney was chosen as the most closely related, available alternative against which to compare age-related trends in cross-reactive antibody levels.

Multiple sequence alignments [13] and BLAST searches [14] generated pairwise identities between F844 gene segments (GenBank accession numbers JQ409334 to JQ409341) and available segments from the following viruses available in GISAID: A(H3N2)v (A/Indiana/10/2011(passage X-1) (Indiana State Department of Health Laboratories, Centers for Disease Control and Prevention, sequence authors B Shu, R Garten, S Emery, A Balish, C Smith, J Barnes, S Lindstrom, A Klimov, N Cox) , A/Wisconsin (passage X-183) (Wisconsin State Laboratory of Hygiene, Centers for Disease Control and Prevention, sequence authors not specified), A/Wuhan/359/1995 (imported from NCBI) and A/Sydney (imported from NCBI).

Haemagglutinin (HA) identity was assessed across the HA1 peptide and antigenic regions defined by one scheme of 59 amino acids [7] and an expanded scheme comprising 130 amino acids [15]. Relatedness was further assessed through phylogenies of HA and neuraminidase (NA) surface proteins of F844, A(H3N2)v, other swine and human influenza A(H3N2) isolates, and TIV components [16]. The authors gratefully acknowledge the 268 originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to GISAID, and recognise in particular the labs who contributed swine and A(H3N2)v sequences highlighted in our phylogenetic analysis: Canadian Food Inspection Agency, Centers for Disease Control and Prevention, Indiana State Department of Health Laboratories, Iowa State Hygienic Laboratory, Kansas Department of Health and Environment, Maine Health and Environmental Testing Laboratory, Minnesota Department of Health, Pennsylvania Department of Health, Public Health Agency of Canada, and University of Pittsburgh Medical Center Microbiology Lab.

Antibody titres were measured in duplicate by haemagglutination inhibition (HI) as previously described [11]. Turkey red blood cells (RBCs) were used for F844 and A/Wisconsin; guinea pig RBCs were used for A/Sydney. Titres <10 were assigned a value of 5. Scatter plots and Pearson correlation coefficients of natural logarithm-transformed titres were explored. Immunogenicity end points included group GMTs, the ratio of post- versus pre-immunisation GMTs (GMTR), the proportion of participants with HI titre ≥40 (by convention considered the sero-protective threshold for evaluating vaccine antigens), and the proportion of sero-converting individuals (those showing four-fold increase in post- compared with pre-immunisation titres or from HI titre <10 pre-immunisation to at least 40 post-immunisation) [17,18]. Linear regression models assessed trends in GMTs by one-year age interval and the chi-square test was used to compare differences in the proportion of participants with HI titre ≥40 by age category.

Results

Participants

Sera from 138 children were included. The mean/median age of paediatric participants was 63/63 months, with a range of 17 to 120 months. Forty-six children never before immunised against seasonal influenza received two doses of 2010/11 TIV, whereas 91 received a single dose. For one child this information was not available. Eighty children (58%) had received at least one prior TIV dose, 24 (17%) had received at least three doses. Sixty-five adults were included, among whom the mean/median age was 40/39 years, with a range of 20 to 59 years. Of these, 58 (89%) had received TIV previously: 41 (71%) a single dose, 16 (28%) twice and one (1.7%) three times previously.

Phylogenetic relatedness

Phylogenetic analysis established F844 to be representative of circulating swH3N2 viruses with zoonotic potential, including A(H3N2)v (Figure 1). BLAST indicated the closest match to each F844 segment originated from swH3N2 viruses isolated in North America between 2005 and 2010 (pairwise identities 97.9–98.9%), with the F844 HA and NA most similar to that of the A/swine/QC/382/2009 virus (98.3% and 99.1% pairwise identity, respectively). The closest human swH3N2 HA was found in A/Iowa/16/2009 (97.4% pairwise identity), and the closest human swH3N2 NA in A/Ontario/1252/2007 (98.9% pairwise identity) (Figure 1).

The human-origin isolate influenza A/Ontario/RV1273/2005 was among the ten isolates most closely related to F844 for all but the NS segment, further reinforcing zoonotic potential of the F844 strain. F844 does not contain the influenza A(H1N1)pdm09 M gene seen in recent A(H3N2)v isolates, however both F844 and A/Indiana/10/2011 (representative A(H3N2)v virus) originate from an identical common ancestor and exhibit 93.3% identity in HA1 peptides and 88% identity in HA antigenic regions (Table 1). Both viruses show comparable identity to A/Sydney in HA1 and antigenic regions, and 27 of the 41 mutations observed in an alignment of F844, A/Indiana/10/2011 and A/Sydney HA1, are common to both F844 and A/Indiana/10/2011.

Immunogenicity and cross-reactivity

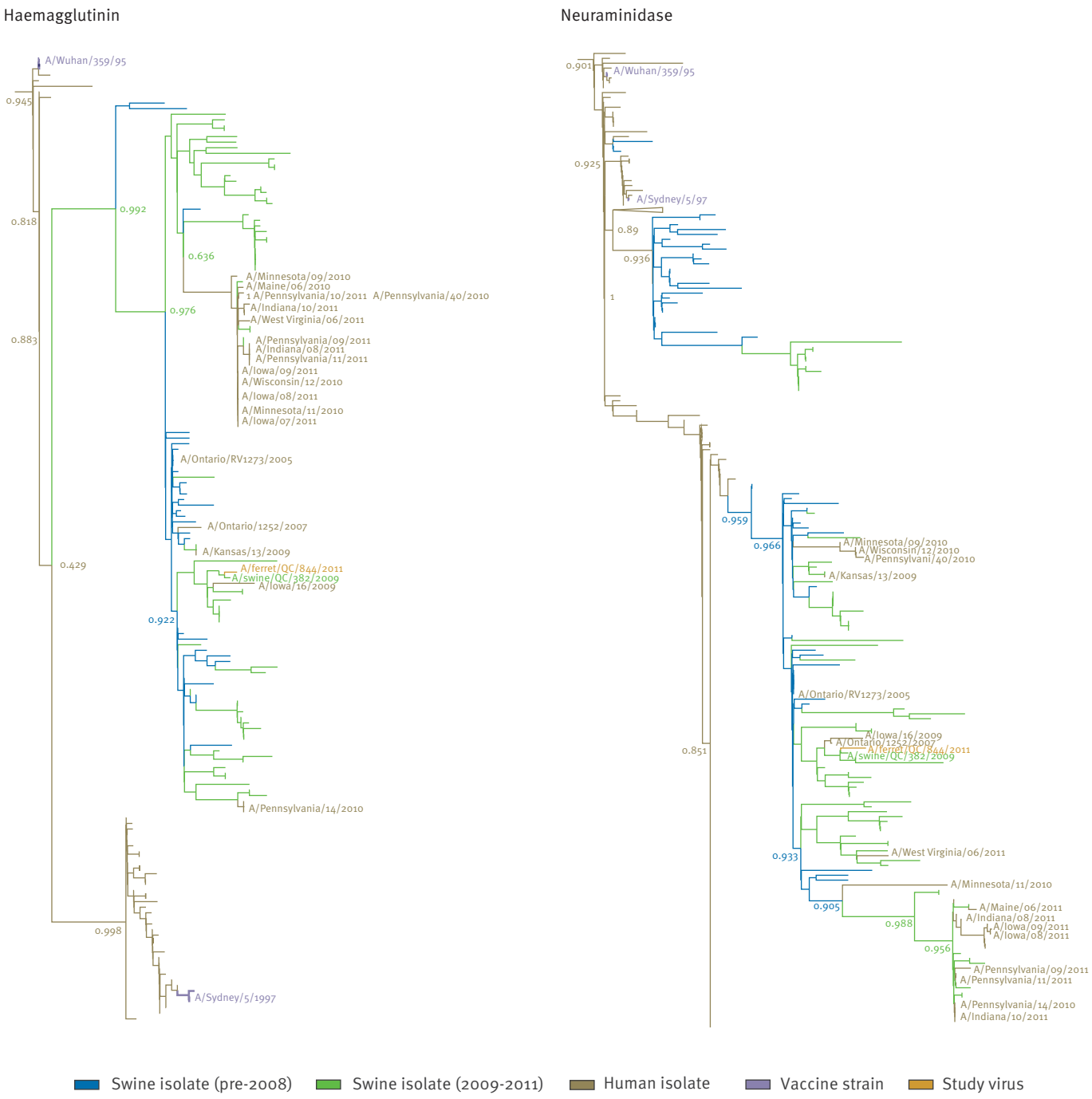
A similar proportion (ca. 35%) of children and adults showed a pre-immunisation HI titre of ≥40 to A/Wisconsin. There was substantial TIV-induced improvement in the level of antibodies to A/Wisconsin, with ca. 90% showing HI titres ≥40 post-immunisation (Table 2). For F844 and A/Sydney, only 1% and 12% of children, respectively, showed HI titres ≥40 pre-immunisation whereas approximately half of the adults showed pre-immunisation titres ≥40 to these viruses. Immunisation with the 2010/11 TIV increased antibody titres to F844 and A/Sydney only marginally in children (8% and 19% sero-converting, respectively) and adults (11% and 15% sero-converting, respectively).

There was a strong correlation between F844 and A/Sydney titres both pre and post immunisation in children (0.74/0.76) and adults (0.68/0.76) (all p<0.001).

The correlation was less strong between F844 and A/Wisconsin in both children (0.24 (p=0.005)/0.43 (p<0.001)) and adults (0.39 (p=0.001)/0.24 (p=0.05)).

Figure 1

Maximum likelihood phylogeny showing location of F844 relative to swine and human swH3N2 viruses and human vaccine strains



F844: influenza A/ferret/QC/844/2011; swH3N2: influenza A(H3N2) of swine origin.

The tree was created with FastTree using the JTT evolutionary model and the Shimodara-Hasegawa test of branch support, using 6,932 swine and human influenza A(H3N2) haemagglutinin sequences and 6,635 swine and human neuraminidase sequences. Certain branches have been collapsed and labels deleted for ease of viewing.

The authors gratefully acknowledge the 268 originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to GISAID, and recognise in particular the labs who contributed swine and A(H3N2)v sequences highlighted in our phylogenetic analysis: Canadian Food Inspection Agency, Centers for Disease Control and Prevention, Indiana State Department of Health Laboratories, Iowa State Hygienic Laboratory, Kansas Department of Health and Environment, Maine Health and Environmental Testing Laboratory, Minnesota Department of Health, Pennsylvania Department of Health, Public Health Agency of Canada, and University of Pittsburgh Medical Center Microbiology Lab.

On linear regression, GMTs to all three antigens increased significantly with age pre and post immunisation in the paediatric cohort; conversely in the adult cohort, GMTs decreased significantly with age for F844 and A/Sydney but not A/Wisconsin (Figure 2A/B).

More children five years and older compared to those under five years of age had HI titres of ≥40, statistically significant for all viruses and time points except the A/Wisconsin virus at the post-immunisation collection (Table 2C). Conversely, antibody titres of ≥40 were less frequent in adults 40 years and older than in younger adults 20–39 years-old, statistically significant for F844 and A/Sydney both before and after immunisation, but not for A/Wisconsin (Table 2C).

Discussion

The intent of this investigation was to assess current vulnerability to emerging zoonotic transmissions of swine influenza H3N2 viruses. With limited early access to the specific US A(H3N2)v, we used a representative swine influenza virus locally acquired from an infected ferret to explore the likelihood of pre-existing and TIV-induced cross-reactive antibody to swine influenza A(H3N2) in children and adults. Although F844 is not a precise match to the specific A(H3N2)v,

Table 1

Amino acid sequence comparison (% pairwise identity) of influenza F844, A(H3N2)v and select human H3N2 vaccine components

	A/ferret/QC/844/2011	A/Indiana/10/2011	A/Wuhan/359/1995	A/Sydney/5/1997
Haemagglutinin HA1 peptide				
A/Indiana/10/2011	93.3			
A/Wuhan/359/1995	91.7	90.0		
A/Sydney/5/1997	89.7	87.8	96.4	
A/Wisconsin/15/2009	87.8	86.0	90.0	90.6
Haemagglutinin antigenic regions (scheme 1)[a]				
A/Indiana/10/2011	88.1			
A/Wuhan/359/1995	81.4	83.1		
A/Sydney/5/1997	76.3	78.0	89.8	
A/Wisconsin/15/2009	71.2	74.6	71.2	74.6
Haemagglutinin antigenic regions (scheme 2)[b]				
A/Indiana/10/2011	88.5			
A/Wuhan/359/1995	82.3	80.0		
A/Sydney/5/1997	78.5	76.2	92.3	
A/Wisconsin/15/2009	76.2	73.1	77.7	80.8
Neuraminidase NA				
A/Indiana/10/2011	93.6			
A/Wuhan/359/1995	93.4	91.3		
A/Sydney/5/1997	93.8	91.7	98.7	
A/Wisconsin/15/2009	92.3	90.8	93.2	N/A
Matrix M1				
A/Indiana/10/2011	93.5			
A/Wuhan/359/1995	94.7	92.9		
A/Sydney/5/1997	95.1	92.1	97.6	
A/Wisconsin/15/2009	95.5	94.8	95.6	95.6
Matrix M2				
A/Indiana/10/2011	83.8			
A/Wuhan/359/1995	82.5	87.0		
A/Sydney/5/1997	82.5	83.3	95.8	
A/Wisconsin/15/2009	81.3	84.8	86.6	88.4

[a] Scheme 1: 59 amino acids [7]

[b] Scheme 2: 130 amino acids [15].

F844: influenza A/ferret/QC/844/2011, a swine influenza A(H3N2) virus isolated from a Quebec ferret.

A/Indiana/10/2011: a representative of the variant swine influenza A(H3N2) virus (A(H3N2)v).

A/Wuhan/359/1995: a human influenza A(H3N2) virus and component of the northern hemisphere vaccines of 1996/97 and 1997/98.

A/Sydney/5/1997: a human influenza A(H3N2) virus and component of the northern hemisphere vaccines of 1998/99 and 1999/00.

A/Wisconsin/15/2009: antigenically equivalent to the influenza A/Perth/16/2009(H3N2) component of the 2010/11 and 2011/12 northern hemisphere vaccines.

The authors acknowledge the Centers for Disease Control and Prevention, the Indiana State Department of Health Laboratories, and the Wisconsin State Laboratory of Hygiene, who submitted sequences used in this table to GISAID.

it is representative of viruses of swine-origin that have infected humans since 2005 and continue to circulate in North American pigs. F844 and A(H3N2)v showed similar pairwise identity to A/Sydney for the most relevant HA surface protein and, in combination, our findings for F844 and A/Sydney may frame major trends in cross-reactive antibody titres to swH3N2 by age. By comparing with antibodies to human viruses that are closely related but ancestral (A/Sydney) and with human strains that are antigenically distant but more recent (A/Wisconsin), we sought to better contextualise and inform age-related observations.

Table 2

Haemagglutination inhibition antibody levels to select influenza A(H3N2) strains before and after 2010/11 trivalent inactivated influenza vaccination, Quebec, August–November 2010 (n=203)

A. Children 17–120 months of age[a] (N=138)

H3N2 virus strain	GMT (95% CI)	Proportion with HI titre ≥40 % (95% CI)	GMTR	Proportion sero-converting % (95% CI)
A/Wisconsin/15/2009[b]				
Pre vaccination	18.7 (14.5–24.3)	37 (29–45)	-	-
Post vaccination	229.7 (176.6–298.8)	89 (84–94)	12.28	80 (74–87)
A/Sydney/5/1997[c]				
Pre-vaccination	8.5 (7–10.2)	12 (7–18)	-	-
Post vaccination	15.5 (12.1–19.8)	30 (23–38)	1.82	19 (12–25)
A/Ferret/QC/844/2011[d]				
Pre vaccination	5.6 (5.3–6)	1 (0–3)	-	-
Post vaccination	7.7 (6.7–8.8)	9 (5–14)	1.38	8 (3–13)

B. Adults 20–59 years of age (N=65)

H3N2 virus strain	GMT (95% CI)	Proportion with HI titre ≥40 % (95% CI)	GMTR	Proportion sero-converting % (95% CI)
A/Wisconsin/15/2009[b]				
Pre vaccination	21.8 (16.2–29.3)	35 (23–47)	-	-
Post vaccination	127.9 (94.4–173.3)	89 (81–97)	5.87	65 (53–77)
A/Sydney/5/1997[c]				
Pre vaccination	24 (18.2–31.5)	45 (32–57)	-	-
Post vaccination	41.7 (31.5–55.3)	62 (49–74)	1.74	15 (6–24)
A/Ferret/QC/844/2011				
Pre vaccination	31.3 (21.9–44.8)	54 (41–66)	-	-
Post vaccination	50.6 (35.9–71.3)	66 (54–78)	1.62	11 (3–19)

C. Proportion with antibody titre ≥40, by age category

	Children aged 17–120 months n (%; 95% CI)			Adults aged 20–59 years n (%; 95% CI)		
H3N2 virus strain	<5 years N=63	≥5 years N=75	P	20-39 years N=33	≥40 years N=32	P
A/Wisconsin/15/2009[b]						
Pre vaccination	14 (22; 12–33)	37 (49; 38–61)	0.001	15 (45; 28–63)	8 (25; 10–40)	0.09
Post vaccination	53 (84; 75–93)	70 (93; 88–99)	0.08	31 (94; 86–100)	27 (84; 71–97)	0.21
A/Sydney/5/1997[c]						
Pre vaccination	0 (0)	17 (23; 13–32)	-	19 (58; 40–75)	10 (31; 15–48)	0.03
Post vaccination	7 (11; 3–19)	35 (47; 35–58)	<0.0001	25 (76; 61–91)	15 (47; 29–65)	0.02
A/Ferret/QC/844/2011						
Pre vaccination	0 (0)	2 (3; 0–6)	-	28 (85; 72–97)	7 (22; 7–37)	<0.0001
Post vaccination	1 (2; 0–5)	12 (16; 8–24)	0.004	31 (94; 86–100)	12 (38; 20–55)	<0.0001

CI: confidence interval; GMT: geometric mean titre; GMTR: ratio of post-/pre-immunisation GMTs; HI: haemagglutination ininhibition.

Sero-conversion defined as four-fold increase in post- versus pre-immunisation titres or from HI titre <10 pre immunisation to at least 40 post immunisation.

[a] Post-immunisation results for age-appropriate dose measured 3–4 weeks following one dose for previously immunised and following two doses for unimmunised children.

[b] Antigenically equivalent to the A/Perth/16/2009(H3N2)-like vaccine component of the 2010/11 and 2011/12 trivalent inactivated influenza vaccine.

[c] Guinea pig red blood cells (RBCs) used; for all other viruses turkey RBCs.

[d] N=137 due to insufficient sera.

The limitations of our approach warrant consideration. Some laboratory variability in influenza antibody assay results is widely recognised. It should also be recognised that titres measured in HI assays, unlike microneutralisation, do not necessarily represent functional antibodies and the threshold of 40 conventionally applied as indicative of sero-protection may not predict immunity to zoonotic infections [17-21]. In general, HI is thought to overestimate cross-reactive heterologous versus homologous responses, and given that A(H3N2)v is slightly more divergent from ancestral strains in its HA1 than is F844, there may be additional reason to consider our results optimistic [18-21]. Conversely, other markers such as cell-mediated immunity may also contribute to protection but were not assessed. We have highlighted that F844 and A(H3N2)v are not precise antigenic matches and that A/Sydney is not the direct precursor of either swH3N2 virus. Differences in absolute titres or proportions by age may be expected although the major age-related trends we highlight should still apply. A specific swH3N2 virus has not yet established sustained community transmission. Further mutation, reassortment or other virus evolution is still possible and we cannot predict which swH3N2 virus may ultimately assert itself in the human population. For the purpose of ongoing risk assessment, it thus remains prudent to consider major trends rather than precise results for a particular swH3N2 virus.

Finally, the small sample size, limited age categories and geographic representation of included sera must also be taken into account in interpreting our findings.

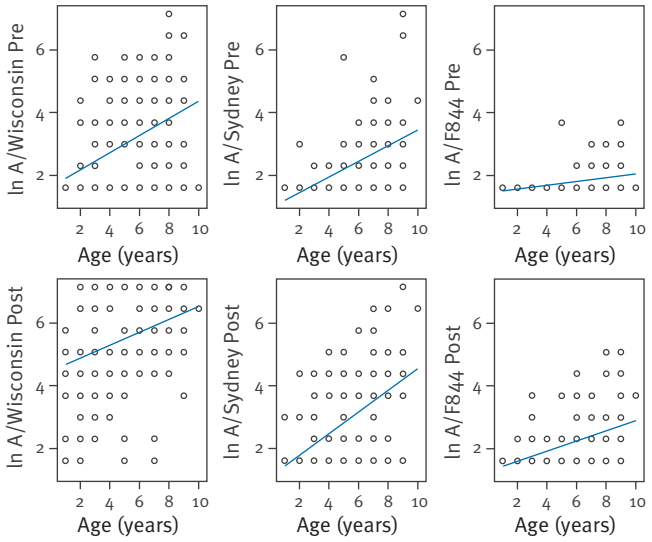
With these caveats in mind, our findings present several signals worthy of further investigation. Only few children younger than 10 years had cross-reactive antibodies to swH3N2 before or after immunisation, and when present, these antibodies were at low levels. We found more adults with cross-reactive antibody titres ≥40 against swH3N2, although GMTs were still not very high. The 2010/11 TIV only marginally increased cross-reactive antibody levels in both children and adults. Given that the same vaccine components have been used in the 2011/12 TIV, these 2010/11 findings will likely also apply to 2011/12 [10]. As noted above, however, the clinical implications of low-level cross-reactive HI antibodies are uncertain.

We observed greater likelihood of cross-reactive antibodies with increasing paediatric age but a paradoxical and unexpected pattern of decrease with increasing age in adults. We cannot address the level of cross-reactive antibodies in children 10 to 19 years of age or the elderly because they were not included in the original immunogenicity trials from which these sera were drawn. However, the pattern of decrease shown across young and middle-aged adults suggests that antibody

Figure 2

Antibody titres pre- and post immunisation with 2010/11 trivalent inactivated influenza vaccine, by age, for influenza A/Wisconsin/15/2009, A/Sydney/5/1997 and A/ferret/QC/844/2011, Quebec, August–November 2010 (n=203)

A. Children



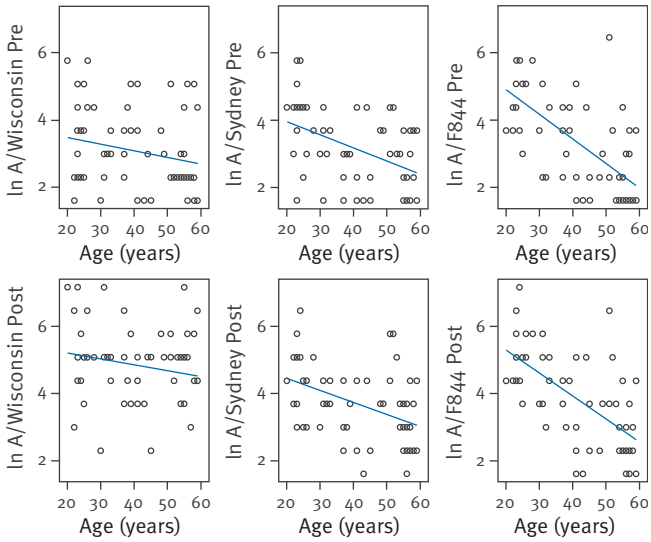
Linear regression β (95% CI) and p value for effect of age on geometric mean titre pre- and post-immunisation, children:

A/Wisconsin pre-immunisation: 1.32 (1.19–1.45), P<0.001; post-immunisation: 1.23 (1.11–1.36), P=0.0002

A/Sydney pre-immunisation: 1.28 (1.2–1.37), P<0.001; post-immunisation: 1.41 (1.3–1.54), P<0.001

A/F844 pre-immunisation: 1.06 (1.04–1.09), P<0.001; post-immunisation: 1.18 (1.12–1.24), P<0.001

B. Adults



Linear regression β (95% CI) and p value for effect of age on geometric mean titre pre-/post-immunisation, adults:

A/Wisconsin pre-immunisation: -1.02 (-1.04 to 1), P=0.08; post-immunisation: -1.02 (-1.04 to 1.01), P=0.14

A/Sydney pre-immunisation: -1.04 (-1.06 to -0.98), P<.0001; post-immunisation: -1.04 (-1.06 to -0.98), P=0.0005

A/F844 pre-immunisation: -1.08 (-1.1 to -0.95), P<.0001; post-immunisation: -1.07 (-1.09 to -0.95), P<.0001

titres may also be low in the elderly and this should prompt further evaluation.

The immuno-epidemiologic reasons for a pattern of declining cross-reactivity despite greater likelihood of cumulative exposure to influenza A(H3N2) viruses with adult age may be worth reflection. The closest ancestor of swH3N2 viruses, including A(H3N2)v, circulated in human populations approximately 15 years ago and is represented in our paper by A/Sydney/5/1997 [7]. Very young participants in this study would not have been exposed to these ancestral viruses in the mid- and late 1990s, and their lack of antibodies is therefore not unexpected; conversely, most adult participants should have been exposed. Allowing for the greatest likelihood of first influenza exposure and infection at pre-school or school age, higher titres to ancestral and related swH3N2 viruses 15 years later in young but not older adults may be consistent with the theory of robust and preferential recall of antibody to first-infecting viruses with subsequent and cumulative infections [22]. A similar phenomenon has been invoked to explain the higher pre-pandemic titres to the influenza A(H1N1)pdm09 virus found in the very old even decades after their priming exposure to a related but historic H1N1 virus in their childhood [23]. Following the same concept, lower titres to A/Sydney and related swH3N2 virus in middle-aged compared with younger adults may signal different original priming experiences. However, it was not the intent of this paper to elucidate immunological mechanisms; these concepts remain speculative and require specific hypothesis testing.

Overall, our results suggest broad susceptibility to swine-origin H3N2 infection in young children, consistent with early epidemiologic features of A(H3N2)v in the US. Susceptibility may also increase with age in adults. Given the recognised potential for children to amplify influenza spread in the community [24], and the greater vulnerability of older adults to severe outcomes of H3N2 infection generally [25], these signals warrant further investigation to guide ongoing risk assessment and response to emerging swH3N2 viruses. The H3N2 subtype of swine-origin influenza may show a different age-related pattern of risk in the human population compared to the H1N1 subtype that caused the 2009 pandemic and recommendations may need to be adjusted accordingly. We observed little TIV-induced improvement in cross-reactive antibodies suggesting that a specific candidate vaccine would be required in the event of further zoonotic transmission and epidemic spread of swH3N2 virus. Additional studies should explore age-related and vaccine-induced effects across a greater age and geographic span, applying multiple immunogenicity assays (HI, microneutralisation, cell-mediated immune markers) and swine-origin influenza A(H3N2) viruses, including A(H3N2)v.

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Competing interests

GD has received research grants from GlaxoSmithKline (GSK) and Sanofi Pasteur, VG from GSK and Merck, MD from GSK, Merck and Wyeth (now Pfizer). GB has received research grant funding from GSK. No other authors have competing interests to declare.

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Outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing *Escherichia coli* O104:H4 among French tourists returning from Turkey, September 2011

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Eight cases of diarrhoea, including two cases of haemolytic uraemic syndrome (HUS), were identified among 22 French tourists who travelled to Turkey in September 2011. A strain of *Escherichia coli* O104:H4 *stx2*-positive, *eae*-negative, *hlyA*-negative, *aggR*-positive, ESBL-negative was isolated from one HUS case. Molecular analyses show this strain to be genetically similar but not indistinguishable from the *E. coli* O104:H4 2011 outbreak strain of France and Germany. Although the source of infection was not identified, we conclude that the HUS cases had probably been infected in Turkey.

On 30 September 2011, the University Hospital of Caen in western France informed the local health authorities of two cases of post-diarrhoeal haemolytic uraemic syndrome (HUS) in adults returning from an organised bus tour in Turkey that had taken place between 4 and 17 September 2011. Both cases were women in their 60s. They had not known each other prior to their journey to Turkey. Diarrhoea onset was 15 September for both cases. They were hospitalised following their return from Turkey and HUS was diagnosed on 20 and 26 September 2011. Initial information obtained from the concerned travel agency indicated the occurrence of additional cases of diarrhoea among other members of this travel group during the trip to Turkey.

Epidemiological investigation

A case was defined as a person with diarrhoea, bloody diarrhoea or HUS, with a date of symptom onset between 4 September and 2 October 2011, who was a member of the group having travelled to Turkey between 4 and 17 September 2011. A case of HUS was defined as acute renal failure and either microangiopathic haemolytic anaemia and/or thrombocytopenia.

The tour operator was contacted to identify any additional cases among individuals of groups who subsequently travelled on the same bus tour.

All 22 travellers of the group were interviewed using a standardised semi-structured questionnaire exploring symptoms, food consumption including sprouts, activities undertaken during the trip to Turkey, and contact with animals or other cases of diarrhoea in the seven days before symptom onset.

Case description

As of 4 October 2011, six additional cases were identified, bringing the total number of cases to eight. Among the eight cases, six were women and two were men with a median age of 64 years (range: 51-71 years). Five cases presented with diarrhoea, one case initially with diarrhoea and a second episode of bloody diarrhoea four days later, and two cases with bloody diarrhoea evolving into HUS. Cases had symptom onset between 6 and 15 September with an initial group of five cases occurring on 6 and 7 September and three cases occurring later during the trip (Figure 1). One case consulted a hospital emergency room for bloody diarrhoea during the trip and was not admitted. Both HUS cases were hospitalised following their return to France. One HUS case was discharged after five days of hospitalisation while the second case, who had a transient ischemic attack, was discharged after nine days of hospitalisation.

The group's two-week bus itinerary in Turkey took them from Istanbul to Ankara, Cappadocia, Aksaray, Konya, Pamukkale, Aphrodesia, Kudsadai, Priene, Miletus, Didymus, Izimir, Selçuk, Pergamum and Bursa. Due to the relatively long stay in Turkey, and the fact that the group stayed in nine different hotels and repeatedly

ate similar foods in numerous restaurants, often with buffet style meals, we were unable to identify a specific food that might have been associated with illness. While group members could usually remember having eaten a certain food item, they could not remember on what date, how often or during which specific meals they had consumed this item. No member of the group reported having eaten sprouts during the trip to Turkey or before departure to Turkey.

Following the initial interview, a second questionnaire focusing specifically on food items eaten at the airport before boarding the plane, during the flight from Paris to Istanbul on 4 September and during the first two days of the tour was undertaken. The objectives were to exclude the hypothesis of contamination before arrival in Turkey and to identify a food consumed during the first 48 hours after arrival in Turkey that could have explained the five initial cases. Information on the menus served during the flight from Paris to Istanbul was obtained from the airline's catering company. Group members reported no common meal or food shared before boarding the plane in Paris and none brought food to the airport to share among the group. Two menus were available during the flight with a choice of poultry or smoked fish as a main course. Neither menu was common to all cases. The group members had difficulty remembering specific foods items served during the 48 hours after arrival in Turkey. These data were not sufficiently robust for further analysis.

Microbiological and serological investigation

Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 possessing the gene *stx2* but not the genes coding for intimin (*eae*) and haemolysin (*hlyA*) was isolated from one of the HUS patients. The strain was also negative for the gene coding EAST1 toxin (*astA*) and positive for the *aggA* gene which encodes the aggregative adherence fimbriae type I (AAF/I). Analyses on the stool of the second HUS patient were negative for STEC O104:H4. The STEC O104:H4 isolate had the following antibiotic resistance profile: ampicillin-resistant (R), streptomycin R, sulphonamide R, trimethoprim R, cotrimoxazole R, tetracycline R, nalidixic acid R, cefotaxime-sensitive (S), ceftazidime S, imipenem S, kanamycin S, gentamicin S, chloramphenicol S, and ciprofloxacin S. Except the absence of an extended-spectrum beta-lactamase, the resistance profile of this isolate was similar to the profile of the strain involved in the recent STEC O104:H4 outbreaks in Germany and Bordeaux in France linked to the consumption of sprouts in May-June 2011 [1,2].

The strain isolated from the HUS case returning from Turkey was compared by pulsed-field gel electrophoresis (PFGE) using *Xba*I and *Not*I [3] to STEC O104:H4 *stx2* strains isolated from two imported cases in France linked to the German 2011 outbreak, ten patients in the Bordeaux outbreak in 2011, and two sporadic cases isolated in France in 2004 and 2009 (Figures 2 and 3). Previous molecular analyses had shown the genetic relatedness of the Bordeaux and German O104:H4 strains [1]. The *Xba*I- and *Not*I-PFGE profiles of the strain isolated from the HUS case returning from Turkey were close but not identical (differences in two bands for *Xba*I and in three bands for *Not*I) to those of the German and Bordeaux O104:H4 2011 outbreak strains. PFGE also showed that the strain isolated from the patient returning from Turkey was unrelated to the two O104:H4 *stx2 aggR agg3A* strains isolated previously in France in 2004 and 2009 [4].

In addition, both HUS cases had a positive serology for *E. coli* O104. Serological testing was performed by a line blot immunoassay using lipopolysaccharides of seven major serogroups of STEC (O26, O91, O103, O111, O128, O145, O157) and of O104 (extracted from a clinical O104:H4 isolate) [5].

Biological samples were not systematically taken from non-HUS cases as they were no longer symptomatic at the time of the investigation. At the initiative of the treating physician, a stool sample was taken from one case and a serum sample from another, both 28 days after the start of diarrhoea. Both analyses were negative.

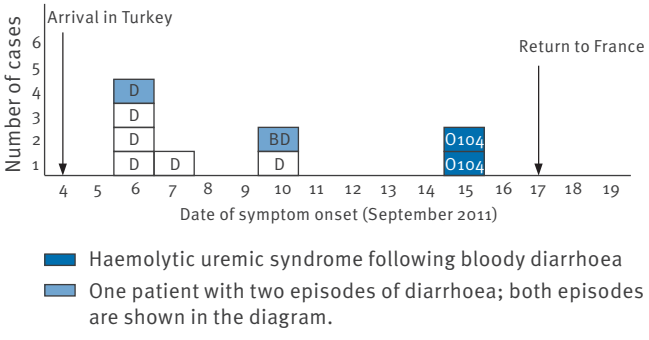
Control measures

Colleagues in other European countries were informed of this outbreak on 4 October via the Epidemic Intelligence Information System (EPIS) of the European Centre of Disease Prevention and Control (ECDC) and Early Warning Response System (EWRS) with the request to report any similar cases.

Germany reported the occurrence of two adult cases of infection with ESBL-negative STEC O104:H4 *stx2* among persons returning from Turkey in July and August 2011. They had developed bloody diarrhoea 11 days and 18

Figure 1

Cases of haemolytic uraemic syndrome due to Shiga toxin-producing *Escherichia coli* O104:H4, diarrhoea or bloody diarrhoea, by date of symptom onset, among French tourists returning from Turkey, September 2011 (n=8)



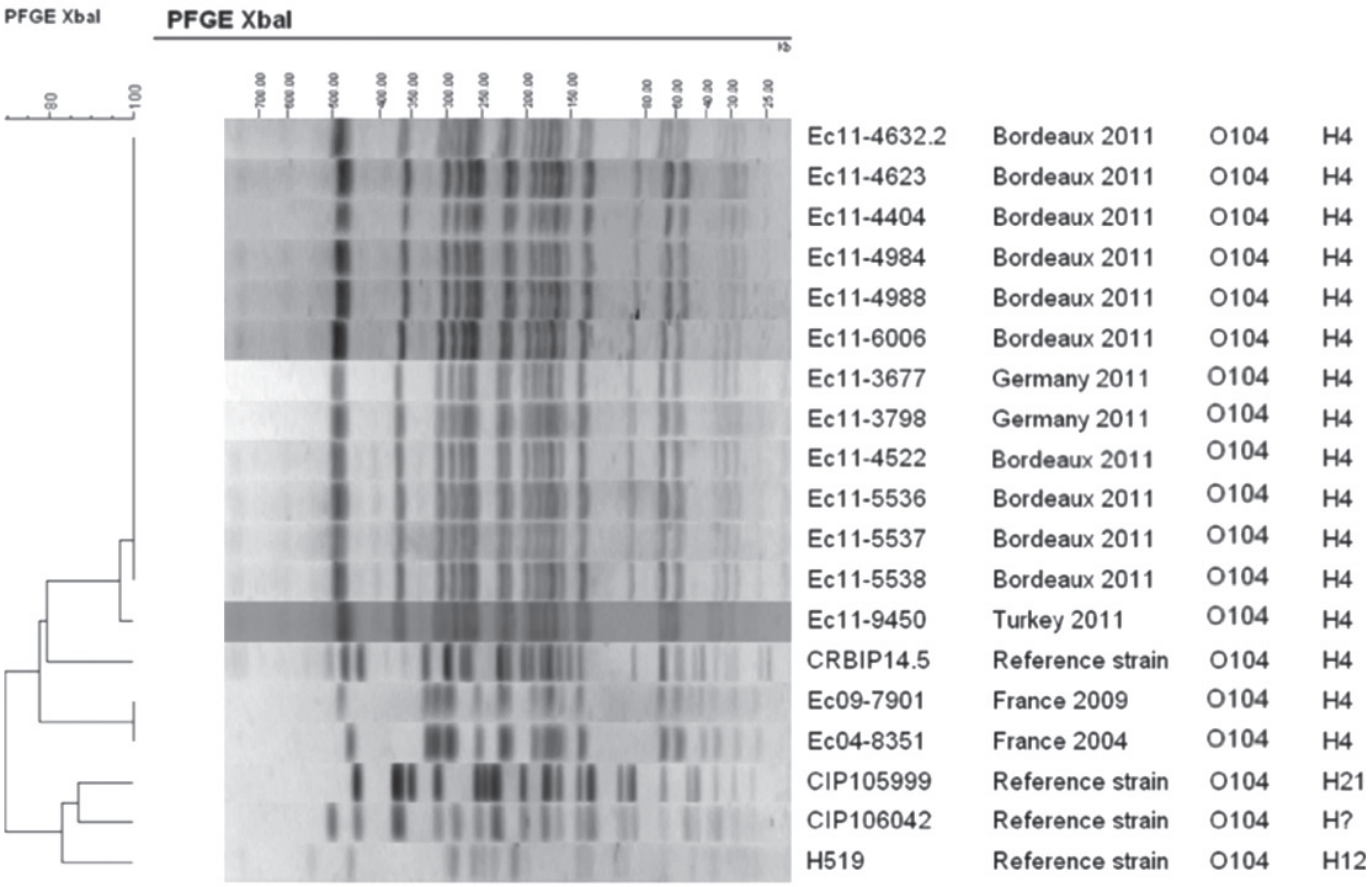
BD: bloody diarrhoea; D: diarrhoea; O104: confirmed *Escherichia coli* O104:H4 infection.

after days after returning from Turkey, where both had stayed in Istanbul and one had additionally spent time at the Black Sea (personal communication, Dirk Werber, January 2012).

Moreover, Danish colleagues reported an STEC O104:H4 *stx2* infection in an adult with diarrhoea onset on 28 September, two days before the end of a month's stay

Figure 2

PFGE profiles (*Xba*I) obtained from one STEC O104:H4 isolate from a French traveller returning from Turkey and other STEC O104:H4 outbreak isolates from France and Germany[a] and from various *Escherichia coli* O104 reference strains



PFGE: pulsed-field gel electrophoresis; STEC: Shiga toxin-producing *Escherichia coli*.

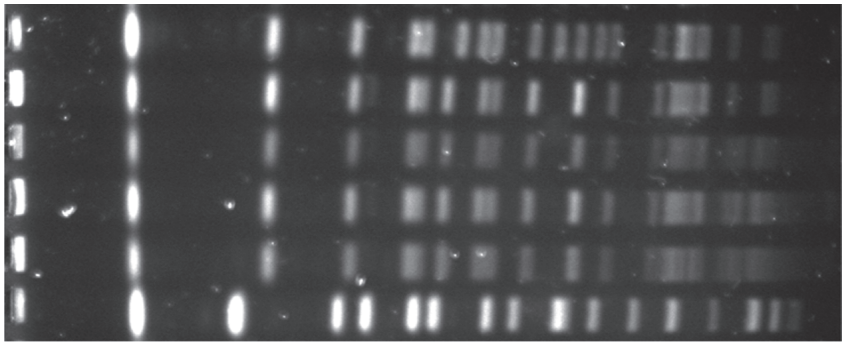
[a] Ten STEC O104:H4 isolates from Bordeaux, France (2011), two STEC O104:H4 isolates from Germany (2011), two STEC O104:H4 isolates from France (2004 and 2009).

The dendrogram generated by BioNumerics version 6.5 software (Applied Maths, Sint-Martens-Latem, Belgium) shows the results of cluster analysis on the basis of PFGE fingerprinting.

Similarity analysis was performed using the Dice coefficient and clustering was done using the unweighted pair-group method with arithmetic averages (UPGMA).

Figure 3

PFGE profiles (*Not*I) obtained from the STEC O104:H4 isolates linked to the outbreak cases, France 2011



PFGE: pulsed field gel electrophoresis; STEC: Shiga toxin-producing *Escherichia coli*.

in private homes in Ankara (personal communication, Charlotte Kjelsø, January 2012).

Contact with the travel agency showed that none of the tourists having subsequently travelled on the same bus tour reported developing diarrhoea. As evidence suggested that this outbreak was limited to tourists from this single travel group, no particular control measures were put in place.

Conclusions

There is no evidence to link this STEC O104:H4 outbreak to the consumption of fenugreek sprouts, as was the case for the German and French outbreaks in May to June 2011 [6-9]. None of the 22 travel group members reported the consumption of sprouts before and during their trip to Turkey.

Microbiological or serological evidence of STEC O104:H4 infection was only obtained for the two HUS cases. Considering that the median incubation period described for STEC O104:H4 is eight to nine days (range: two to 18 days) [10] and that these cases developed their symptoms 11 days after their arrival in Turkey, it is probable that they were infected during their stay in Turkey. In addition, these cases did not know each other before their trip to Turkey, they do not live in the same town and they consumed no common foods before or during their flight to Turkey, which provides further evidence in favour of this hypothesis. No source of contamination could be identified for these cases.

The fact that the six initial diarrhoea cases did not share a common food before or during the flight to Turkey suggests that they were infected following their arrival in Istanbul. However, their reported incubation period was much shorter than that of the HUS cases. Moreover, none were confirmed as STEC O104:H4 infection. Thus, this cluster may have been due to another pathogen and may have been a distinct event not linked to the HUS cases.

Turkey is among several destinations where European tourists had previously travelled before developing STEC O104 infection between 2004 and 2009 (n=4), along with Afghanistan, Egypt and Tunisia [11]. This outbreak supports data suggesting that the STEC serogroup O104 circulates in these areas. Further evidence is provided by the three additional cases that were subsequently identified in Germany and Denmark among persons also returning from Turkey within the same approximate time frame. Public health authorities and clinicians should be vigilant for possible STEC O104 infection in individuals returning from these areas who present with post-diarrhoeal HUS.

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Two invasive mosquito species, *Aedes albopictus* and *Aedes japonicus japonicus*, trapped in south-west Germany, July to August 2011

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Adult females of two invasive species, *Aedes albopictus* and *Aedes japonicus japonicus*, were collected for the first time in July and August 2011 in Germany. Previously, only immature stages of these species had been found in the country. Repeated detection of these species reveals the Upper Rhine Valley in south-west Germany to be a particularly sensitive region for the introduction and establishment of exotic mosquito species that needs careful observation.

As part of nationwide mosquito monitoring activities, two invasive mosquito species, *Aedes albopictus* and *Ae. japonicus japonicus*, were trapped in the Upper Rhine Valley in south-west Germany (federal state of Baden-Württemberg), in the summer of 2011. Neither of these culicid species had been previously collected in Germany as adult specimens.

Various exotic mosquito species such as *Ae. albopictus*, *Ae. j. japonicus*, *Ae. atropalpus*, *Ae. koreicus* and *Ae. aegypti* have recently invaded Europe [1]. In a few instances, eradication has been possible but *Ae. albopictus* and *Ae. j. japonicus* have become established and continue to spread [2,3]. Autochthonous human cases of chikungunya in northern Italy and southern France and of dengue in southern France and Croatia have been attributed to the presence of the vector *Ae. albopictus* [4-6]. This, together with the demonstration of several pathogenic viruses in field-collected mosquitoes in Germany [7-9], prompted the German authorities to initiate nationwide mosquito monitoring activities in 2011.

Background

Ae. albopictus is a most efficient vector of numerous arboviruses [10]. After its introduction into Italy in the late 1980s, it is now widely distributed in the Mediterranean region and continues to spread [11]. While this species actively moves within short distances, the most important mode of long-distance dispersal is passive transportation by vehicles [1]. Although *Ae. j. japonicus* has been found carrying West Nile virus in the field and its vector competence has been demonstrated in the laboratory for several viruses, the role of this species in the natural transmission of pathogens is unclear [1].

Trapping strategy

To search for invasive mosquito species, BG-Sentinel traps (Biogents, Germany) were set up in southern Germany at various possible portals of entry for exotic mosquitoes, i.e. along public transportation routes close to borders with neighbouring countries. The traps were operated permanently from the beginning of July to the end of August 2011, with a sample collection interval of seven days. During the whole season, the traps were equipped with BG-Lure (Biogents), a proven attractant for several exotic mosquito species. To increase the catching efficacy, carbon dioxide (CO_2) was added as an additional attractant for the last 24 hours of the weekly collection period. It was supplied from gas bottles at a rate of approximately 20 g/h and released through a nozzle 20 cm above the trap. Collected mosquitoes were morphologically identified using the identification keys of Schaffner et al. [12] and Becker et al. [13]. Genetic confirmation was performed by cytochrome c oxidase subunit I (COI) barcode region PCR amplification [14] and DNA sequencing following standard protocols. Sequence analysis was carried out using the COI species identification tool of the Barcode of Life Data Systems [15].

Mosquitoes trapped

A total of 10 female specimens of *Ae. j. japonicus* and one single female specimen of *Ae. albopictus* were identified in a trap operated behind a rest area on the A5 motorway entering Germany from Switzerland (N 47° 36 '03.5'', E 07° 36' 18.7'') (Figure). The *Ae. j. japonicus* females were collected from mid-July to the end of

August, while the *Ae. albopictus* female was trapped in late July (Table).

In a second trap, set up in a cemetery in Freiburg (Figure), close to a truck-railway transshipment station (N 48° 00' 39.7'', E 07° 50' 27.8''), a female *Culiseta longiareolata* was detected in mid-August (Table).

In addition to the three mosquito species mentioned, several female specimens of indigenous species were collected in the two traps, in particular *Culex hortensis* at the site in Weil am Rhein and *Cx. pipiens* or *Cx. torrentium* at the site in Freiburg (Table). No male specimens of any mosquito species were trapped.

Implications of the findings

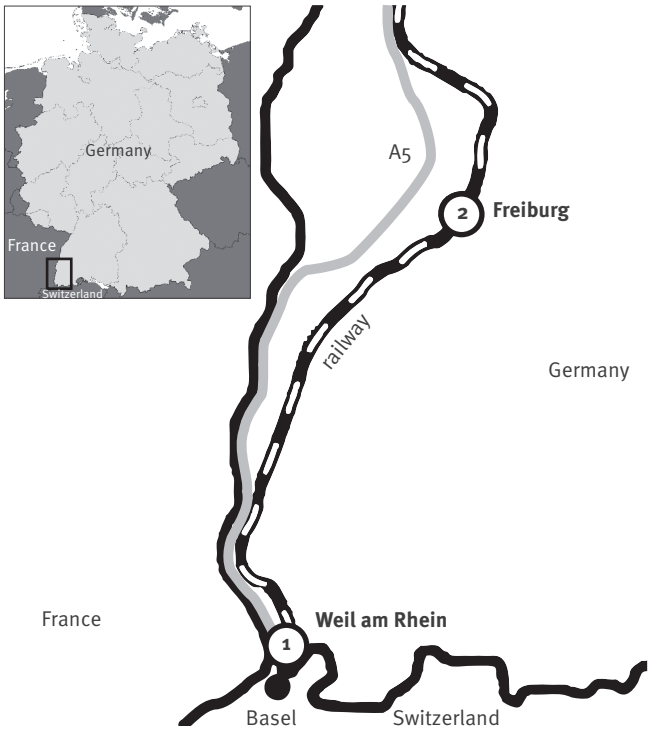
After the earlier finding of five eggs of *Ae. albopictus* in an ovitrap in September 2007 [16] and the finding of numerous preimaginal stages of *Ae. j. japonicus* in 2008, 2009 and 2010 [2,17,18], the females described in this study are the first adult mosquitoes of these species trapped in Germany. For *Ae. albopictus*, it is only the second time that this species has been observed in the country. Although monitoring of adult mosquitoes should not replace the monitoring of immature stages, it has the advantage that breeding sites need not be searched for and arduously examined. Also, species identification in adults is much easier and quicker, so that response times for control can be accelerated. The finding of adults may indicate directions of dispersal and, if introduction can be excluded, show that environmental conditions are adequate to complete the developmental cycle.

As the most important mode of long-distance dispersal of *Ae. albopictus* is passive transportation by vehicles, the A5 motorway, entering Germany from Switzerland, represents one of the most likely portals of entry for the introduction of this species by ground vehicles from southern Europe. Indeed, the only demonstration of *Ae. albopictus* stages in Germany before this study, namely five eggs in an ovitrap in 2007, was associated with a parking area on this motorway close to the Swiss border [16].

After various reports from other central European countries, *Ae. j. japonicus* was first detected in Germany in the German–Swiss border zone during a Swiss study in 2008 [2]. Due to its demonstrated wide distribution in the sampled region, this species is thought to have been present unnoticed for several years. However, it could not be found in Weil am Rhein and the adjacent municipalities at that time. It was only in 2009 that monitoring in south-western Germany revealed the widespread occurrence of *Ae. j. japonicus* immature stages in Germany, including in the Weil am Rhein

Figure

Trap location (1) where two culicid mosquito species (*Aedes albopictus* and *Ae. japonicus japonicus*) were collected, Baden-Württemberg, Germany, July–August 2011



A second trap, in Freiburg, is indicated (2), where a female *Culiseta longiareolata* was detected.

Table

Adult (female) mosquitoes trapped at two sites, Baden-Württemberg, Germany, July–August 2011

Mosquito species	Number of adult mosquites trapped															
	Trap site 1 (Weil am Rhein)								Trap site 2 (Freiburg)							
	Calendar week July–August 2011															
	27	28	29	30	31	32	33	34	27	28	29	30	31	32	33	34
Aedes albopictus	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
Aedes japonicus japonicus	–	2	3	3	–	1	–	1	–	–	–	–	–	–	–	–
Culiseta annulata	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Culiseta longiareolata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–
Culex hortensis	4	1	–	3	2	–	1	5	–	–	–	–	–	–	–	–
Culex pipiens and/or *Culex torrentium*	–	–	–	1	–	1	2	1	5	4	6	4	2	5	4	4

region [17]. Thus, our new data from 2011 confirm the persistence of this species in southern Germany.

Before summer 2011, *Cs. longiareolata* – a thermophilic mosquito species that in Europe is endemic to the Mediterranean – had never been collected in Germany. Around the same time as our study, larvae and pupae of *Cs. longiareolata* were found in another area of south-western Germany [19], some 140 km north of our collection site and also adjacent to the A5 motorway. In our study, the adult was caught near a truck-railway transshipment station, a destination of numerous trucks from southern Europe. The vector capacity of this bird-biting species is unknown.

In our study, the mosquito species were caught using suction traps for adults. As the traps were operated for two months only and the climatic conditions in southern Germany in 2011 were relatively bad for mosquitoes, the trapping of the three species is probably due to a combination of a highly sensitive trapping system and the selection of suitable trap positions. The BG-Sentinel trap has been shown in a variety of studies to be superior to other traps for collecting some exotic Aedes species, and in combination with CO_2, it is at least as efficient as other CO_2 traps for the collection of other culicid species [e.g. 20]. Due to our particular collection regimen, however, the contribution of CO_2 to the collection success is not clear. In addition to the trap efficacy, the selection of the trap position is an important factor influencing the collection result. We carefully inspected possible sites for the release of imported mosquitoes from vehicles entering Germany and placed the traps within flight distance (a few hundred metres) at sites on non-public premises protected from wind, sun and rain.

In summary, our study provides evidence of a second introduction of *Ae. albopictus* into Germany and the persistence of *Ae. j. japonicus* in south-western Germany. Our findings confirm that the German Upper Rhine Valley is a suitable area for the introduction and establishment of invasive species [3], further highlighted by our finding of an adult *Cs. longiareolata*. It is characterised by a very mild climate likely to offer suitable climatic conditions for the establishment of thermophilic exotic mosquito species. Our results call for further search for mosquito adults and immature stages, particularly of *Ae. albopictus*, in 2012 along the major traffic axes in south-western Germany through intensified monitoring. Should additional adults or even immature stages of *Ae. albopictus* be found, control measures such as insecticiding, reduction of potential breeding sites and public health education should immediately be implemented. The further spread of *Ae. j. japonicus* in southern Germany can probably only be prevented by extensive public education on the developmental demands of this species and appeals to the public to avoid producing artificial man-made breeding sites.

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Influenza A(H1N1)pdm09 vaccination policies and coverage in Europe

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In August 2010 the Vaccine European New Integrated Collaboration Effort (VENICE) project conducted a survey to collect information on influenza A(H1N1) pdm09 vaccination policies and vaccination coverage in the European Union (EU), Norway and Iceland. Of 29 responding countries, 26 organised national pandemic influenza vaccination and one country had recommendations for vaccination but did not have a specific programme. Of the 27 countries with vaccine recommendations, all recommended it for healthcare workers and pregnant women. Twelve countries recommended vaccine for all ages. Six and three countries had recommendations for specific age groups in children and in adults, countries for specific adult age groups. Most countries recommended vaccine for those in new risk groups identified early in the pandemic such as morbid obese and people with neurologic diseases. Two thirds of countries started their vaccination campaigns within a four week period after week 40/2009. The reported vaccination coverage varied between countries from 0.4% to 59% for the entire population (22 countries); 3% to 68% for healthcare workers (13 countries); 0% to 58% for pregnant women (12 countries); 0.2% to 74% for children (12 countries). Most countries identified similar target groups for pandemic vaccine, but substantial variability in vaccination coverage was seen. The recommendations were in accordance with policy advice from the EU Health Security Committee and the World Health Organization.

Introduction

In late April 2009, the World Health Organization (WHO) received reports of sustained person-to-person transmission of infection with a previously unreported influenza A(H1N1) virus in Mexico and the United States. The virus quickly spread to multiple countries in Europe, the Americas and the Far East. After transmission had been established on more than one continent, the WHO declared a pandemic on 11 June 2009 [1].

Based on the epidemiologic characterisation of the groups most affected during the early phase of the pandemic, WHO's Strategic Advisory Group of Experts (SAGE) and the European Union (EU) Health Security Committee (HSC) [2] issued similar recommendations on target groups for pandemic vaccination.

The WHO SAGE on immunisation recommended that 'All countries should immunise their healthcare workers as a first priority to protect the essential health infrastructure.' The committee also suggested that countries should consider prioritising vaccination of other groups in the following order, but noted that countries needed to determine their order of priority based on country-specific conditions: (i) pregnant women, (ii) individuals aged › six months with one of several chronic medical conditions, including asthma and morbid obesity (body mass index (BMI) ≥40 kg/m^2), (iii) healthy young adults (aged ›15 years and ‹49 years), (iv) healthy children, (v) healthy adults aged ›49 years and ‹65 years, (vi) healthy adults aged 65 years and older [3].

The representatives of the EU Members States (MS) in the HSC with the scientific support of the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) recommended three priority groups to be vaccinated first, if limited amounts of vaccine were available: (i) all persons ≥ six months with underlying chronic conditions increasing

the risk for severe disease, starting with the ones who have a severe underlying condition (e.g. severe asthma, unstable coronary heart disease, uncompensated heart failure), (ii) pregnant women, and (iii) healthcare workers (HCWs). After the priority groups had been vaccinated, the vaccination could continue according to national recommendations [4-6].

The HSC priority policy focussed on vaccination of priority groups. Based on estimates of the proportion of those under 65 years of age in risk groups (8.5%) and estimation of the proportion of the population in HCWs (3%) it was estimated that approximately 12% of the population should be vaccinated [7,8].

Prior to the 2009 pandemic almost all EU/European Economic Area (EEA) countries had included pandemic vaccine as a component of their plans for mitigation or control [9]. Rapid central authorisation had been planned for using a 'mock-up vaccine' strategy [10] and following vaccine authorisation by EMA and the Commission (or by corresponding national regulatory bodies) vaccination plans were implemented across the majority of countries.

To document the policies and enactment of the pandemic vaccination, ECDC requested the Vaccine European New Integrated Collaboration Effort (VENICE) consortium to undertake a survey among MS with the aim of describing the policies, practises and performance of the national programmes. The specific objectives of this paper are to describe the vaccination policies including specific groups targeted for vaccination and to present obtained estimated vaccination coverage rates of pandemic vaccine among EU/EEA countries during the 2009 pandemic.

Methods

The VENICE project undertook a web-based survey covering 27 EU MS and two EEA countries (Norway and Iceland) (hereafter- VENICE participating countries). The WHO Regional Office for Europe was invited to collaborate in order to avoid redundant surveys in the EU. All WHO European region countries were invited to participate in the one survey. A joint report from WHO and VENICE will be presented separately on these compiled data. This paper includes data from the EU MS, Norway and Iceland only.

The survey was conducted in August 2010. The questionnaire was placed on the VENICE website platform and was available for all assigned representatives from each VENICE participating country [11]. Non-responders were followed up with two reminders in early September. Data were gathered through national 'gatekeepers' (nominated vaccination experts with delegated responsibility to enact VENICE surveys for their country). Gatekeepers were particularly asked to collaborate with the national members of the EU HSC, influenza section in order to validate survey responses. Data were collected using a standardised questionnaire seeking information on population groups recommended for pandemic vaccine (age groups, chronic diseases and underlying conditions, occupation or other social groups), programme funding, logistics associated with the national programmes (doses of vaccine purchased and distributed in each country), vaccination coverage rates achieved and factors influencing vaccination coverage. Countries were also asked to report the order of priority in which target groups were being offered vaccination. Due to different dates of vaccination initiation in MS, arbitrary country-specific phases of the 2009 pandemic were created: early, middle and late phase, not reflecting identical calendar time periods. This paper describes part of collected data on vaccination policy, recommendations and vaccination coverage results. We have also included data obtained from ECDC summarising the vaccines available for use in Europe during the pandemic as background information.

Results

Vaccination policy and recommendations

All 29 EU/EEA countries participating in the VENICE project responded to the survey (data from the United Kingdom (UK) were provided only for England). Twenty-six countries reported implementing pandemic vaccination programmes. Latvia and Poland reported they did not have such programmes and Bulgaria reported it had vaccination recommendations but did not enact its programme because vaccine was not available until after the pandemic subsided. Twenty-five countries published an official document (policy, guidelines) on vaccination recommendations for their population. Nearly all countries with programmes had the same policy across the country, only Sweden reported having different regional strategies.

Vaccines used within the European Union/ European Economic Area countries

Vaccines available to EU/EEA MS included eight vaccines, three of which were centrally authorised by the European Commission (Focetria, Pandemrix, Celvapan) with additional (n=5) vaccines receiving national authorisation. All vaccines (all inactivated) were based on the initial isolate of the new pandemic virus strain, A/California/7/2009(H1N1). An overview of the vaccines used is detailed in Table 1 and describes the vaccine product description, the culture medium, haemagglutinin content, adjuvant emulsion and number of doses, as recommended in December 2009.

Age groups

Twelve countries recommended vaccine for individuals of all ages. Six countries had recommendations for varying age groups in children, and three countries recommended pandemic vaccine to varying adult age groups (Table 2).

Established and new risk groups

Chronic diseases and conditions (Table 2) were considered as indications for pandemic vaccine. All countries

with recommendations for vaccination with pandemic vaccine (n=27) recommended vaccine for those with chronic respiratory, cardiovascular or renal diseases; 26 countries recommended vaccination of those with neurologic and metabolic disorders; 25 countries recommended pandemic vaccine for those with chronic liver diseases or immunosuppression due to disease or treatment; however only 16 recommended vaccination for individuals with morbid obesity (defined as body mass index (BMI) ≥40 kg/m²).

Pregnant women

All 27 countries recommended vaccine to pregnant women: 25 countries to all pregnant women. Bulgaria and Romania recommended vaccine only for those pregnant women with an additional risk condition. Twelve countries recommended pandemic vaccine at any stage in pregnancy and 14 during either the second or third trimester. Twelve countries also recommended vaccine for postpartum women if not already vaccinated (Table 2).

Occupational groups

All 27 countries recommended HCWs should be offered vaccine (Table 2). Sixteen countries recommended vaccine to all HCWs and 11 to some (those having close contact with patients, or for staff with no contact with patients, but contact with potentially contaminated material e.g. in laboratories).Vaccine was recommended for some other occupational essential service groups: police in 12 countries, military in 11 countries, firemen in nine countries and staff in the educational sector in seven countries. In Luxembourg vaccination was recommended only to educational staff working with very young children.

Other social groups

Twelve countries followed a 'cocooning strategy' recommending vaccination of household contacts of children of six months of age or under (who were too young to be vaccinated) and nine countries recommended vaccination of household contacts of at risk individu-

Table 1

Overview of vaccines against influenza A(H1N1)pdm09 available in the European Union in December 2009

Name, producer	Product description	Culture medium	Haemagglutinin content	Adjuvant emulsion	Number of doses
Celvapan, Baxter	Whole virion, wild-type A/ California/7/2009 (H1N1), inactivated	Vero cell- derived	7.5 µg	None	All › 6 months 2 x 0.5 mL
Pandemrix, GSK	Split-virion, reassortant A/ California/7/2009 (H1N1)-like strain, inactivated, adjuvanted	Egg-derived	3.75 µg (per full dose)	AS03	Adults, adolescents and children ≥ 10 years 1 x 0.5 mL
			1.87 µg (per half dose)		Children 6 months – 9 years 2 x 0.25 mL
Focetria, Novartis	Surface-antigens (haemagglutinin and neuraminidase), reassortant, A/ California/7/2009 (H1N1)-like strain, inactivated, adjuvanted	Egg-derived	7.5 µg	MF59C.1	Adults, adolescents and children ≥ 9 years 1 x 0.5 mL
					Children 6 months – 8 years 2 x 0.5 mL
Fluval P, Omnivest	Whole virion, reassortant A/ California/7/2009 (H1N1)-like strain, inactivated, adjuvanted	Egg-derived	6 µg (per full dose)	Aluminium phosphate	Adults and adolescents › 12 years 1 x 0.5 mL
			3 µg (per half dose)		Children 12 months –12 years 1 x 0.25 mL
Panenza, Sanofi Pasteur	Split-virion, reassortant A/ California/7/2009 (H1N1)-like strain, inactivated	Egg-derived	15 µg (per full dose)	None	Adults, adolescents and children › 8 years 1 x 0.5 mL Elderly › 60 years and children 3 – 8 years 2 x 0.5 mL
			7.5 µg (per half dose)		Children 6 – 35 months 2 x 0.25 mL
Celtura, Novartis	Surface-antigens (haemagglutinin and neuraminidase), reassortant, A/ California/7/2009 (H1N1)-like strain, inactivated, adjuvanted	MDCK cell-derived	3.75 µg	MF59C.1	Adults 18 – 40 years, children 3 – 17 years 1 x 0.25 mL
					Adults › 40 years 2 x 0.25 mL
PanvaxH1N1, CSL	Split-virion, reassortant A/ California/7/2009 (H1N1)-like strain, inactivated	Egg-derived	15 µg	None	Adults, adolescents and children › 9 years 1 x 0.5 mL
CANTGRIP, Cantacuzino	Split-virion, reassortant A/ California/7/2009 (H1N1)-like strain, inactivated	Egg-derived	15 µg	None	Adults ≥ 18 years 1 x 0.5 mL

Number of doses is as recommended in December 2009 but in some countries the number of doses and dosage changed over time.
Source: European Centre for Disease Prevention and Control (ECDC) data.

als. Vaccination was also recommended for residents of long-term care facilities in 14 countries (Table 2).

Implementation of vaccination - prioritisation by groups and entire population

Of the 26 countries with pandemic vaccination programmes that reported when they started and finished (not all reported finish date) immunisation, the first began in week 40 of 2009 (week starting 28 September 2009) and by week 44 (end of week 1 November 2009) more than two thirds of the countries had commenced their programmes. However there was a long 'tail' with some countries not able to start until near the end of 2009 (Figure).

Of the 27 countries with vaccination recommendations, vaccine was reported to be prioritised within recommended groups in 22 countries: Austria, Belgium, Cyprus, Czech Republic, Denmark, England, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Norway, Portugal, Romania, Slovakia, Slovenia and Sweden. In contrast Bulgaria, Estonia, Lithuania, the Netherlands and Spain did not report prioritising any group.

Among the 22 countries that prioritised there was considerable standardisation (Table 3). In the early phase of the pandemic vaccination programme, most countries prioritised HCWs, individuals with chronic diseases or underlying conditions and pregnant women. Some countries also implemented a 'cocooning strategy' approach during this phase. In the middle phase, nine countries prioritised vaccination of the population according to age groups; by the late phase vaccination was offered to the entire population in seven countries.

Vaccination monitoring and coverage

Twenty-two countries provided population-wide data on pandemic influenza vaccination coverage (range 0.4% to 59%). The highest reported population vaccination coverage was reached in the Netherlands and the Nordic countries (Denmark did not report total population coverage) (range 30% to 59%).

Table 2

Population groups recommended for pandemic influenza vaccine in the European Union Member States and European Economic Area countries that had vaccination recommendations during the 2009 pandemic, influenza A(H1N1)pdm09 vaccination survey, August 2010 (n=27 countries)

Population groups	Number of countries
Age[a]	
Children	
All (≥6 months – <18 years)	13
Some age groups[b]	6
Only in risk groups/underlying conditions	7
Adults	
All (≥18 years)	13
Some age groups[c]	3
Only in risk groups/underlying conditions	10
All ages	
All age groups	12
Chronic diseases and underlying conditions	
Respiratory	27
Cardiovascular	27
Renal	27
Neurological /neuromuscular	26
Metabolic (including diabetes)	26
Hepatic	25
Immunosuppression due to disease or treatment	25
Any condition compromising respiratory function	21
Hematologic	18
Haemoglobinophathies	16
Morbid obesity (Body Mass Index >40 kg/m²)	16
Pregnant women	27
All	25
Only with additional risk condition	2
Any trimester[a]	12
Either second or third trimester	14
Postpartum if not vaccinated	12
Occupations	
Healthcare	27
Police	12
Military	11
Firefighters	9
Border control	7
Educational	7
Public transport	6
Energy	7
Finance /banking	3
Other populations	
Close contacts (cocooning strategy)[d] of:	
Infants ≤6 months of age	12
Individuals in risk groups	9
Residents of long term care facilities	14

[a] One country did not answer this question.

[b] Some children (n=6): >1 year–2 years (Estonia); 6 months–5 years (England); 6 months–4 years (Netherlands); 12 months–18 years (Hungary); 6 months–12 years (Portugal); >16–17 years (Romania).

[c] Some adults: >60 years (Netherlands); 18–27 years (Italy); ≥65 years (England).

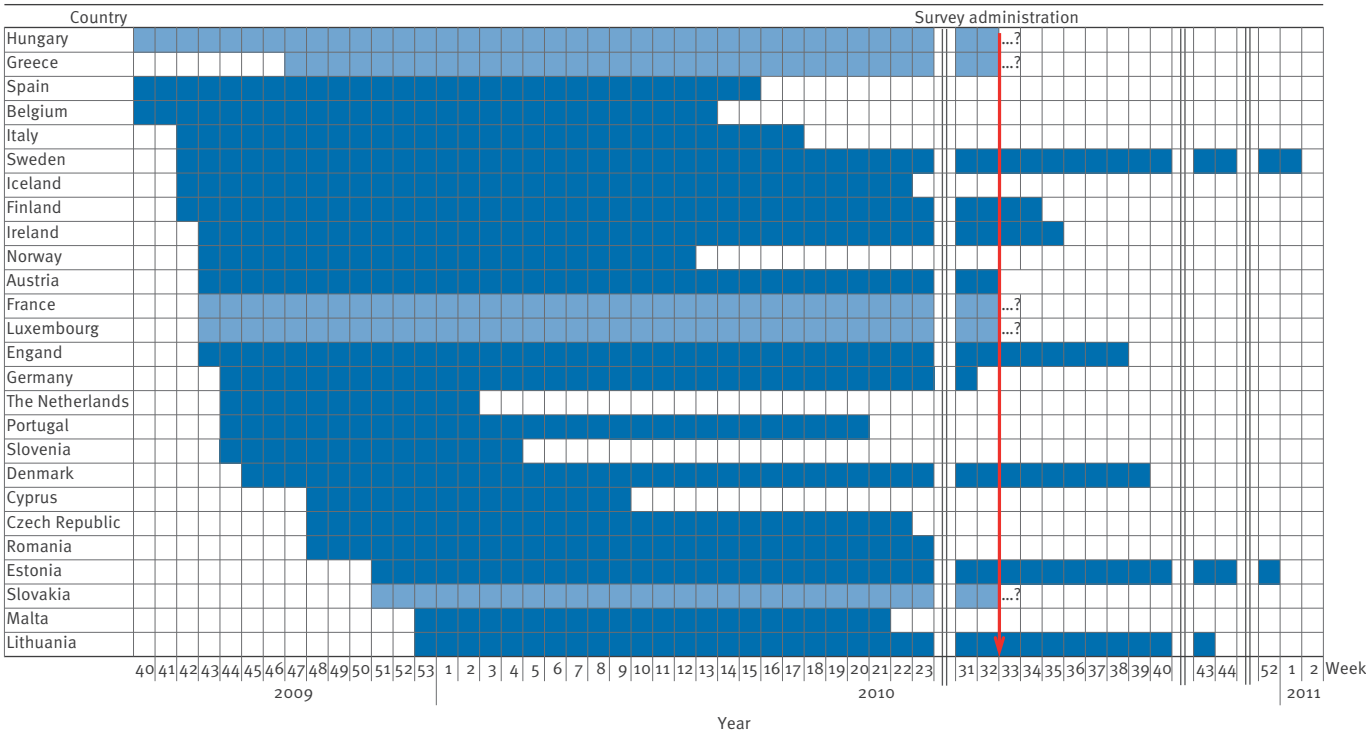
[d] Definition and rationale for "cocooning": Infants ≤6 months of age having little if any immunity to influenza if their mothers were not vaccinated during pregnancy are at higher risk of influenza-related complications. To ensure infant protection, immediate household contacts (representing its cocoon) should be vaccinated against influenza A(H1N1)pdm09 so they will not transmit the virus to the infant. The same concept applies to individuals with some chronic diseases (e.g., patients with hematopoietic stem cell transplants) since the immune response to the vaccine may be inadequate, vaccination of contacts (household members, healthcare workers, and other individuals) is recommended.

Vaccination coverage data for HCWs were available in 13 countries (range 3% to 68%), with the highest coverage reported in the Netherlands, Romania and Hungary (range 50% to 68%).

Vaccination coverage in pregnant women and children (age groups targeted among children varied by country) was provided by 12 countries (range 0% to 58% and 0.2% to 74% respectively). The highest vaccination coverage among pregnant women was reported by the Netherlands and Ireland. The highest coverage among children among those providing data was achieved in the Netherlands, Finland and Norway (Table 4).

Figure

Vaccination programmes for pandemic influenza vaccine in the European Union Member States and European Economic Area countries that organised national pandemic influenza vaccination during the 2009 pandemic, influenza A(H1N1) pdm09 vaccination survey, August 2010 (n=26 countries)



The figure covers the period from 28 Sep 2009 to 9 Jan 2011.
Due to lack of space in the figure there are breaks between weeks in the year 2010.

Table 3

Pandemic vaccination of priority groups and entire population in the European Union Member States and European Economic Area countries that prioritised vaccination within recommended groups during the 2009 pandemic, influenza A(H1N1)pdm09 vaccination survey, August 2010 (n=22 countries)

	Initial priority (Number of countries)	Middle priority (Number of countries)	Late priority (Number of countries)	Total (Number of countries)
Healthcare workers	21	1	0	**22**
Chronic diseases and underlying conditions	14	7	1	**22**
Pregnant women	14	7	0	**21**
Cocooning strategy	5	4	1	**10**
Age groups	2	9	2	**13**
Entire population	1[a]	3	7	**11**

[a] Vaccination was recommended to priority groups, but nobody was excluded if individuals wanted to be vaccinated.

Data on vaccination coverage among persons ≥ six months with chronic diseases and underlying conditions (risk groups varied between countries) were provided by nine countries (range 8% to 72%) with the highest coverage in the Netherlands and Ireland.

Twenty-four of the 26 EU/EEA countries with pandemic vaccination programmes measured pandemic vaccination coverage using administrative methods. Three of these countries (France, Germany and Ireland) also used surveys to estimate vaccination coverage. Although some countries were unable to provide coverage data at the time of the survey and reported that they may be able to report it at a future date.

Potential factors influencing vaccination coverage

Countries reported that a number of public perception factors may have negatively influenced vaccination coverage rates. These included varying levels of

Table 4

Pandemic vaccination coverage among specific groups of population by countries in European Union and European Economic Area during the 2009 pandemic, influenza A(H1N1)pdm09 vaccination survey, August 2010 (n=22 countries)

	Vaccination coverage (%)				
Countries	Overall[a] (n=22)	≥ 6 months of age with chronic diseases and underlying conditions (n=9)	Pregnant women[b] (n=12)	Children[c] (n=12)	Healthcare workers[d] (n=13)
Austria	3	NA	NA	NA	NA
Cyprus	3	NA	NA	NA	NA
Czech Republic	0.6	NA	0	NA	7
Denmark	NA	20	NA	NA	NA
England	NA	38	15	24	40
Estonia	3	21	5	NA	21
Finland	50	NA	NA	74	NA
France	8	NA	23	10	NA
Germany[e]	8	12	9	NA	16
Greece	3	NA	NA	NA	NA
Hungary	27	NA	9	NA	68
Iceland	46	NA	NA	45	NA
Ireland	23	48	32	46	31
Italy	4	13	12	0.3	15
Luxembourg	6	8	NA	7	NA
Malta	23	NA	NA	NA	40
Netherlands	30	72	58	74	50
Norway	45	NA	NA	55	NA
Portugal	6	NA	18	15	35
Romania	9	NA	NA	NA	51
Spain	27	24	9	NA	12
Sweden[f]	59	NA	NA	NA	NA
Slovenia	5	NA	1	1	NA
Slovakia	0.4	NA	NA	0.2	3

[a] Some countries recommended pandemic vaccine for some population groups but calculated overall vaccination coverage.

[b] Pregnant women: all countries that provided vaccination coverage recommended vaccination to all pregnant women (with or without risk indication).

[c] Groups for which vaccination coverage were measured: France, Iceland, Italy, Norway and Slovenia (n=5), ≥6months–<18years of age; England, ≥6 months–<5 years of age; Finland, ≤15 years of age; Ireland, >6months–<15years or age; Luxembourg, at risk; Netherlands, ≥6 months–4years of age; Portugal, ≥6 months–12 years of age.

[d] Healthcare workers: Czech Republic, England, Malta, Netherlands, Portugal (n=5) recommended pandemic vaccine to only healthcare workers with close contact with patients; Estonia recommended for healthcare workers with close contact with patient and with no contact with patients, but contact with potentially contaminated material; Hungary, Malta, Romania, Spain, Sweden and Slovakia (n=6) recommended pandemic vaccine to all healthcare workers.

[e] Data for age groups ≥14 years.

[f] In Sweden - more recent data reported higher vaccination coverage from four regions, suggesting that vaccination coverage may have been higher than reported at time of survey. The vaccination coverage was on average 67 % for children and adolescents under the age of 20 and 51% for adults in four regions (with immunisation registries) in Sweden. These four regions have around 5.3 million inhabitants (the whole of Sweden is 9.1 million), which corresponds roughly to 57 % of the Swedish population [12].

NA: Data not available or not provided for this specific population group at the time of survey.

Vaccination coverage figures in this table were rounded.

concern about vaccine safety (n=13), confidence in the need for the vaccine (n=23), concerns about thiomersal (n=12), or adjuvant in the vaccine (n=18), accelerated licensing process (n=16). Comparison with VENICE surveys for seasonal influenza showed that on the whole countries where there was usually little use of seasonal influenza vaccines vaccinated fewer people with pandemic vaccine and their pandemic vaccine programmes started later. However not all countries that used seasonal influenza vaccines routinely for risk groups immunised many people in the pandemic and there were a number of countries that experienced particular difficulties which usually immunised substantial proportions of their older population [13].

Discussion

These results demonstrate that European countries' recommendations and implementation of their pandemic vaccination programmes broadly followed both the EU/WHO recommendations issued during the summer of 2009 [3,5]. A large majority of countries recommended vaccination of those ≥ six months of age with chronic conditions, pregnant women and HCWs. What differences there were between the EU and SAGE positions probably reflected that the former represented a consensus between Ministries of Health, and therefore was a pragmatic choice based partially on the amounts of vaccines countries had ordered. In contrast the SAGE recommendation was a less constrained expert opinion. A number of EU countries which had ordered larger amounts of vaccine went on beyond the HSC recommendation to other population groups, age-groups, or entire populations. This was done with the stepwise approach as recommended by the WHO [3].

As the pandemic spread, a number of new clinical risk group categories emerged, and recommendations for vaccination were adjusted by a number of countries. However, early in the pandemic, severe disease was reported among this group and approximately half the countries then included people with morbid obesity in their recommended groups [14]. Subsequent published studies have reported morbid obesity to be an independent risk factor for severe influenza associated with increased odds of death [15]. Pregnant women were another group added to those recommended for vaccination during the pandemic prompted by American evidence of a severe influenza among pregnant and postpartum women early in the pandemic [16]. An additional benefit of vaccination of the mother during pregnancy is that it directly and indirectly protects infants during their vulnerable first months of life when they cannot be immunised [17-21]. Countries recommending vaccination of pregnant women increased from 10 in 2008-09 to all 27 countries in the pandemic (two countries recommended vaccination only for pregnant women with other established risk conditions) [13].

Children posed a difficulty for policy makers. At high risk of infection, they had the highest hospitalisation and age-specific attack rates. Some children (e.g. less than two years of age or with chronic disease) were at particular risk of severe complications. Children spread influenza easily, facilitated by poorer respiratory etiquette and close contact with each other and family members [22,23]. Additionally, they excrete the virus longer than adults [24]. Despite the fact that childhood vaccination was not recommended by the HSC, 19 MS recommended pandemic vaccination for children (Table 1) due to observed highest transmission of influenza A(H1N1)pdm09 virus among schoolchildren [4]. Some countries focussed on vaccinating the vulnerable very young children in particular [4,24]. In the previous seasonal VENICE survey conducted in 2008 only six countries recommended vaccine for children but in the pandemic 19 countries recommended this: 13 as part of the overall population, and six for specific age groups (age groups varied between countries) [13,25,26].

All countries with vaccination programmes recommended vaccinating HCWs with the same rationale as in any influenza season. Most countries recommended vaccinating all HCWs, but some only for staff with patient contact. It was also considered that protecting HCWs at risk of infection during the course of their work was important to maintain morale and defend essential health services during any influenza season [27-30]. This was particularly so during the pandemic when demand on health services was in places intense [31,32].

Many countries reported that the fact that the pandemic was less severe than anticipated in their planning proved to be a mixed blessing. The case for vaccination outside the risk groups was weakened in the view of the public and professionals who sometimes felt they had been promised something worse [33]. The fact that the pandemic severity could worsen at any point was true but not persuasive [34]. For example it meant that recommendations to vaccinate individuals working in essential services became irrelevant outside the health sector. European countries here showed pragmatism since although more than a third of the countries had recommendations to vaccinate essential service staff (11 and 12 countries recommended vaccination of the military and police respectively) most did not do so except as part of whole population policies [4].

A particular problem is how to measure success. It is tempting but misleading to use whole population coverage (Table 3) since a minority of countries aimed to vaccinate the entire population. Countries like the Netherlands, Ireland and England, which adopted a risk group approach, may have done equally well despite lower population coverage. The problem was that the vaccine strategies, protecting the vulnerable versus reducing transmission, were not stated explicitly by the MS. In comparison to presented European data, the estimated population coverage for the United States was 27% with a non-adjuvanted pandemic vaccine and 41% in Canada, with mostly adjuvanted vaccines [35,36]. This is lower than in Nordic countries and the

Netherlands, similar to Spain and Hungary and higher than in the remaining EU/EEA countries [37]. However this comparison should be interpreted with caution as different methods for vaccination coverage measurement were used.

The lack of efficient vaccination coverage assessment mechanisms that allowed measuring vaccination coverage in risk groups during the course of the pandemic prevented MS in accurate monitoring of these interventions. In that sense the systems in the United States were superior as they enabled the monitoring of concerns and problems with vaccination coverage and report these publicly and quickly [38]. No EU country produced such data in real time through some monitored attitudes during the pandemic [39]. Reliability of reported vaccination coverage data also depend on methods used to measure vaccination coverage data. All countries used administrative data and some also used surveys. However, administrative methods used varied between countries limiting the comparability of presently collected data. Comparison of vaccine coverage may be misleading also when different sources for numerators and denominators are used among countries. Comparisons are also difficult due to the different starting date of indication for different target groups. Population-based surveys are valuable tools to assess vaccination coverage rapidly and to obtain additional information such as reasons for vaccination or non-vaccination without causing additional administrative burden to the healthcare system. Additionally they provide an alternative method for validating data obtained from official monitoring sources. However, only three countries used this methodology to augment their administrative methods.

Some new vaccines were more immunogenic than anticipated so that for most vaccines only a single dose was required [40]. Also many older people possessed some immunity from exposure to a similar virus that had circulated before the 1960s [4,41]. However, the mild nature of the pandemic meant that demand and acceptance was less than expected in some countries and this was further complicated by allegations of excessive influence of pharmaceutical companies in policy making [42] and concerns about the safety of the vaccine.

This survey identified similarity across countries in groups most commonly recommended and prioritised for pandemic vaccine as well as marked variability in vaccination coverage rates. Multiple reasons for these discrepancies could be identified, related to the complexity of the communications, public perception and vaccine availability. The results from this survey also demonstrate that countries responded to and changed vaccination policy and recommendations in response to the pandemic, advice from expert groups and the changing epidemiology of the disease.

Based on the results of this survey more work is needed to see how recommendations (at national or international level) can be effectively translated into higher vaccination coverage.

Furthermore in order to improve influenza vaccination coverage countries have to strive to strengthen and/or implement the influenza vaccination coverage monitoring systems in place for most common population groups for whom vaccination is recommended (by age, chronic diseases, occupations including HCWs, pregnant women). In order to make comparison of vaccination coverage at EU/EEA level annual population based surveys conducted using the same or similar methodology may be useful [43,44].

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