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Effectiveness of seasonal 2012/13 vaccine in preventing laboratory-confirmed influenza infection in primary care in the United Kingdom: mid-season analysis 2012/13

J McMenamin (jim.mcmenamin@nhs.net)[1], N Andrews[2], C Robertson[1,3,4], D M Fleming[5], H Durnall[5], B von Wissmann[1], J Ellis[6], A Lackenby[6], S Cottrell[7], B Smyth[8], M Zambon[2], C Moore[7], J M Watson[2], R G Pebody[2]

1. Health Protection Scotland, Glasgow, United Kingdom
2. Health Protection Agency Health Protection Services – Colindale, London, United Kingdom
3. University of Strathclyde, Glasgow, United Kingdom
4. International Prevention Research Institute, Lyon, France
5. Royal College of General Practitioners Research and Surveillance Centre, Birmingham, United Kingdom
6. Health Protection Agency Microbiology Services – Colindale, London, United Kingdom
7. Public Health Wales, Cardiff, United Kingdom
8. Public Health Agency Northern Ireland, Belfast, United Kingdom

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The early experience of the United Kingdom (UK) is that influenza B has dominated the influenza 2012/13 season. Overall trivalent influenza vaccine (TIV) adjusted vaccine effectiveness (VE) against all laboratory-confirmed influenza in primary care was 51% (95% confidence interval (CI): 27% to 68%); TIV adjusted VE against influenza A alone or influenza B alone was 49% (95% CI: -2% to 75%) and 52% (95% CI: 23% to 70%) respectively. Vaccination remains the best protection against influenza.

Background

In common with many countries the United Kingdom (UK) experienced unusually late influenza activity in 2011/12, with activity peaking only in week 8 of 2012 [1] in a season dominated by influenza A(H3N2) and characterised by excess in all-cause mortality and the occurrence of influenza outbreaks in nursing home settings [2,3]. Trivalent seasonal influenza vaccine (TIV) provided only moderate initial protection against A(H3N2) infection in primary care in 2011/12 with subsequent significant intra-seasonal waning of protection [1]. Overall in 2011/12 TIV VE against confirmed A(H3N2) infection, adjusted for age, surveillance scheme and month was 23% (95% confidence interval (CI): -10% to 47%).

These results, coupled with virological data on emerging strain types, supported the rationale for the change to the World Health Organization (WHO) recommendation for composition of the TIV for the northern hemisphere season for 2012/13 [4] in which the A/California/7/2009 (H1N1)pdm09-like virus was retained, and the A(H3N2) vaccine strain was updated to an A/Victoria/361/2011 (H3N2)-like virus. Additionally, the B/Victoria lineage influenza B vaccine component was replaced with an influenza B strain of the B/Yamagata lineage (B/Wisconsin/1/2010-like virus). Compared to season 2011/12, the UK has experienced a relatively early influenza season in 2012/13, which has been dominated by influenza B circulation, but also with some A(H3N2) circulation [5]. The 2012/13 intra-seasonal estimation of vaccine uptake in individuals who are in clinical groups at increased clinical risk of complications [6] has indicated similar levels compared to the same time point in 2011/12 [5]. The UK has an established surveillance scheme to produce interim and end of season estimation of the effectiveness of the influenza vaccine programme [1,7] and this paper presents the interim evaluation of the effectiveness of the 2012/13 vaccine.

Estimation of influenza vaccine effectiveness

Study population and period

Data were derived from five primary care influenza sentinel surveillance schemes in England (two schemes), Northern Ireland, Scotland and Wales. Details of the Royal College of General Practitioners (RCGP), Health Protection Agency (HPA) Specialist Microbiology Network (SMN), Public Health Wales, Public Health Agency of Northern Ireland and Health Protection Scotland (HPS) swabbing schemes have been presented previously [1,7].

The study period ran from 1 October 2012 to 4 January 2013. Cases were defined, as previously [1,7], as persons presenting during the study period in a participating general practitioner (GP) practice with an acute influenza-like illness (ILI) who were swabbed and then tested positive for influenza A or influenza B. ILI was

defined as an individual presenting in primary care with an acute respiratory illness with either history of fever or documented temperature >38°C or complaint of feverishness [1,7]. Patients were swabbed as part of clinical care, with verbal consent. Controls were individuals presenting with ILI in the same period that were swabbed and tested negative for influenza.

A standardised questionnaire collecting demographic, clinical and epidemiological information from cases and controls including date of birth, sex, defined underlying clinical risk group [1,7], date of onset of respiratory illness, date of specimen and influenza vaccination status for 2012/13 with vaccination dates was completed by the patient's responsible GP at the time of swabbing.

Laboratory methods

Laboratory confirmation was undertaken using real-time polymerase chain reaction (RT-PCR) assays for circulating influenza A viruses, influenza B viruses and other respiratory viruses [8,9]. Samples in England were sent to the HPA Microbiology Services, Colindale (RCGP scheme) or one of the specialist HPA microbiology laboratories (SMN scheme). Samples in Wales were sent to the Public Health Wales Specialist Virology Centre and in Scotland to the West of Scotland Specialist Virology Centre (HPS scheme) for molecular testing. In Northern Ireland samples were sent to the Regional Virus Laboratory, Belfast. All participating UK laboratories are a designated WHO National Influenza Centre (NIC) and participate in WHO and UK quality assurance programmes. This aids RT-PCR assays to be comparable between laboratories.

Statistical methods

Persons with a date of onset between 1 October 2012 and 4 January 2013 were available for this analysis. If date of onset of symptoms was missing then the date the swab was taken was used to define the time of ILI. Persons were defined as vaccinated if the date of vaccination with the 2012/13 TIV was 14 or more days before onset of illness. Those in whom the period between vaccination and onset of illness was less than 14 days were excluded, as immunity is unknown. Patients were also excluded if the date of vaccination was missing, and samples with a delay greater than 29 days between onset of illness (where known) and sample collection were excluded as the sensitivity of the polymerase chain reaction (PCR) test reduces for long intervals between onset and sampling [10,11].

VE was estimated as 1-(odds ratio) using multivariable logistic regression models with influenza A or influenza B PCR results as outcomes and seasonal vaccination status as the linear predictor. In the analyses evaluating VE in preventing influenza A infection, samples positive for influenza B were excluded and vice versa. In the multivariable analysis the known confounders age (coded into five standard age groups, <5 years, 5–14 years, 15–44 years, 45–64 years and ≥65 years) and month of ILI onset (or swab taken if onset was unknown) were included as well as sex and surveillance scheme (HPS, RCGP, SMN, Northern Ireland, Wales). Effect modification by age and scheme was assessed by likelihood ratio tests.

All statistical analyses were carried out in Stata version 12 (StataCorp, College Station, Texas).

2012/13 influenza vaccine effectiveness

This report has information on 1,865 individuals from whom samples were collected during the study period. Of these, 957 samples were collected through the RCGP surveillance scheme, 293 through the SMN scheme, 511 through the HPS scheme, 41 through the Public Health Wales scheme and 63 in Northern Ireland. Table 1 shows the distribution and completeness of the baseline characteristics of the study participants according to whether they were cases or controls.

Those excluded from the study because of late swabbing, a time of less than 14 days between vaccination and onset of symptoms and missing information on vaccination are summarised in Table 2. There were therefore 1,324 persons (i.e. 121 influenza A cases and 1,203 controls) for whom data on both vaccination status and influenza A infection were available. Similarly, there were 1,580 persons (i.e. 377 influenza B cases and 1,203 controls) included in the estimation of trivalent vaccine for prevention of influenza B.

Vaccine effectiveness in prevention of influenza

Table 3 shows the number of samples positive and negative for influenza A, influenza B and the combined influenza A or B virus according to vaccination status. Crude and adjusted vaccine effectiveness are also shown.

The adjusted VE estimates (Table 3) were 49% (95% CI: -2% to 75%) for influenza A, 52% (95% CI: 23% to 70%) for influenza B and 51% (95% CI: 27% to 68) for influenza A and B combined. As seen in previous years, age and month of onset were associated with positivity and vaccination status and were therefore confounding variables. Risk group was missing for 158/1,865 (8.5%) and this variable was not included in the model as it was not significantly associated with swab positivity when added to the multivariable model and analyses in previous years had shown that this was not a confounding variable [1,7]. Sex and surveillance scheme were retained in the model but did not change the VE estimates. When looking at effect modification there was no evidence that VE varied by scheme (p=0.26) or age (p=0.50).

Discussion

The early experience of the influenza season in the UK [5] and in a number of European Union (EU) Member States, the United States (US) and Canada [12] presents an opportunity for the generation of interim

Table 1

Details for influenza A and B cases and controls originally considered for the mid 2012/13 season trivalent seasonal influenza vaccine effectiveness analysis, United Kingdom, 1 October 2012–4 January 2013 (n=1,865)

Variable	Controls n (%) (N=1,340)	Influenza B cases n (%) (N=399)	Influenza A cases n (%) (N=126)
Age group (years)			
<5	159 (12)	29 (7)	8 (6)
5–14	91 (7)	101 (25)	9 (7)
15–44	589 (44)	166 (42)	80 (63)
45–64	324 (24)	89 (22)	25 (20)
≥65	172 (13)	14 (4)	4 (3)
Missing	5 (0)	0 (0)	0 (0)
Sex			
Male	552 (41)	167 (42)	55 (44)
Female	774 (58)	223 (56)	70 (56)
Missing	14 (1)	9 (2)	1 (1)
Month of sample collection			
October	230 (17)	8 (2)	13 (10)
November	553 (41)	49 (12)	23 (18)
December	543 (41)	333 (83)	90 (71)
January	14 (1)	9 (2)	0 (0)
Surveillance scheme			
RCGP	638 (48)	271 (68)	48 (38)
SMN	216 (16)	48 (12)	29 (23)
HPS	424 (32)	40 (10)	47 (37)
Wales	22 (2)	18 (5)	1 (1)
Northern Ireland	40 (3)	22 (6)	1 (1)
Risk Group			
No	923 (69)	315 (79)	98 (78)
Yes	301 (22)	56 (14)	14 (11)
Missing	116 (9)	28 (7)	14 (11)
Interval between onset and sampling (days)			
0–1	148 (11)	31 (8)	19 (15)
2–4	495 (37)	202 (51)	63 (50)
5–7	325 (24)	92 (23)	25 (20)
8–14	197 (15)	23 (6)	9 (7)
15–29	59 (4)	7 (2)	1 (1)
≥29	21 (2)	6 (2)	1 (1)
Missing onset date	95 (7)	38 (10)	8 (6)
Vaccination status (TIV 2012/13)			
Unvaccinated	996 (74)	354 (89)	110 (87)
Vaccinated 0–13 days ago[a]	37 (3)	1 (0)	0 (0)
Vaccinated ≥14 days ago[a]	179 (13)	23 (6)	9 (7)
Vaccinated timing not known	47 (4)	6 (2)	2 (2)
Missing	81 (6)	15 (4)	5 (4)

HPS: Health Protection Scotland; RCGP: Royal College of General Practitioners' surveillance scheme; SMN: Health Protection Agency (HPA) Specialist Microbiology Network; TIV: trivalent seasonal influenza vaccine.

[a] This refers to the time interval between vaccination time and time of symptom onset or swab.

assessment of seasonal influenza vaccine effectiveness. This can be used to inform those countries yet to experience a significant season and to add to the evidence base around choice of vaccine composition for the northern hemisphere influenza season of 2013/14. Unlike the pattern in the current season in North America, where influenza activity has been dominated by influenza A(H3N2) followed by influenza B, the early influenza season experienced across the UK has been dominated by influenza B cases with noted homogeneity in this pattern of laboratory detection in each country (with the exception of the Scottish scheme in which influenza A(H3N2) cases appear to be in slight excess). Influenza B viruses from both the B/Yamagata

Table 2

Selecting participants with known symptom onset of influenza-like illness (n=1,865) for the 2012/13 trivalent seasonal vaccine effectiveness analysis, United Kingdom, 1 October 2012–4 January 2013

Participants	Controls	Influenza B cases	Influenza A cases	Total
With known date of symptom onset[a]	1,340	399	126	1,865
With interval from symptom onset to sampling >29 days	-21	-6	-1	-28
Missing vaccination history[b]	-80	-15	-4	-99
Vaccinated 0–13 days before symptom onset[c]	-36	-1	-0	-37
Remaining for vaccine effectiveness analysis	**1,203**	**377**	**121**	**1,701**

The '-' sign indicates that the respective participant numbers were excluded from the vaccine effectiveness analysis.

[a] When the symptom onset date was missing the date when the participant was swabbed was used.

[b] Numbers exclude the participants with missing vaccination history who additionally had an interval from symptom onset to sampling >29 days.

[c] Numbers exclude the participants vaccinated 0–13 days before symptom onset who additionally had an interval from symptom onset to sampling >29 days and a missing vaccination history.

Table 3

Number of cases versus controls for influenza A and/or B according to 2012/13 trivalent seasonal influenza vaccine vaccination status and vaccine effectiveness (crude and adjusted[a]) estimates, United Kingdom, 1 October 2012–4 January 2013

Type of influenza	Vaccination status	Cases/controls	Crude VE % (95% CI)	Adjusted[a] VE % (95% CI)
A and B	Unvaccinated	459/979	63 (47 to 74)	51 (27 to 68)
	Vaccinated	39/224		
A	Unvaccinated	110/979	56 (17 to 77)	49 (-2 to 75)
	Vaccinated	11/224		
B	Unvaccinated	349/979	65 (47 to 77)	52 (23 to 70)
	Vaccinated	28/224		

VE: Vaccine effectiveness.

[a] Adjusted for age-group, sex, month and surveillance scheme.

and B/Victoria lineages have co-circulated during the 2012/13 influenza season in the UK, with the majority of influenza B isolates antigenically characterised to date belonging to the B/Yamagata lineage [5].

This is the fifth season in which the UK pooled estimation of TIV VE has been undertaken [1,7]. The data quality for the pooled analysis even at this interim analysis stage is deemed high with few missing data field entries.

This observational study of interim influenza VE for TIV against laboratory-confirmed influenza infection in primary care in the UK 2012/13 winter season, which would appear at this juncture to be a medium intensity influenza season with influenza B the dominant circulating strain, has two key findings: reassuringly the northern hemisphere 2012/13 TIV appears to offer moderate protection against the circulating influenza B strain; the point estimate for the TIV VE against influenza A is based on smaller numbers and, though not statistically significant at this stage, suggests a similar moderate level of protection.

These UK interim results which are adjusted for age, sex and calendar month within the season are consistent with the crude TIV VE reported in recent weeks in Morbidity and Mortality Weekly Report (MMWR) from this season's US experience to date [13] in which their season has been dominated by influenza A(H3N2) and with the overall adjusted VE estimate reported from Canada [14]. Indeed the UK crude VE estimates prior to adjustment for age appear near identical to those in the US.

While the TIV VE for influenza B is statistically significant one should keep in mind that there is a reliance on a trivalent vaccine with only one influenza B component, which in any given influenza season may offer limited protection against another influenza B lineage not targeted by the vaccine [15]. The availability of quadrivalent seasonal influenza vaccines licensed for use in the EU [16] would mean that this can be potentially averted. Work needs to be undertaken to demonstrate whether introduction of these vaccines would be cost-effective.

In conclusion, this study undertaken mid-season provides good evidence that this season's TIV provides protection against laboratory-confirmed influenza B infection and more limited evidence of likely protection against laboratory-confirmed influenza A infection

in the patients attending their GP with influenza like illness in the UK. It is important to note that more precision in this estimate will be available at the end of the season, together with the ability to obtain age-stratified estimates. Vaccination with the seasonal influenza vaccine remains the best protection against influenza. The results are consistent with a protective benefit from seasonal influenza vaccine. Within the UK and beyond, particularly in those countries who are either still early in their influenza season or who have evidence of continuing influenza transmission, it is important to stress that it is not too late to be vaccinated this season and individuals in clinical groups eligible for vaccination who have yet to be vaccinated should be encouraged to get vaccinated.

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Conflicts of interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that DM Fleming has received funding to attend influenza related meetings and has received consultancy fees from influenza vaccine manufacturers who might have an interest in the submitted work in the previous 3 years. In addition, the Virus Reference Department of the Health Protection Agency receives funding from a variety of vaccine manufacturers who might have an interest in the submitted work. All other authors declare they have no conflicts of interest.

Authors' contributions

Jim McMenamin led the writing of the rapid communication. All authors provided contribution to the rapid communication and approved the final version. Nick Andrews and Chris Robertson undertook the statistical analysis on which the rapid communication is based. Richard Pebody, Nick Andrews, Douglas Fleming, John Watson, Jim McMenamin and Chris Robertson were involved in the original methodological design but all other authors have had a role in modification of this design over the years.

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Interim estimates of influenza vaccine effectiveness in 2012/13 from Canada's sentinel surveillance network, January 2013

D M Skowronski (danuta.skowronski@bccdc.ca)[1,2], N Z Janjua[1,2], G De Serres[3,4,5], J A Dickinson[6], A-L Winter[7], S M Mahmud[8,9], S Sabaiduc[1], J B Gubbay[7,10], H Charest[3], M Petric[1,2], K Fonseca[6,11], P Van Caeseele[12], T L Kwindt[1,2], M Krajden[1,2], A Eshaghi[7], Y Li[9,13]

1. British Columbia Centre for Disease Control, Vancouver, Canada
2. University of British Columbia, Vancouver, Canada
3. Institut National de Santé Publique du Québec (National Institute of Health of Quebec), Québec, Canada
4. Laval University, Quebec, Canada
5. Centre Hospitalier Universitaire de Québec (University Hospital Centre of Quebec), Québec, Canada
6. University of Calgary, Calgary, Canada
7. Public Health Ontario, Toronto, Canada
8. Winnipeg Regional Health Authority, Winnipeg, Canada
9. University of Manitoba, Winnipeg, Canada
10. University of Toronto, Toronto, Canada
11. Alberta Provincial Laboratory, Calgary, Canada
12. Cadham Provincial Laboratory, Winnipeg, Canada
13. National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

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The 2012/13 influenza season in Canada has been characterised to date by early and moderately severe activity, dominated (90%) by the A(H3N2) subtype. Vaccine effectiveness (VE) was assessed in January 2013 by Canada's sentinel surveillance network using a test-negative case–control design. Interim adjusted-VE against medically attended laboratory-confirmed influenza A(H3N2) infection was 45% (95% CI: 13–66). Influenza A(H3N2) viruses in Canada are similar to the vaccine, based on haemagglutination inhibition; however, antigenic site mutations are described in the haemagglutinin gene.

Background

The 2012/13 influenza season in North America has shown moderately severe activity, spiking over the December/January holiday period, with influenza A(H3N2) viruses predominating among typed/subtyped viruses to date in both Canada (about 90%) and the United States (US) (about 70%) [1,2].

The updated 2012/13 A(H3N2) reference strain recommended by the World Health Organization as vaccine component for the northern hemisphere (A/Victoria/361/2011-like) is antigenically distinct from that recommended for the previous season (A/Perth/16/2009-like) [3], with 11 amino acid (AA) residue differences at antigenic sites of the haemagglutinin (HA) surface protein [4].

Vaccine effectiveness (VE) in Canada was assessed by the country's sentinel surveillance network in January 2013. Here we report the interim 2012/13 VE estimates against the dominant circulating influenza A(H3N2) subtype in the context of antigenic and genetic characterisation of circulating strains.

Estimating influenza vaccine effectiveness

As previously described [5-11], a test-negative case–control design was used to estimate VE, whereby a patient presenting with influenza-like illness (ILI) testing positive for influenza virus was considered a case and a person testing negative was considered a control.

Several hundred community-based practitioners in sentinel surveillance sites across participating provinces (British Columbia, Alberta, Manitoba, Ontario and Quebec) may offer nasal or nasopharyngeal swabbing to any patient presenting within seven days of symptom onset of ILI – defined as acute onset of respiratory illness with fever and cough and one or more of the following: sore throat, arthralgia, myalgia or prostration.

The VE analysis period included specimens collected from 1 November 2012 (week 44: 28 October 2012–3 November 2012) to 23 January 2013 (week 4: 20–26 January 2013), taking into account onset of influenza activity (Figure 1) and an immunisation campaign that started in October. Epidemiological information was obtained from consenting patients or their parents/guardians using a standard questionnaire at the time of specimen collection, before testing. Ethics review boards in each participating province approved this study.

Specimens were tested for influenza viruses A (to subtype level) and B at provincial reference laboratories by real-time reverse-transcription polymerase chain reaction according to provincial protocols [4,11]. Odds ratios (OR) for influenza vaccination among cases versus controls were estimated by multivariable logistic regression. VE against medically attended laboratory-confirmed influenza was calculated as [1 – OR] × 100. Patients for whom the timing of vaccination was unknown or was less than two weeks before symptom onset were excluded from the primary VE analysis but explored in sensitivity analyses. Those with unknown comorbidity were included and further explored in sensitivity analyses.

Genetic characterisation of sentinel influenza A(H3N2) viruses

Sequencing of the HA1 gene of a convenience sample (n=82) of available influenza A(H3N2) viruses, spanning the season so far but with emphasis on more recent activity, was undertaken for each province to identify AA substitutions within the 131 residues of antigenic sites A–E [11,12]. These were expressed as percentage identity and relatedness compared with the vaccine reference strain (A/Victoria/351/2011). Pairwise identities were calculated from alignments of translated protein sequences generated in Geneious Pro v4.8.5 using a MUSCLE multiple sequence alignment algorithm. The approximate likelihood method was used to generate the phylogenetic tree of aligned nucleotide sequences in Geneious Pro v4.8.5.

HA sequences from reference strains used in the phylogenetic analysis were obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

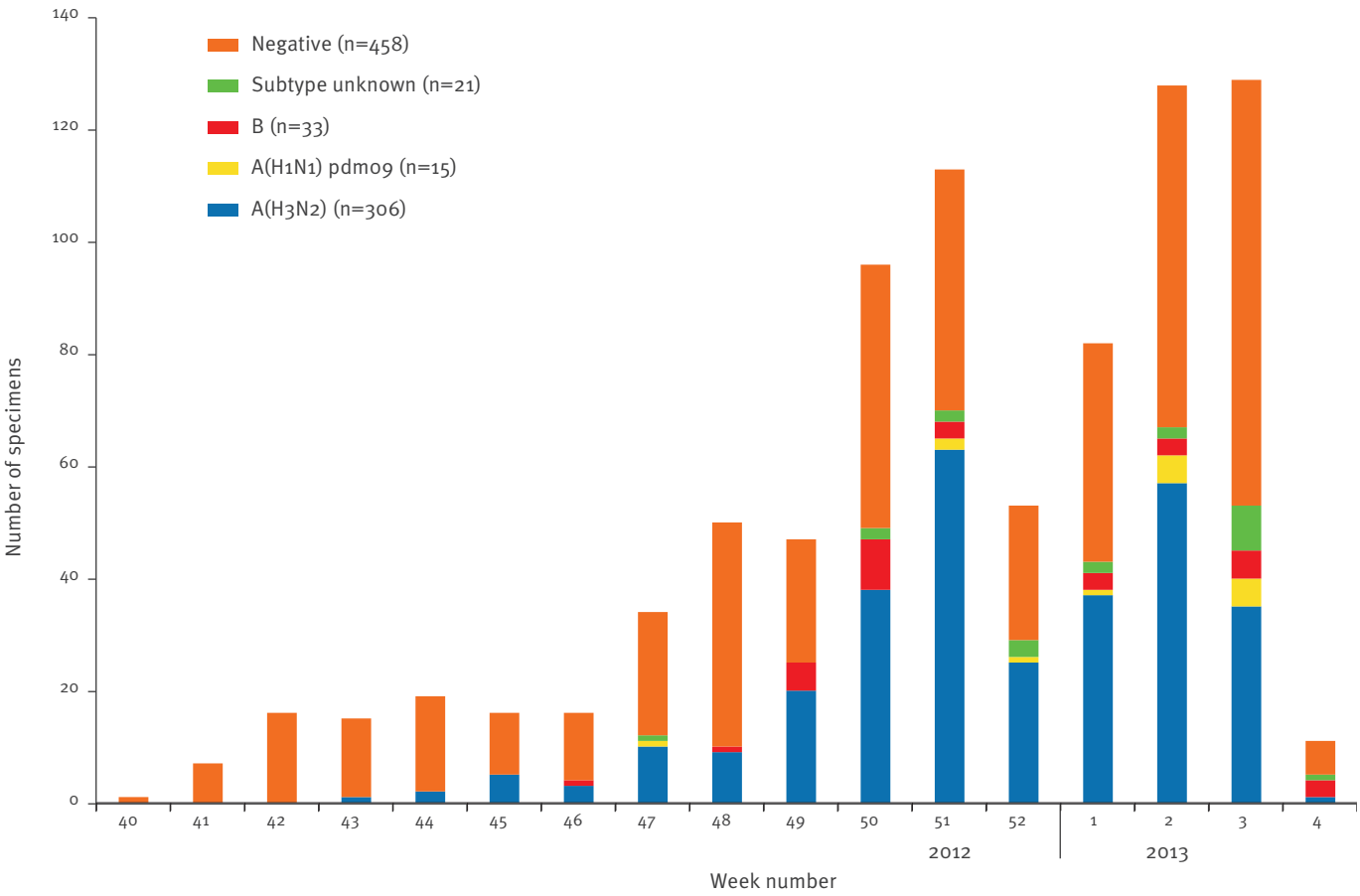
Interim estimates of influenza vaccine effectiveness

Participants

A total of 939 specimens were submitted from sentinel surveillance sites between 1 November 2012 and 23 January 2013. After exclusion criteria were applied (Figure 2), 739 participants contributed to overall VE analysis: their profile was similar to that seen in VE

Figure 1

Laboratory detection of influenza by week and virus subtype, Canada, 2012/13 sentinel surveillance system (n=833)



Of 999 nasal or nasopharyngeal specimens collected between 1 October 2012 (week 40: 30 September–6 October 2012) and 23 January 2013 (week 4: 20–26 January 2013), we excluded from the epidemic curve specimens from the following patients: those failing to meet the influenza-like illness (ILI) case definition or for whom it was unknown (n=24); those whose specimens were collected more than seven days after symptom onset or for whom the interval was unknown (n=132); those whose age was unknown (n=1) and those for whom influenza test results were unavailable or indeterminate (n=9). Specimens were included regardless of the patient's vaccination status or timing of vaccination; specimens from patients with unknown comorbidity were also included.

Table 1

Reference haemagglutinin sequences used in phylogenetic analysis, Canada, 2012/13 sentinel surveillance, January 2013

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI302231	Norway	2010-Dec-03	A/Norway/1330/2010	WHO National Influenza Centre	National Institute for Medical Research	
EPI302235	Iraq	2011-Jan-02	A/Iraq/7-FSS/2011	National Influenza Centre of Iraq	National Institute for Medical Research	
EPI287084	United States	2010-Jul-13	A/Alabama/05/2010	Centers for Disease Control and Prevention	National Institute for Medical Research	
EPI319241	United States	2010-Dec-30	A/Iowa/19/2010	WHO Collaborating Centre for Reference and Research on Influenza	National Institute for Medical Research	
EPI358885	Greece	2012-Feb-01	A/Athens GR/112/2012	Institut Pasteur Hellenique	National Institute for Medical Research	
EPI279881	Hong Kong	2010-Jul-05	A/Hong Kong/2121/2010	Government Virus Unit	National Institute for Medical Research	
EPI331093	Hong Kong	2011-May-19	A/Hong Kong/3969/2011	Government Virus Unit	National Institute for Medical Research	
EPI269899	Australia	2009-Jun-02	A/Victoria/210/2009	Victorian Infectious Diseases Reference Laboratory	National Institute for Medical Research	
EPI232453	Australia	2009-Jun-02	A/Victoria/208/2009	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	
EPI326139	Sweden	2011-Mar-28	A/Stockholm/18/2011	Swedish Institute for Infectious Disease Control	National Institute for Medical Research	
EPI407333	United States	2012-Nov-14	A/Colorado/20/2012	Colorado Department of Health Lab	Centers for Disease Control and Prevention	
EPI407120	United States	2012- Nov-06	A/Louisiana/11/2012	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention	
EPI407309	United States	2012- Nov-19	A/Kentucky/17/2012	Kentucky Division of Laboratory Services	Centers for Disease Control and Prevention	
EPI407282	United States	2012- Nov-02	A/Texas/83/2012	Houston Department of Health and Human Services	Centers for Disease Control and Prevention	
EPI406054	United States	2012-Nov-05	A/Massachusetts/07/2012	Massachusetts Department of Public Health	Centers for Disease Control and Prevention	
EPI407405	United States	2012-Nov-19	A/Idaho/24/2012	State of Idaho Bureau of Laboratories	Centers for Disease Control and Prevention	
EPI404911	United States	2012-Nov-12	A/Iowa/15/2012	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI407103	United States	2012-Nov-08	A/Ohio/92/2012	Ohio Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI408613	United States	2012-Nov-26	A/Nebraska/11/2012	Nebraska Public Health Lab	Centers for Disease Control and Prevention	
EPI404956	United States	2012-Nov-08	A/Indiana/162/2012	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI407109	United States	2012-Nov-11	A/Maryland/33/2012	Maryland Department of Health and Mental Hygiene	Centers for Disease Control and Prevention	
EPI405064	Sweden	2012-Nov-08	A/Stockholm/39/2012		Swedish Institute for Infectious Disease Control	
EPI408113	United Kingdom	2012-Nov-08	A/England/586/2012	Health Protection Agency	National Institute for Medical Research	
EPI406927	Spain	2012-Nov-30	A/Madrid/323/2012	Instituto de Salud Carlos III	Instituto de Salud Carlos III	Pozo F; Cuevas MT; Calderon A; Gonzalez-Esguevillas M; Molinero M; Moreno S. Casas I.
EPI408107	Denmark	2012-Nov-08	A/Denmark/71/2012	Statens Serum Institut	National Institute for Medical Research	
EPI407146	Japan	2012-Nov-16	A/Yamaguchi/31/2012	Yamaguchi Prefectural Institute of Public Health and Environment	National Institute of Infectious Diseases	Fujisaki Seiichiro; Kim Namhee; Aya Sato; Tashiro Masato; Odagiri Takato
EPI413220	Japan	2012-Nov-01	A/Sapporo/125/2012	Sapporo City Institute of Public Health	National Institute of Infectious Diseases	Fujisaki Seiichiro; Kim Namhee; Aya Sato; Tashiro Masato; Odagiri Takato

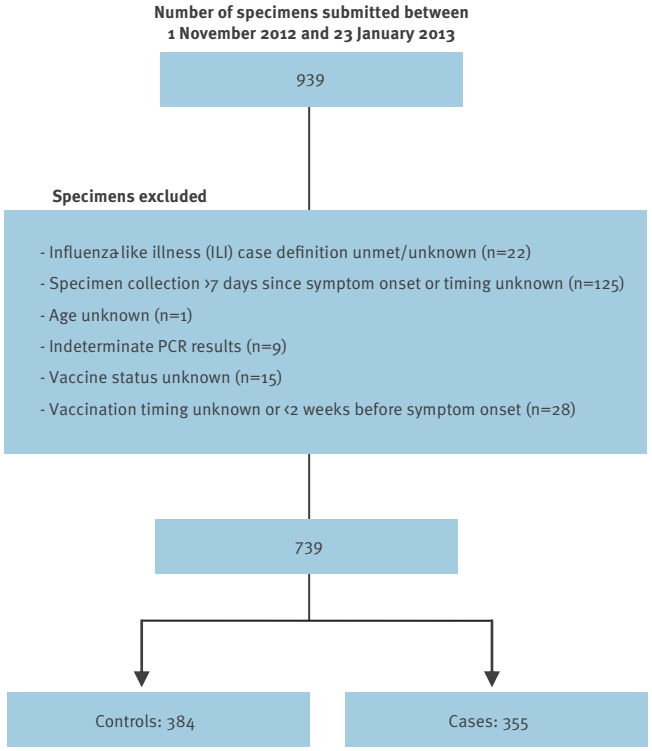
analyses of previous seasons [4,8,9,11]. Those aged 20–49 years contributed most to the analysis (43%) and the median interval between symptom onset and specimen collection was three days (Table 2).

About half (355/739) of the specimens were positive for influenza, of which 86% (287/334) of subtyped viruses were A(H3N2) (Table 3), a predominance similar to that noted elsewhere for Canada (Figure 1) [1]. The 2012/13 vaccine was received by 27% (108/402) controls (i.e. test-negative) and 17% (61/365) cases (i.e. test-positive) ($p<0.001$) (Table 2). Of those with information available for both 2011/12 and 2012/13 (n=682), 136/150 (91%) of those immunised in 2012/13 were also immunised in 2011/12. The proportion of controls reporting immunisation for 2012/13 and earlier seasons was comparable to that in previous sentinel and other survey reports for Canada (about 30%) [4,7-9,11,14] and was also similar for influenza A(H1N1) pdm09 immunisation: 48% compared with previous Canadian surveys (41%) [11]. The proportion of samples from patients with comorbidity was comparable to previous sentinel system estimates (14–23%) and other reports for Canada (15–20%) [4,7-11,15].

The overall crude (unadjusted) VE against influenza A(H3N2) virus was 39% (95% CI: 10–59) and against any influenza was 45% (95% CI: 20–63) (Table 4). Fully adjusted VEs were 45% (95% CI: 13–66) for A(H3N2) and 52% (95% CI: 25–69) overall. The overall VE estimate reflects the predominance of influenza A(H3N2) virus, with little contribution from influenza B or A(H1N1) viruses, precluding reliable estimates for those components.

Figure 2

Specimen exclusion for interim influenza vaccine effectiveness analysis, Canada, 2012/13 sentinel surveillance system



Virus characterisation

All influenza A(H3N2) isolates to date this season characterised in Canada by the haemagglutination inhibition assay have been considered antigenically similar to the 2012/13 vaccine component, although characterisation so far includes few (n=3) of the sentinel viruses described here [1]. HA1 sequences of a subset of 82 (29%) sentinel A(H3N2) viruses were thus assessed for substitutions potentially contributing to suboptimal VE (Figure 3, Table 5). Sequencing was based on original specimens from British Columbia (n=15), Alberta (n=25), Manitoba (n=4) and Ontario (n=11) and virus isolates from Quebec (n=27).

Of the 82 sequences, 75 clustered within the European Centre for Disease Prevention and Control (ECDC)-described Clade 3C, which includes the A/Victoria/361/2011 vaccine strain (Figure 3) [16]. There were, however, four to eight AA mutations (93.9–96.9% vaccine identity) in HA1 antigenic sites compared with the A/Victoria/361/2011 vaccine reference strain as follows: 2/82 with four AA mutations (from specimens collected mid-November and mid-December); 19/82 with five (October–January); 22/82 with six (October–January); 29/82 with seven (November–January) and 3/82 with eight mutations (late-December). Of note, the 32/82 viruses with seven or eight AA mutations included loss of glycosylation through T128A substitution in antigenic site B. The remaining seven sentinel sequences (collected mid-November to early January) clustered within ECDC Clade 6 (A/Iowa/19/2010-like) with 6/82 showing 11AA mutations (91.6% vaccine identity) and one exhibiting 12 AA mutations (90.8% vaccine identity) relative to the A/Victoria/361/2011 vaccine strain (Figure 3, Table 5). These Clade 6 viruses also included loss of glycosylation at position N45S, a non-antigenic site mutation.

Discussion

Mid-season reporting of virus evolution, vaccine relatedness and VE can support real-time risk communication and mitigation. Our interim 2012/13 VE results show that vaccination reduced the risk of medically attended laboratory-confirmed influenza due to the predominant A(H3N2) virus subtype by about half.

Our estimates are comparable to, if somewhat lower than, interim 2012/13 VE estimates recently reported by the US indicating 62% VE overall, 55% for influenza A and 70% for influenza B [17]. The proportion of influenza A viruses contributing to interim VE analysis in the US study setting (57%) is different from the profile for the rest of the US (about 70%) or Canada (about 90%); influenza A(H3N2) viruses have so far predominated in both countries [1,2]. Participant profiles were not presented and multivariable adjustment was also not undertaken in the interim US analysis. Although

Table 2

Profile of participants included in primary analysis, interim 2012/13 influenza vaccine effectiveness evaluation, Canada

Characteristics	Control (test-negative) N=384 n (%)	Case (test-positive) N=355 n (%)	Total N=739 n (%)
Age group (years)			
1–8	59 (15)	67 (19)	126 (17)
9–19	38 (10)	46 (13)	84 (11)
20–49	166 (43)	149 (42)	315 (43)
50–64	80 (21)	61 (17)	141 (19)
≥65	41 (11)	32 (9)	73 (10)
Median (range)	37 (‹1–92)	32 (‹1–90)	35 (‹1–92)
Sex			
Female	228 (59)	206 (58)	434 (59)
Comorbidity[a]			
No	270 (70)	271 (76)	541 (73)
Yes	81 (21)	61 (17)	142 (19)
Unknown	33 (9)	23 (6)	56 (8)
Received 2012/13 TIV[b,c]			
Any immunisation[d]	108/402 (27)	61/365 (17)	169/767 (22)
≥2 weeks before symptom onset	90 (23)	51 (14)	141 (19)
Among those with comorbidity	28 (35)	20 (33)	48 (34)
Among those without comorbidity	55 (20)	29 (11)	84 (16)
Received 2011/12 TIV[e]			
No	227 (69)	240 (73)	467 (71)
Yes	104 (31)	88 (27)	192 (29)
Received 2010/11 TIV[f]			
No	204 (64)	217 (70)	421 (67)
Yes	113 (36)	91 (30)	204 (33)
Received adjuvanted A(H1N1)pdm09 vaccine[g]			
No	156 (52)	147 (51)	303 (52)
Yes	144 (48)	140 (49)	284 (48)
Specimen collection interval (days)			
≤4	282 (73)	293 (83)	575 (78)
5–7	102 (27)	62 (17)	164 (22)
Median (range)	3 (0–7)	3 (0–7)	3 (0–7)

TIV: trivalent influenza vaccine.

[a] Chronic medical conditions that place individuals at higher risk of serious complications (hospitalisation or death) from influenza as defined by Canada's National Advisory Committee on Immunization [13], including heart, pulmonary (including asthma), renal, metabolic (such as diabetes), blood, cancer, immune compromising conditions or those that compromise the management of respiratory secretions and increase the risk of aspiration or morbid obesity. Questionnaire was answered as 'yes', 'no' or 'unknown' to any one or more of these conditions without specifying.

[b] Vaccine status was based on self/parental/guardian report. Detail related to special paediatric dosing requirements was not sought. Immunised participants were predominantly offered split (non-adjuvanted) 2012/13 trivalent inactivated influenza vaccine during the regular autumn immunisation campaign. In British Columbia and Quebec, influenza vaccine is provided free of charge to high-risk groups [13]. Others are encouraged to receive vaccine but must purchase it. In Ontario, Alberta and Manitoba, the vaccine is provided free of charge to all citizens aged ≥6 months.

[c] In Canada, adjuvanted vaccine is approved for people aged ≥65 years and live-attenuated vaccine by nasal administration is approved for those aged 2–59 years [13]; their use, however, remains infrequent. Of the 47 people aged ≥65 years who were considered immunised in this study, 14 reported that they received adjuvanted vaccine and 19 did not know, while the rest would have received non-adjuvanted vaccine. Overall, 5/141 immunised participants and 5/18 immunised children aged ≤10 years reported intranasal administration. Vaccine effectiveness analysis was not stratified on that basis.

[d] Immunised people who received the vaccine ‹2 weeks before symptom onset or for whom this was unknown were excluded from the primary vaccine effectiveness analysis. They were included for assessing 'any' immunisation regardless of timing and for comparison with other sources of vaccine coverage. The denominator is therefore shown for 'any' immunisation.

[e] Children ‹2 years-old in 2012/13 were excluded from 2011/12 vaccine uptake analysis as they may not have been age-eligible in autumn 2011.

[f] Children ‹3 years-old in 2012/13 were excluded from 2010/11 vaccine uptake analysis as they may not have been age-eligible in autumn 2010.

[g] Children ‹4 years-old in 2012/13 were excluded from influenza A(H1N1)pdm09 vaccine uptake analysis as they may not have been age-eligible in autumn 2009.

Table 3

Laboratory profile of specimens included in primary analysis, interim 2012/13 influenza vaccine effectiveness evaluation, Canada

Specimen included	Alberta N=225 n (%)	British Columbia N=156 n (%)	Manitoba N=63 n (%)	Ontario[a] N=108 n (%)	Quebec N=187 n (%)	Total N=739 n (%)
Influenza negative	120 (53)	92 (59)	46 (73)	48 (44)	78 (42)	384 (52)
Influenza positive	105 (47)	64 (41)	17 (27)	60 (56)	109 (58)	355 (48)
A positive	89 (85)	57 (89)	14 (82)	59 (98)	104 (95)	323 (91)
B positive	16 (15)	7 (11)	3 (18)	1 (2)	5 (5)	32 (9)
Influenza A positive						
H3N2	81 (91)	54 (95)	4 (29)	54 (92)	94 (90)	287 (89)
(H1N1)pdm09	4 (5)	2 (4)	1 (7)	4 (7)	4 (4)	15 (5)
Subtype unknown	4 (5)	1 (2)	9 (64)	1 (2)	6 (6)	21 (7)

[a] Ontario was delayed while awaiting ethics board review, diminishing its contribution to this interim analysis.

Table 4

Interim 2012/13 influenza A(H3N2) and overall influenza vaccine effectiveness, Canada

Analysis scenarios	A(H3N2)[a] VE (95% CI)	Number Total (Cases; Vac) [Controls; Vac]	Any Influenza VE (95% CI)	Number Total (Cases; Vac) [Controls; Vac]
Primary analysis				
Crude (unadjusted)[b,c]	39 (10–59)	671 (287; 45) [384; 90]	45 (20–63)	739 (355; 51) [384; 90]
Adjusted for:[b,c]				
Age in years (1–8, 9–19, 20–49, 50–64, ≥65)	38 (4–60)		46 (18–64)	
Comorbidity (yes/no)[b]	38 (7–58)		43 (17–61)	
Province (BC, AB, MB, ON, QC)	46 (18–64)		50 (26–66)	
Specimen collection interval (≤4 d/5–7 d)	40 (10–60)		46 (20–63)	
Week of specimen collection	39 (9–59)		45 (20–63)	
Age, comorbidity	38 (3–60)		45 (17–64)	
Age, comorbidity, province	45 (13–65)		51 (24–68)	
Age, comorbidity, province, interval	46 (14–66)		52 (26–69)	
Age, comorbidity, province, interval, week	45 (13–66)		52 (25–69)	
Sensitivity analysis				
Vaccination defined without regard to interval to symptom onset				
Crude	38 (11–57)	699 (297; 55) [402; 108]	45 (22–62)	767 (365; 61) [402; 108]
Fully adjusted[d]	38 (5–60)		47 (21–65)	
Those vaccinated within 2 weeks of symptom onset considered as				
Unvaccinated; Crude	38 (8–58)	699 (297; 45) [402; 90]	44 (18–61)	767 (365; 51) [402; 90]
Unvaccinated; Fully adjusted[d]	42 (9–63)		48 (20–66)	
Vaccinated; Crude	38 (11–57)	699 (297; 55) [402; 108]	45 (22–62)	767 (365; 61) [402; 108]
Vaccinated; Fully adjusted[d]	38 (5–60)		47 (21–65)	
Those with unknown comorbidity				
Re-coded 'Yes' for comorbidity; Fully adjusted[d]	45 (13–66)	671 (287; 45) [384; 90]	51 (25–69)	739 (355; 51) [384; 90]
Excluded from analysis				
Crude	38 (6–59)	617 (266; 43) [351; 83]	44(17–62)	683 (332; 49) [351; 83]
Fully adjusted[d]	44 (9–65)		51(23–69)	
Restricted to those with no comorbidity				
Crude	48 (14–69)	484 (214; 25) [270; 55]	53 (24–71)	541 (271; 29) [270; 55]
Fully adjusted[e]	60 (27–78)		65 (39–80)	

AB: Alberta; BC: British Columbia; MB: Manitoba; ON: Ontario; QC: Quebec; Vac: vaccinated – i.e. number of (cases) or [controls] vaccinated; VE: vaccine effectiveness.

[a] Those with influenza A of unknown subtype were excluded from the A(H3N2)-specific analysis.

[b] For the primary analysis, those with unknown comorbidity were coded as 'No' but explored in the sensitivity analysis as shown.

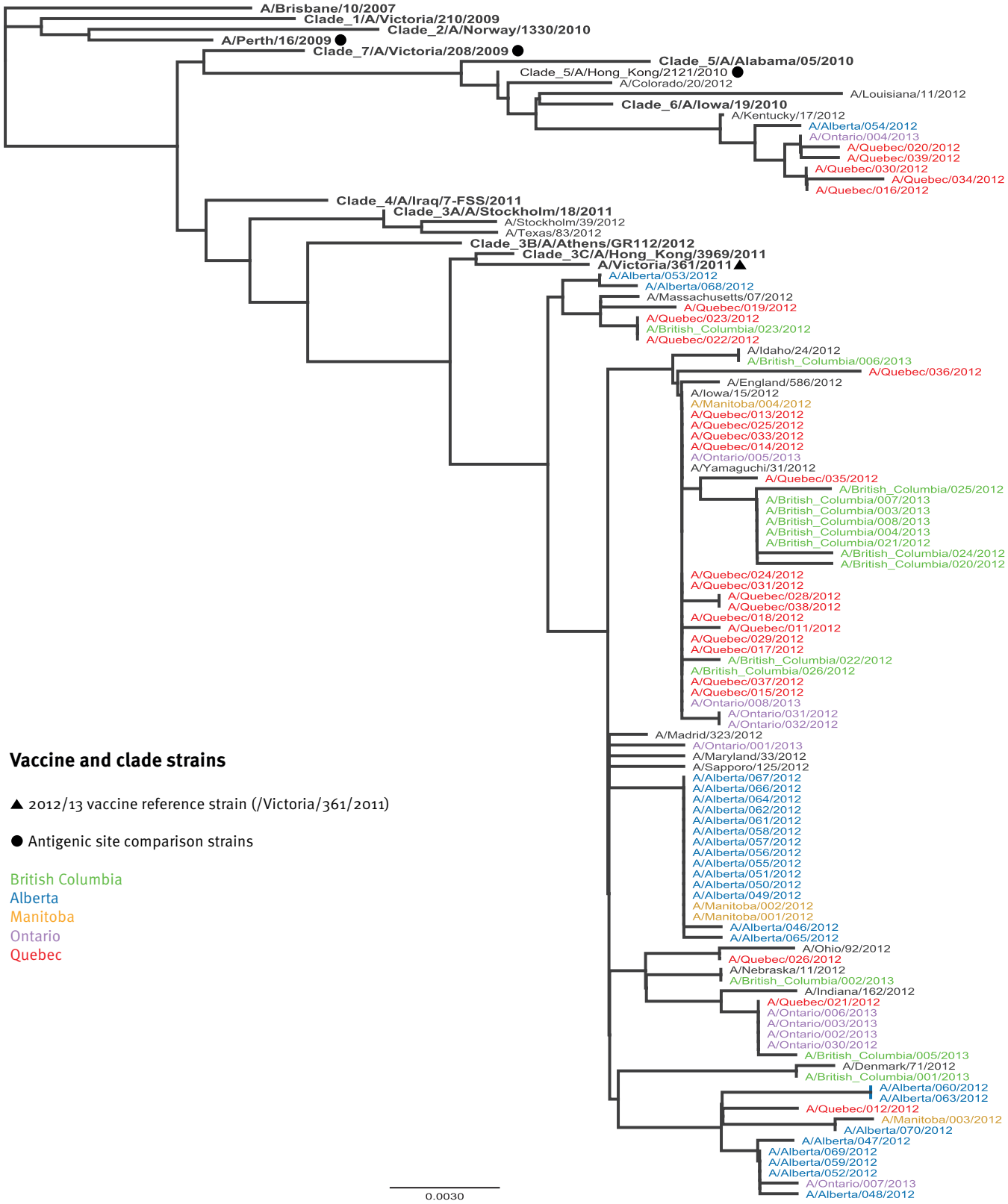
[c] Those immunised <2 weeks before symptom onset or from whom a specimen was collected >7 days since symptom onset (or for whom these were unknown) were excluded but explored in the sensitivity analysis as shown.

[d] Adjusted for age, comorbidity, province, interval, week.

[e] Adjusted for age, province, interval, week.

Figure 3

Phylogenetic tree of influenza A(H3N2) viruses, Canada, 2012/13 sentinel surveillance system



The phylogenetic tree was created by aligning the 82 Canadian sentinel sequences against sequences representative of emerging viral clades as described by the European Centre for Disease Prevention and Control (ECDC) [16] (n=10), A(H3N2) sequences collected globally between 1 November 2012 and 18 January 2013 (n=17), and recent vaccine strains (n=3). The global sequences were downloaded from Global Initiative on Sharing Avian Influenza Data (GISAID) by searching for human influenza A(H3N2) haemagglutinin sequences collected in the specified period (Table 1).

Table 5

Changes in amino acid sequence encoded by haemagglutinin (HA1) gene (antigenic regions)[a] for subset of 2012/13 Canadian sentinel influenza A(H3N2) strains relative to reference strains[b]

Antigenic site		C				E				D	A	B	A		B					D		C			
Amino acid number HA1		45	48	53	54	62	67	88	94	121	124	128	142	145	156	157	186	192	198	219	230	278	280	304	312
A/Perth/16/2009		**S**	**T**	D	S	**K**	I	V	Y	N	S	T	R	N	**H**	L	**G**	I	**A**	**S**	I	N	E	A	**N**
A/Victoria/208/2009		**S**	**T**	D	S	E	I	V	Y	N	S	T	R	N	**H**	L	**G**	I	**A**	**S**	I	N	E	A	**N**
A/Hong Kong/2121/2010		**S**	**T**	**N**	S	E	I	V	**H**	N	S	T	R	N	**H**	L	**G**	I	**A**	**S**	**V**	N	**A**	A	**N**
A/Victoria/361/2011[c]		N	I	D	S	E	I	V	Y	N	S	T	R	N	Q	L	V	I	S	Y	I	N	E	A	S
British Columbia	n																								
A/British Columbia/020/2012[d]	11											**A**	**G**	**S**	**H**		**G**			**S**		**K**			
A/British Columbia/023/2012[d]	1														**H**	**S**	**G**			**S**		**K**			
A/British Columbia/002/2013[d]	1													**S**	**H**		**G**			**S**		**K**			
A/British Columbia/001/2013[d]	1										**N**			**S**	**H**		**G**			**S**		**K**			
A/British Columbia/005/2013[d]	1					**K**								**S**	**H**		**G**			**S**		**K**			
Alberta	n																								
A/Alberta/046/2012[d]	14						**V**							**S**	**H**		**G**			**S**		**K**			
A/Alberta/047/2012[d]	6													**S**	**H**		**G**			**S**		**K**			
A/Alberta/053/2012[d]	2														**H**		**G**			**S**		**K**			
A/Alberta/060/2012[d]	2				**G**									**S**	**H**		**G**			**S**		**K**			
A/Alberta/054/2012[e]	1	**S**	**T**	**N**					**H**						**H**		**G**		**A**	**S**	**V**		**A**	**D**	**N**
Manitoba	n																								
A/Manitoba/001/2012[d]	2						**V**							**S**	**H**		**G**			**S**		**K**			
A/Manitoba/003/2012[d]	1													**S**	**H**		**G**			**S**		**K**			
A/Manitoba/004/2012[d]	1											**A**	**G**	**S**	**H**		**G**			**S**		**K**			
Ontario	n																								
A/Ontario/030/2012[d]	5													**S**	**H**		**G**			**S**		**K**			
A/Ontario/005/2013[d]	2											**A**	**G**	**S**	**H**		**G**			**S**		**K**			
A/Ontario/031/2012[d]	2										**R**	**A**	**G**	**S**	**H**		**G**			**S**		**K**			
A/Ontario/001/2013[d]	1									**S**				**S**	**H**		**G**			**S**		**K**			
A/Ontario/004/2013[e]	1	**S**	**T**	**N**					**H**						**H**		**G**		**A**	**S**	**V**		**A**		**N**
Quebec	n																								
A/Quebec/011/2012[d]	14											**A**	**G**	**S**	**H**		**G**			**S**		**K**			
A/Quebec/028/2012[d]	2											**A**	**G**	**S**	**H**		**G**	**V**		**S**		**K**			
A/Quebec/019/2012[d]	3														**H**	**S**	**G**			**S**		**K**			
A/Quebec/021/2012[d]	2													**S**	**H**		**G**			**S**		**K**			
A/Quebec/012/2012[d]	1							**I**						**S**	**H**		**G**			**S**		**K**			
A/Quebec/020/2012[e]	2	**S**	**T**	**N**					**H**						**H**		**G**		**A**	**S**	**V**		**A**		**N**
A/Quebec/016/2012[e]	2	**S**	**T**	**N**					**H**						**H**		**G**		**T**	**S**	**V**		**A**		**N**
A/Quebec/034/2012[e]	1	**S**	**T**	**N**					**Q**						**H**		**G**		**T**	**S**	**V**		**A**		**N**

Bold font signifies amino acid substitution compared with the 2012/13 northern hemisphere vaccine reference strain.

All sequences were deposited into GenBank (accession numbers: KC526204-KC526214; KC535019-KC535064; and KC539112-KC539136).

[a] Antigenic regions A–E comprise 131 amino acid residues [12]. Only the 24 positions in those 131 residues showing mutations in the present study are displayed. British Columbia, Alberta, Manitoba and Ontario sequencing was performed on original specimens; Quebec performed the sequencing on virus isolates.

[b] 2012/13 northern hemisphere vaccine reference strain (A/Victoria/361/2011) and other recent vaccine and variant reference strains.

[c] 2012/13 northern hemisphere vaccine reference strain.

[d] A total of 75 sentinel sequences clustered within Clade 3C, which also includes the 2012/13 A/Victoria/361/2011 vaccine strain ([16] and Figure 3). Common to each of these 75 sentinel sequences however, were antigenic site mutations compared with the A/Victoria/361/2011 vaccine strain as shown in this table and summarised as follows, with the antigenic site shown in parentheses: Q156H (B), V186G (B), Y219S (D), N278K (C). Of these 75 sequences, 69 also showed N145S (A) while the other four included L157S (B). Of these 69 sequences, 14/22 Alberta and 2/4 Manitoba sequences additionally showed I67V (E) and 11/14 British Columbia, 1/4 Manitoba, 4/10 Ontario and 16/19 Quebec sequences included T128A causing loss of glycosylation site (B) as well as R142G (A) mutations.

[e] Seven sequences clustered within Clade 6 (A/Iowa/19/2010-like; see [16] and Figure 3) with antigenic site mutations compared with the A/Victoria/361/2011 vaccine strain as shown in this table and additional loss of glycosylation at non-antigenic site N45S (not shown).

our own adjusted VE estimates did not substantially differ (less than 5–10%) from our unadjusted VE estimates, assessment of bias and confounding has to be separately undertaken for each dataset. Nevertheless, suboptimal VE for the influenza A(H3N2) component of the vaccine in both Canada and the US is inconsistent with haemagglutination inhibition characterisation indicating good vaccine match to circulating A(H3N2) viruses [1,2]. Such discordance between conventional in vitro characterisation of vaccine match by haemagglutination inhibition and epidemiological measures of VE has been noted in previous seasons' estimates from our sentinel network [6,7,11], highlighted also in a recent meta-analysis of other studies, including randomised controlled trials [18].

Molecular markers of virus mutation may offer more insight. It has previously been suggested that a change of at least four AA in two or more HA antigenic sites heralds emergence of virus drift, potentially compromising antibody binding [19]. However, HA antigenic-site maps have been updated and more studies are needed to correlate genetic variation in circulating viruses with epidemiological variation in measured VE [12,20]. Not only the number but also the nature and location of AA substitutions are likely to be relevant. Furthermore, hypotheses to explain the variable efficacy of repeat immunisation have included positive and negative interference from pre-existing antibody, with differential effects depending on the antigenic distance across successive vaccine components and circulating strains [21]. We note that a high proportion of participants (91%) who were immunised this season had also received vaccine the previous season. These virological, host and other factors potentially contributing to suboptimal VE warrant more in-depth evaluation.

Limitations of this surveillance approach to VE estimation have been described previously [6-11]. For our interim analysis, we draw particular attention to small sample size, resulting in wide confidence intervals and variability around the point estimate. Age-specific VE analyses (e.g. children and elderly people) would be of additional important interest – our estimates primarily reflect the prominent contribution of adults 20–49 years of age. However, stratification of VE analysis by age would further reduce the statistical power and precision of estimates in this interim report. The slightly higher VE with restriction to participants without comorbidity (Table 4) may similarly reflect such variability. End-of-season analysis will further expand upon these interim findings and may better support stratified analyses. Although we have assessed vaccine relatedness through gene sequencing of community-based sentinel viruses available from each province and across the season to date, in this interim assessment the sampling frame for specimen selection was not random or systematic. Bias may result from the preferential inclusion of specimens that demonstrate low cycle threshold values (high RNA levels) or successful virus isolation. These, however, are issues for all laboratory-based influenza surveillance. Finally, in reviewing participant profiles, we identified no obvious signals of bias and in our analysis we adjusted for recognised potential confounders, but ultimately, given the observational design, we cannot rule out other unrecognised influences on the VE estimates.

In summary, our interim findings indicate that the 2012/13 vaccine shows a substantial but suboptimal protection. As such, adjunct protective measures (e.g. antivirals) may be warranted for those at high risk of influenza complications, whether they are vaccinated or not. Interim virus monitoring and VE results may also inform vaccine reformulation for subsequent seasons. Ultimately, however, better understanding of the factors affecting annual influenza VE is needed for improved product development and immunisation programme acceptance in the long term.

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Conflict of interest

GDS has received research grants from GlaxoSmithKline (GSK) and Sanofi Pasteur and participated in an ad hoc GSK advisory board meeting for an unrelated issue for which travel expenses were reimbursed. SMM has received research grants from GSK, Pfizer and Sanofi Pasteur. JBG has received research grants from GSK and Hoffmann-LaRoche for antiviral resistance studies. MK has received research grants from Roche, Merck, Gen-Probe and Siemens. Salaries

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Authors' contributions

Principal investigator (epidemiology): DMS (National and British Columbia); GDS (Quebec); JAD (Alberta); ALW (Ontario); SMM (Manitoba). Principal investigator (laboratory): JBG (Ontario); HC (Quebec); MPP and MK (British Columbia); KF (Alberta); PVC (Manitoba), YL (national). National database coordination: TLK. Data analysis: NZJ and DMS (epidemiology); SS and AE (phylogenetic). Data interpretation: all. Preparation of first draft: DMS. Draft revision and approval: all.

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Decline in influenza vaccine effectiveness with time after vaccination, Navarre, Spain, season 2011/12

J Castilla (jcastilc@navarra.es)[1,2], I Martínez-Baz[1,2], V Martínez-Artola[3], G Reina[4], F Pozo[5], M García Cenoz[1,2], M Guevara[1,2], J Morán[6], F Irisarri[1,2], M Arriazu[1,2], E Albéniz[6], C Ezpeleta[3], A Barricarte[1,2], Primary Health Care Sentinel Network[7], Network for Influenza Surveillance in Hospitals of Navarre[7]

1. Instituto de Salud Pública de Navarra (Public Health Institute of Navarre), Pamplona, Spain
2. Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP; Network of Biomedical Research Centers Epidemiology and Public Health), Spain
3. Complejo Hospitalario de Navarra (Hospital complex of Navarre), Pamplona, Spain
4. Clínica Universidad de Navarra (University Clinic of Navarre), Pamplona, Spain
5. National Centre of Microbiology (World Health Organization National Influenza Centre - Madrid), Instituto de Salud Carlos III, Majadahonda, Spain
6. Primary Healthcare Directorate, Navarre Health Service, Pamplona, Spain
7. The members of these networks are listed at the end of the article

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This study evaluates the influenza vaccine effectiveness (VE) in preventing laboratory-confirmed cases in Navarre, Spain, in the 2011/12 season in which the peak was delayed until week 7 of 2012. We conducted a test-negative case–control study. Patients with influenza-like illness in hospitals and primary healthcare were swabbed for testing by reverse transcription-polymerase chain reaction. Influenza vaccination status and other covariates were obtained from healthcare databases. The vaccination status of confirmed cases and negative controls was compared after adjusting for potential confounders. VE was calculated as (1-odds ratio)x100. The 411 confirmed cases (93% influenza A(H3)) were compared with 346 controls. Most characterised viruses did not match the vaccine strains. The adjusted estimate of VE was 31% (95% confidence interval (CI): -21 to 60) for all patients, 44% (95% CI: -11 to 72) for those younger than 65 years and 19% (95% CI: -146 to 73) for those 65 or older. The VE was 61% (95% CI: 5 to 84) in the first 100 days after vaccination, 42% (95% CI: -39 to 75) between 100 and 119 days, and zero thereafter. This decline mainly affected people aged 65 or over. These results suggest a low preventive effect of the 2011/12 seasonal influenza vaccine, and a decline in VE with time since vaccination.

Introduction

Influenza is an important health problem that can lead to serious complications in persons with risk factors [1,2]. Annual vaccination is the primary measure for preventing influenza and its complications [3]. Because the influenza vaccine composition is adapted each season to the viruses in circulation, its effectiveness varies [4].

Observational studies are the main way to evaluate vaccine effectiveness (VE) in each season, however, possible biases affecting comparability between vaccinated and unvaccinated persons must be overcome [5-8]. Studies with non-specific outcomes tend to underestimate the VE [6], a problem that is resolved by analysing virologically confirmed cases [4,9]. A design that compares confirmed influenza cases with test-negative controls ensures good comparability and is easy to carry out, thus this type of study has come to be widely used [4,9].

Song et al., in an immunogenicity study of the influenza vaccine, found that the antibody levels decline progressively, beginning in the first months after vaccine administration [10]. In addition, people with higher risk of complications due to influenza may have a weaker immune response due to the immunodepression associated with some chronic diseases or to the immunosenescence associated with aging [11,12].

In Spain, influenza circulation in the 2011/12 season reached a peak in week 7 of 2012, the second latest peak in the past 15 seasons, after the 2005/06 influenza wave [13]. Influenza A(H3N2) was the predominant virus in circulation, and a certain degree of vaccine–virus mismatch was observed [13]. The objective of this study was to describe the effectiveness of the influenza vaccine in the 2011/12 season in preventing laboratory-confirmed influenza, including both outpatients and hospitalised patients.

Methods

Study population

The present study was based on electronic clinical records in the region of Navarre, Spain in the 2011/12 season. The Navarre Ethical Committee for Medical Research approved the study protocol. The Navarre Health Service provides healthcare, free at point of

service, to 97% of the 642,051 inhabitants of the region (private companies provide healthcare to the remaining 3% of the population). The clinical records have been computerised since the year 2000 and include reports from primary care, hospital admissions, vaccination register, and laboratory test results.

The seasonal vaccination campaign took place from 10 October to 25 November 2011. In Navarre the trivalent inactivated non-adjuvanted vaccine was recommended and offered free of charge to people aged 60 years or older and to those with risk factors or major chronic conditions [14]. Other people can also be vaccinated if they pay for the vaccine. In the 2011/12 season, the vaccine included the strains A/California/07/2009(H1N1)-like, A/Perth/16/2009(H3N2)-like and B/Brisbane/60/2008-like virus [15]. Precise instructions for registering each dose were given to all vaccination points [14].

Influenza surveillance was based on automatic reporting of cases of influenza-like illness (ILI) from all primary healthcare centres and hospitals. Following the European Union case definition, ILI was considered to be the sudden onset of any general symptom (fever or feverishness, malaise, headache or myalgia) in addition to any respiratory symptom (cough, sore throat or shortness of breath) [16]. A sentinel network composed of a representative sample of 76 primary healthcare physicians and paediatricians, covering 15% of the population, was asked to take nasopharyngeal and pharyngeal swabs, after obtaining verbal informed consent, from all their patients diagnosed with ILI whose symptoms had begun preferably less than five days previously. An agreed protocol of care for influenza cases was applied in hospitals, which specified early detection and nasopharyngeal swabbing of all hospitalised patients with ILI. Swabs were analysed by reverse transcription polymerase chain reaction (RT-PCR), and influenza-positive samples were subsequently typed/subtyped as influenza A(H1 and H3), A(H1N1)pdm09 and B. About one in four positive swabs was randomly selected each week and sent to the National Influenza Centre–Madrid laboratory for genetic characterisation.

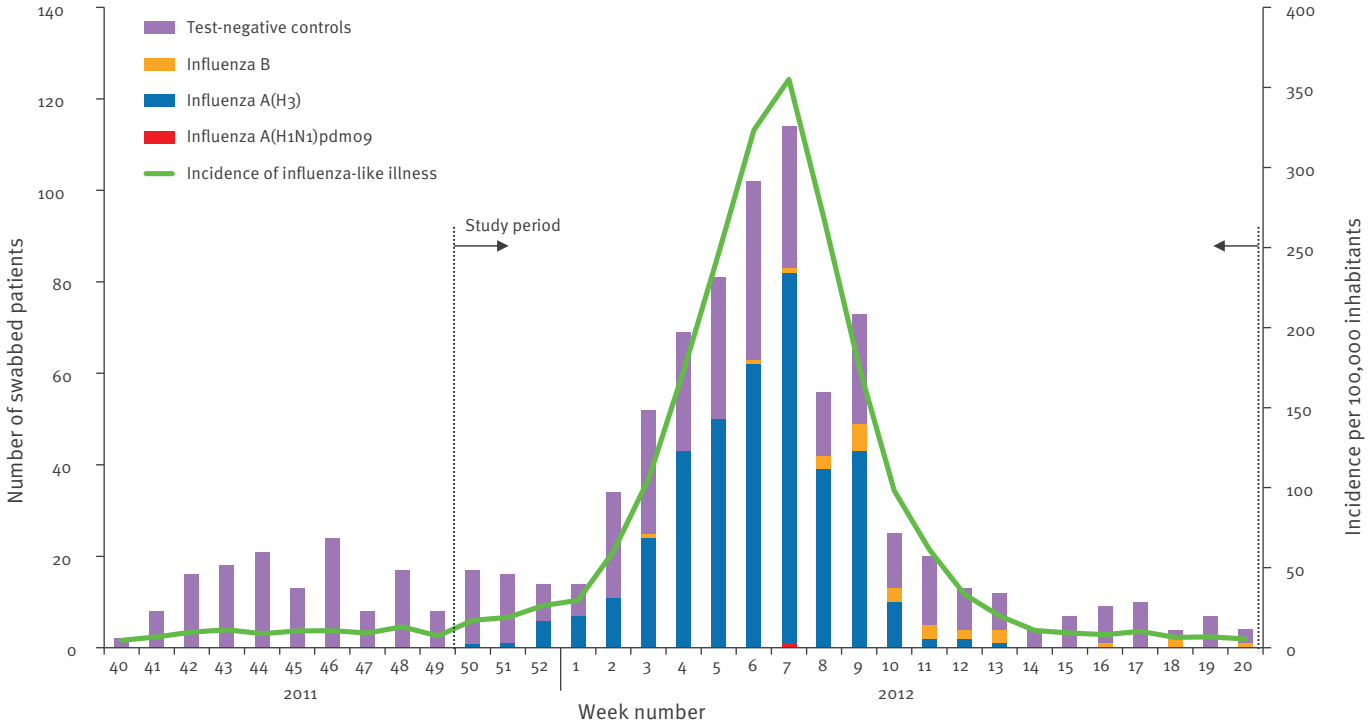
Study design and statistical analysis

We carried out a case–control study nested in the cohort of the population covered by the Navarre Health Service. Healthcare workers, persons living in nursing homes and children under six months of age were excluded. The study began in the first week in which influenza virus was detected, 12 December 2011 (week 50), and ended in the last week in which ILI patients tested positive for influenza, 20 May 2012 (week 20).

The cases were patients diagnosed with ILI in primary care or in hospitals who were confirmed for influenza virus by RT-PCR. The controls were patients with ILI in primary healthcare or in hospitals who were negative for influenza virus. Their vaccination status for the trivalent 2011/12 seasonal influenza vaccine was obtained from the online regional vaccination register [17]. Subjects were considered to be protected starting 14 days after vaccine administration.

Figure

Number of influenza cases and test-negative controls, and incidence of influenza-like illness by week, Navarre, 3 Oct 2011–20 May 2012



From the electronic healthcare records we obtained the following baseline characteristics: sex, age, district of residence, migrant status (country of birth other than Spain has been related to a different pattern of use of healthcare services [18]), major chronic conditions (heart disease, lung disease, renal disease, cancer, diabetes mellitus, cirrhosis, dementia, stroke, immunodeficiency, rheumatic disease and body mass index of 40 kg/m2 or greater), hospitalisation in the previous 12 months and outpatient visits in the previous 12 months.

Vaccination status was compared between cases and controls. Different analyses were done: (i) comparing cases of each type of influenza with negative controls, (ii) including only patients in whom influenza vaccination was indicated because they were 60 years or older or had a major chronic condition, (iii) considering only patients in primary care, and (iv) including only swabs taken in the first four days after symptom onset. VE was also evaluated separately in two age strata (<65 and ≥65 years; a cut-off age different from that of the vaccination target population was chosen to match the one commonly used in similar studies), in two periods (weeks 50/2011 to week 8/2012 and weeks 9/2012 to 20/2012) and in three strata according to time since vaccination (<100, 100–119 and ≥120 days).

Percentages were compared by chi-square test. Logistic regression techniques were used to calculate the odds ratios (OR) with their 95% confidence intervals (CI). ORs were adjusted for potential confounders including healthcare setting, and for date of diagnosis grouped into four-week periods. VE was estimated as (1-OR)x100.

Results

Description of cases and controls

The weekly number of swabbed patients followed the pattern of ILI incidence in the population (Figure). During the study period, 757 ILI patients were swabbed, 588 in primary healthcare and 169 in hospitals. Some 411 (54.3%) were confirmed for influenza virus: 382 for influenza A(H3), 28 for influenza B and one for influenza A(H1N1)pdm09.

Compared with confirmed cases of influenza, the group of test-negative controls had a higher proportion of males, of persons under the age of five years, people who had consulted a physician five or more times in the past year, who had major chronic conditions, and who were treated in hospital. There were no significant differences regarding migrant status or urban/rural residence, and these variables were therefore not included in the multivariate analysis (Table 1). Vaccine coverage in controls (18.8%) was slightly higher than in the overall population cohort in which the study was nested (14.2%, p=0.015).

Effectiveness of the 2011/12 seasonal influenza vaccine

Compared with test-negative controls, a smaller proportion of confirmed influenza cases had received the 2011/12 seasonal influenza vaccine (OR: 0.60; 95% CI: 0.40 to 0.89; p=0.012). In the adjusted analysis, the VE was 31% (95% CI: -21 to 60; p=0.200). The VE was somewhat higher between weeks 50/2011 and 8/2012

Table 1

Characteristics of laboratory-confirmed influenza cases (n=411) and test-negative controls (n=346), Navarre, 12 Dec 2011–20 May 2012

	Laboratory-confirmed influenza cases n (%)	Test-negative controls n (%)	p value
Age groups (years)			<0.001
<5	28 (6.8)	56 (16.2)	-
5–14	50 (12.2)	40 (11.6)	-
15–44	164 (39.9)	123 (35.5)	-
45–64	115 (28.0)	74 (21.4)	-
≥65	54 (13.1)	53 (15.3)	-
Sex			0.006
Male	188 (45.7)	194 (56.1)	-
Female	223 (54.3)	152 (43.9)	-
Residence			0.164
Rural	103 (25.1)	103 (29.8)	
Urban	308 (74.9)	243 (70.2)	
Migrant status			0.097
No	381 (92.7)	308 (89.0)	-
Yes	30 (7.3)	38 (11.0)	-
Major chronic conditions			0.002
No	301 (73.2)	217 (62.7)	-
Yes	110 (26.8)	129 (37.3)	-
Hospitalisation in the previous year			<0.001
No	384 (93.4)	292 (84.4)	-
Yes	27 (6.6)	54 (15.6)	-
Outpatient visits in the previous year			0.016
0	44 (11.4)	32 (9.3)	-
1 to 5	195 (47.5)	133 (38.4)	-
>5	172 (41.9)	181 (52.3)	-
Health care setting			<0.001
Primary healthcare	378 (92.0)	210 (60.7)	-
Hospital	30 (7.3)	119 (34.9)	-
Emergency rooms	3 (0.7)	17 (4.9)	-
Period			<0.001
Weeks 50/2011 to 8/2012 (12 Dec 2011–26 Feb 2012)	332 (80.8)	237 (68.5)	-
Weeks 9/2012 to 20/2012 (27 Feb–20 May 2012)	79 (19.2)	109 (31.5)	-
Seasonal influenza vaccine 2011/12			<0.015
No	361 (87.8)	281 (81.2)	-
Yes	50 (12.2)	65 (18.8)	-
Total	**411 (100)**	**346 (100)**	**-**

(37%; 95% CI: -18 to 67), and lower between weeks 9/2012 and 20/2012 (19%; 95% CI: -176 to 76). The estimates of VE were very similar in the analyses that were restricted to cases of influenza A(H3) (29%; 95% CI: -26 to 60), to subjects with an indication for vaccination (30%; 95% CI: -34 to 63), to patients in primary care (31%; 95% CI: -32 to 64), and to patients swabbed within the first four days after symptom onset (29%; 95% CI: -38 to 63). The point estimate of the VE was higher in subjects under the age of 65 years (44%; 95% CI: -11 to 72) than in those aged 65 years or older (19%; 95% CI: -146 to 73), although these differences did not reach statistical significance. In none of all the analyses was the VE statistically significant (Table 2).

The VE was 61% (95% CI: 5 to 84) in the first 100 days after vaccination, dropping to 42% (95% CI: -39 to 75) between days 100 and 119, and ceasing to confer any protection after 120 days (-35%, 95% CI: -211 to 41) (Table 3). Persons vaccinated more than 120 days before diagnosis versus those vaccinated less than 100 days before diagnosis were at an increased risk for contracting influenza, with an OR of 3.45 (95% CI: 1.10 to 10.85; p=0.034).

Table 2

Influenza vaccine effectiveness in preventing laboratory-confirmed influenza by patient characteristic, comparisons of influenza-positive cases (n=411) and test-negative controls (n=346), Navarre, 12 Dec 2011–20 May 2012

	Cases/controls	Crude vaccine effectiveness % (95% CI)	p value	Adjusted vaccine effectiveness % (95% CI)[a]	p value
All swabbed patients					
Unvaccinated	361/281	Reference	-	Reference	-
Vaccinated	50/65	40 (11 to 60)	0.012	31 (-21 to 60)	0.200
Weeks 50/2011 to 8/2012 (12 Dec 2011–26 Feb 2012)					
Unvaccinated	298/195	Reference	-	Reference	-
Vaccinated	34/42	47 (14 to 67)	0.011	37 (-18 to 67)	0.151
Weeks 9/2012 to 20/2012 (27 Feb 2012–20 May 2012)					
Unvaccinated	63/86	Reference	-	Reference	-
Vaccinated	16/23	15 (-94 to 54)	0.888	19 (-176 to 76)	0.741
Influenza A(H3)[b]					
Unvaccinated	335/281	Reference	-	Reference	-
Vaccinated	47/65	39 (9 to 60)	0.011	29 (-26 to 60)	0.247
Influenza B[b]					
Unvaccinated	25/281	Reference	-	Reference	-
Vaccinated	3/65	48 (-77 to 85)	0.295	54 (-102 to 90)	0.301
Target population for vaccination[c]					
Unvaccinated	112/98	Reference	-	Reference	-
Vaccinated	43/54	30 (-13 to 57)	0.143	30 (-34 to 63)	0.286
Primary healthcare patients					
Unvaccinated	337/185	Reference	-	Reference	-
Vaccinated	41/25	10 (-53 to 47)	0.697	31 (-32 to 64)	0.262
Hospitalised patients					
Unvaccinated	21/85	Reference	-	Reference	-
Vaccinated	9/34	-7 (-157 to 65)	0.877	9 (-212 to 73)	0.879
Primary care patients swabbed <5 days after symptom onset					
Unvaccinated	334/182	Reference	-	Reference	-
Vaccinated	41/24	7 (-59 to 45)	0.793	29 (-38 to 63)	0.319
Patients aged <65 years					
Unvaccinated	337/258	Reference	-	Reference	-
Vaccinated	20/35	56 (22 to 75)	0.005	44 (-11 to 72)	0.095
Patients aged ≥65 years					
Unvaccinated	24/23	Reference	-	Reference	-
Vaccinated	30/30	4 (-106 to 55)	0.913	19 (-146 to 73)	0.710

CI: confidence interval.

[a] Vaccine effectiveness adjusted for sex, age (<5; 5–14; 15–44; 45–64; ≥65 years), major chronic conditions, outpatient visits in the previous year, hospitalisation in the previous year, healthcare setting, and period of diagnosis.

[b] There was one case of influenza A(H1N1)pdm09, not shown in this table.

[c] Target population for vaccination includes people ≥60 years-old and people with major chronic conditions.

Table 3

Influenza vaccine effectiveness in preventing laboratory-confirmed influenza by vaccination status and time after vaccination, comparison of influenza-positive cases (n=411) and test-negative controls (n=346), Navarre, 12 Dec 2011–20 May 2012

	Cases/controls	Crude vaccine effectiveness % (95% CI)	p value	Adjusted vaccine effectiveness % (95% CI)[a]	p value
All swabbed patients					
Unvaccinated	361/281	Reference	-	Reference	-
<100 days after vaccination	11/24	64 (26 to 83)	0.006	61 (5 to 84)	0.039
100–119 days after vaccination	15/16	27 (-50 to 64)	0.392	42 (-39 to 75)	0.222
≥120 days after vaccination	24/25	25 (-34 to 58)	0.326	-35 (-211 to 41)	0.476
Influenza A(H3) cases					
Unvaccinated	335/281	Reference	-	Reference	-
<100 days after vaccination	11/24	62 (20 to 81)	0.010	61 (4 to 84)	0.040
100–119 days after vaccination	15/16	21 (-62 to 62)	0.514	39 (-48 to 74)	0.275
≥120 days after vaccination	21/25	29 (-29 to 61)	0.255	-55 (-283 to 37)	0.342
Target population for vaccination[b]					
Unvaccinated	112/98	Reference	-	Reference	-
<100 days after vaccination	7/21	71 (28 to 88)	0.007	69 (6 to 90)	0.038
100–119 days after vaccination	15/14	6 (-104 to 57)	0.871	39 (-49 to 75)	0.277
≥120 days after vaccination	21/19	3 (-90 to 51)	0.923	-51 (-298 to 42)	0.397
Primary healthcare patients					
Unvaccinated	337/185	Reference	-	Reference	-
<100 days after vaccination	10/13	58 (2 to 82)	0.045	63 (2 to 96)	0.045
100–119 days after vaccination	14/5	-54 (-333 to 45)	0.417	-2 (-215 to 67)	0.971
≥120 days after vaccination	17/7	-33 (-227 to 46)	0.530	-2 (-194 to 65)	0.971
Hospitalised patients					
Unvaccinated	21/85	Reference	-	Reference	-
<100 days after vaccination	1/10	59 (-234 to 95)	0.401	65 (-277 to 97)	0.383
100–119 days after vaccination	1/9	55 (-275 to 95)	0.460	78 (-126 to 98)	0.202
≥120 days after vaccination	7/15	-89 (-522 to 32)	0.220	-182 (-1,219 to 40)	0.187
Primary care patients swabbed <5 days after symptom onset					
Unvaccinated	334/182	Reference	-	Reference	-
<100 days after vaccination	10/12	55 (-7 to 81)	0.072	58 (-12 to 84)	0.084
100–119 days after vaccination	14/5	-53 (-330 to 46)	0.425	0 (-211 to 68)	0.999
≥120 days after vaccination	17/7	-32 (-225 to 46)	0.541	0 (-189 to 66)	0.991
Week 50/2011 to 8/2012 (12 Dec 2011–26 Feb 2012)					
Unvaccinated	298/195	Reference	-	Reference	-
<100 days after vaccination	10/24	73 (42 to 87)	0.001	65 (11 to 86)	0.027
100–119 days after vaccination	13/16	47 (-13 to 75)	0.101	48 (-32 to 79)	0.168
≥120 days after vaccination	11/2	-260 (-1,541 to 21)	0.098	-375 (-2,513 to 14)	0.073
Patients aged <65 years					
Unvaccinated	337/258	Reference		Reference	
<100 days after vaccination	6/13	65 (6 to 87)	0.038	47 (-63 to 83)	0.269
100–119 days after vaccination	6/7	34 (-98 to 78)	0.454	54 (-55 to 86)	0.210
≥120 days after vaccination	8/15	59 (2 to 83)	0.044	32 (-111 to 78)	0.503
Patients aged ≥65 years					
Unvaccinated	24/23	Reference		Reference	
<100 days after vaccination	5/11	56 (-145 to 87)	0.176	85 (-8 to 98)	0.059
100–119 days after vaccination	9/9	4 (-184 to 68)	0.939	24 (-224 to 82)	0.715
≥120 days after vaccination	16/10	-53 (-307 to 42)	0.390	-208 (-1,563 to 43)	0.192

CI: confidence interval.

[a] Vaccine effectiveness adjusted for sex, age (<5; 5–14; 15–44; 45–64; ≥65 years), major chronic conditions, outpatient visits in the previous year, hospitalisation in the previous year, healthcare setting, and period of diagnosis.

[b] Target population for vaccination includes people ≥60 years-old and people with major chronic conditions.

The point estimates of the influenza VE ranged between 61% and 69% during the first 100 days after vaccination in the analyses restricted to cases of influenza A(H3), to persons with an indication for influenza vaccination, to primary care patients, and to hospitalised patients, although this last result was not statistically significant. However, in all these analyses the vaccine had practically zero effectiveness at 120 or more days after vaccination (Table 3). In persons under 65 years of age the VE declined little with time since vaccination, whereas in those aged 65 years or older the OR for the risk of influenza was 20.81 (95% CI: 2.14 to 202.71; p=0.009) for those vaccinated more than 120 days previously versus those vaccinated less than 100 days previously.

Genetic characterisation

In total 102 isolates obtained from the confirmed cases, were further characterised by phylogenetic analysis of the HA1 sequence of the haemagglutinin gene in the National Influenza Centre - Madrid laboratory: 90 were influenza A(H3N2), 11 influenza B and one was influenza A(H1N1)pdm09. The strains most frequently identified were similar to A/Victoria/361/2011(H3N2) (41.2%), A/England/259/2011(H3N2) (24.5%), A/Iowa/19/2010(H3N2) (20.6%) and B/Bangladesh/3333/2007(Yamagata) (9.8%). The proportions of strains were similar in the two periods from week 50/2011 to 8/2012 (12 December 2011 to 26 February 2012) and from week 9/2012 to 20/2012 (27 February to 20 May 2012), except for a reduced proportion of characterisations of strain A/England/259/2011(H3N2) and an increase of B/Bangladesh/3333/2007(Yamagata) (Table 4).

Discussion

The results of this study suggest that on average, the seasonal influenza vaccine had a low protective effect in preventing laboratory-confirmed influenza during the 2011/12 season in Navarre. Most of the strains we characterised showed reduced reactivity with post-infection ferret antiserum raised against the vaccine viruses, suggesting a certain degree of vaccine-virus mismatch [19]. Although the confidence intervals were wide, similar estimates were obtained in analyses restricted to the target population for vaccination, to primary healthcare patients, or to patients swabbed within the first four days after symptom onset, which strengthens the conclusion and rules out possible biases. Evaluation of VE in preventing cases of influenza A(H3) only, also yielded similar estimates.

The early estimates of influenza VE for the first part of the season were higher than what we found for the complete season [20,21], which suggests a decline in VE over time. Two possible mechanisms, or a combination of both, could explain this reduced VE. The first is a change in the viruses circulating during the season, either due to appearance of another virus type or due to antigenic drift of circulating viruses, resulting in a loss of the match with the vaccine viruses. Our results do not support this mechanism, since the only relevant change in the circulating viruses was an increase in influenza B viruses, and low VE was also observed when we evaluated the effectiveness of the vaccine against influenza A(H3) only.

The second possible mechanism is waning immunity in those who received the vaccine. It has been reported that antibody levels begin to fall one month after

Table 4

Distribution of influenza cases by type of virus and distribution of cases with characterisation by strains in two calendar periods. Navarre, Spain, 2011-2012

	Week 50/2011 to 8/2012 (12 Dec 2011–26 Feb 2012) n (%)	Week 9/2012 to 20/2012 (27 Feb –20 May 2012) n (%)	p value
All cases			
Influenza A(H3)	325 (97.9)	57 (72.2)	<0.001
Influenza A(H1N1)pdm09	1 (0.3)	0 (0)	1.000
Influenza B	6 (1.8)	22 (17.8)	<0.001
Total	332 (100)	79 (100)	-
Cases with characterisation			
Influenza A/Victoria/361/2011(H3N2)	34 (42.5)	8 (36.4)	0.635
Influenza A/England/259/2011(H3N2)	24 (30.0)	1 (4.5)	0.012
Influenza A/Stockholm/18/2011(H3N2)	2 (2.5)	0 (0)	1.000
Influenza A/Iowa/19/2010(H3N2)	17 (21.3)	4 (18.2)	1.000
Influenza B/Bangladesh/3333/2007(Yamagata)	2 (2.5)	8 (36.4)	<0.001
Influenza B/Brisbane/60/2008(Victoria)	0 (0)	1 (4.5)	0.216
Influenza A/St Petersburg/100/2011(H1N1) pdm09	1 (1.3)	0 (0)	1.000
Total	**80 (100)**	**22 (100)**	**-**

administration of the influenza vaccine [10]. This loss of immune response is more pronounced in older persons [10-12]. The results of our study show a decline in the VE beginning 100 days after vaccination, primarily in persons aged 65 years or older. This finding could be explained by an immunosenescence phenomenon, aggravated by the long time between vaccination and virus circulation, which was longer than in most other seasons [13], and the limited match between vaccine and circulating strains [20,21].

Longer time between symptom onset and swabbing has been associated with reduced sensitivity in virus detection, which could underestimate VE [6]. We controlled for this effect mainly in the design of our study, since 99% of the swabs from primary healthcare patients were taken within the first four days after symptom onset. Moreover, we repeated the analysis after eliminating the cases swabbed after the first four days, and no relevant changes in the estimate of VE were found.

The present study included both outpatient and hospital cases systematically recruited in a previously defined population. Primary care patients made up the bulk of subjects in our study and, when the analysis was limited to these patients, the VE was maintained. The number of cases treated in hospitals was small, which did not allow us to obtain a specific estimate of the VE in preventing hospitalised cases.

Although institutionalised patients were not included in this study, several influenza outbreaks in nursing homes with high vaccination coverage were detected in Navarre in the 2011/12 season [22]. This may be considered another consequence of the low VE.

This case–control analysis included only laboratory-confirmed cases and compared them with test-negative controls recruited in the same healthcare settings before either patient or physician knew the laboratory result, a fact that provides better comparability and reduces selection bias [6]. This type of design has been used in other studies that have evaluated influenza VE [20,21,23,24]. The case¬–control study was nested in a population cohort for which extensive and reliable databases were available, and which was treated in hospitals and primary healthcare by physicians trained to detect and swab ILI patients, all of which can prevent unmeasured confounding [25].

In interpreting the results, some limitations must be kept in mind. The study size was insufficient to demonstrate a VE under 40%, which was reflected in wide confidence intervals that included zero. It may not be possible to generalise the results and apply them to other geographical areas, although other published studies in the same influenza season are consistent with our data [20,21]. Although RT-PCR has high sensitivity for the detection of influenza virus, we cannot completely rule out some false negative results, which would cause a small underestimation of the VE. Although all the analyses were adjusted for the commonly recognised confounding factors, some residual confusion is possible [6].

These results suggest that VE may vary throughout the influenza season. The early estimates of influenza VE obtained in mid-season may drop during the season. This situation should be kept in mind given its implications for clinical practice and public health; it should not be interpreted as an error in the estimates, but as a description of reality. These early estimates remain enormously useful in redirecting preventive strategies during the influenza season and because they can aid the selection of strains to be included in the following season's vaccine [20,21].

The description of situations in which influenza VE is low should serve as a stimulus to design better influenza vaccines [26], to improve the selection of strains contained in the vaccine, to choose the most appropriate time for vaccination in each area, to encourage vaccination of caregivers of high-risk individuals, and to highlight the importance of other preventive measures that complement vaccination in high-risk populations, such as promotion of basic hygiene measures and avoidance of contact with influenza cases [27]. Early treatment with antiviral drugs should be considered in persons diagnosed with influenza who have a high risk of complications, regardless of vaccination status [28]. In seasons when influenza starts late, it may be useful to revaccinate persons with a high risk of complications, especially those who may have a reduced immune response due to immunosenescence or immunodepression.

Even in seasons in which the effectiveness of influenza vaccine is low, it may appreciably reduce the number of cases and hospitalisations in high-risk persons. In the 2011/12 season in Navarre, the vaccine managed to avoid almost one third of the influenza cases in the vaccinated at-risk population; while not entirely satisfactory, this result is important in terms of individual and public health.

Conclusions

Our results support a low protective effect of the 2011/12 seasonal vaccine in Navarre and suggest a decline in VE in the elderly with time since vaccination. Even under these conditions, annual immunisation of high-risk populations against influenza remains of interest, although it should be complemented with other preventive initiatives such as basic hygiene measures, vaccination of caregivers and avoidance of contact with influenza cases.

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Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection

R G Pebody (richard.pebody@hpa.org.uk)[1], N Andrews[1], J McMenamin[2], H Durnall[3], J Ellis[4], C I Thompson[4], C Robertson[5,6], S Cottrell[7], B Smyth[8], M Zambon[4], C Moore[7], D M Fleming[3], J M Watson[1]

1. Health Protection Agency Health Protection Services – Colindale, London, United Kingdom
2. Health Protection Scotland, Glasgow, United Kingdom
3. Royal College of General Practitioners Research and Surveillance Centre, Birmingham, United Kingdom
4. Health Protection Agency Microbiology Services – Colindale, London, United Kingdom
5. University of Strathclyde, Glasgow, United Kingdom
6. International Prevention Research Institute, Lyon, France
7. Public Health Wales, Cardiff, United Kingdom
8. Public Health Agency Northern Ireland, Belfast, United Kingdom

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The 2011/12 season was characterised by unusually late influenza A (H3N2) activity in the United Kingdom (UK). We measured vaccine effectiveness (VE) of the 2011/12 trivalent seasonal influenza vaccine (TIV) in a test-negative case–control study in primary care. Overall VE against confirmed influenza A (H3N2) infection, adjusted for age, surveillance scheme and month, was 23% (95% confidence interval (CI): -10 to 47). Stratified analysis by time period gave an adjusted VE of 43% (95% CI: -34 to 75) for October 2011 to January 2012 and 17% (95% CI: -24 to 45) for February 2012 to April 2012. Stratified analysis by time since vaccination gave an adjusted VE of 53% (95% CI: 0 to 78) for those vaccinated less than three months, and 12% (95% CI: -31 to 41) for those vaccinated three months or more before onset of symptoms (test for trend: p=0.02). For confirmed influenza B infection, adjusted VE was 92% (95% CI: 38 to 99). A proportion (20.6%) of UK influenza A(H3N2) viruses circulating in 2011/12 showed reduced reactivity (fourfold difference in haemagglutination inhibition assays) to the A/Perth/16/2009 2011/12 vaccine component, with no significant change in proportion over the season. Overall TIV protection against influenza A(H3N2) infection was low, with significant intraseasonal waning.

Introduction

Following the 2009 influenza pandemic and the first post-pandemic influenza season which was dominated by influenza A(H1N1)pdm09 virus activity, the United Kingdom (UK) experienced unusually late influenza activity in 2011/12, peaking only in week 8/2012 [1]. The dominant circulating influenza virus in 2011/12 was influenza A(H3N2), with the disease burden falling particularly on the elderly population, as evidenced by an increase in excess all-cause mortality and influenza outbreaks in nursing home settings. A number of these end-of-season outbreaks occurred in populations highly vaccinated with influenza vaccine [1]. Influenza B also circulated throughout the 2011/12 season, particularly in January and February 2012.

In 2011/12, the UK, like many other countries, utilised non-adjuvanted trivalent seasonal influenza vaccines (TIV) targeted at all those over 65 years of age and at those under the age of 65 years falling into a clinical risk group. The 2011/12 TIV contained the three influenza strains A/California/7/2009 (H1N1) pdm09-like virus, A/Perth/16/2009 (H3N2)-like virus, B/Brisbane/60/2008-like virus, as recommended by the World Health Organization (WHO) for the 2011/12 winter season in the northern hemisphere [1]. The vaccination programme started in September 2011 and reached an uptake of 74% in those over 65 years of age and 51.6% in those under 65 years of age falling into a clinical at-risk group by the end of January 2012 in England [2]. Early 2011/12 season estimates suggested a low to moderate VE against influenza A(H3) of 43% (95% CI: -0.4 to 67.7). The occurrence of late season outbreaks led to questions about whether protection had waned following the 2011/12 vaccination programme earlier in the season [3,4].

This study presents the end-of-season vaccine effectiveness (VE) for the 2011/12 seasonal TIV in preventing medically attended confirmed influenza A(H3N2) and B infection. It also examines the protective effect of vaccination at different points during the season and by time since vaccination, to determine if there is any evidence of intraseasonal waning protection. The results

are put into context with available antigenic data for circulating A(H3N2) viruses.

Methods

Study population and period

Data were derived from five primary care influenza sentinel surveillance schemes in England (two schemes), Scotland, Wales and Northern Ireland. Details of the Royal College of General Practitioners (RCGP), Health Protection Agency (HPA) Specialist Microbiology Network (SMN), Public Health Wales, Public Health Agency (PHA) of Northern Ireland and Health Protection Scotland (HPS) swabbing schemes have been presented previously [5].

The study period ran from 1 October 2011 to 16 April 2012. Cases were defined, as persons presenting during the study period in a participating GP practice with an acute influenza-like illness (ILI) who were swabbed and then tested positive for influenza A(H3N2) or B. A case of ILI was defined as an individual presenting in primary care with an acute respiratory illness with fever or complaint of feverishness. Patients were swabbed as part of clinical care, with verbal consent. Controls were individuals presenting with ILI in the same period who were swabbed and tested negative for influenza. Individuals testing positive for other influenza A types (including A(H1N1)pdm09) were excluded from the study.

A standardised questionnaire collected demographic, clinical and epidemiological information from cases and controls including date of birth, sex, defined underlying clinical risk group, date of onset of respiratory illness, date of specimen collection, and influenza vaccination status for 2011/12 with vaccination dates completed by the patient's responsible general practitioner.

Laboratory methods

Laboratory confirmation was undertaken using real-time polymerase chain reaction (RT-PCR) assays for circulating influenza A viruses, influenza B viruses and other respiratory viruses [6,7]. Samples in England were sent to the HPA Microbiology Services, Colindale (RCGP scheme) or one of the specialist HPA microbiology laboratories (SMN scheme). Samples in Wales were sent to the Public Health Wales Specialist Virology Centre and in Scotland to the West of Scotland Specialist Virology Centre (HPS scheme) for molecular testing. In Northern Ireland samples were sent to the Regional Virus Laboratory, Belfast. Influenza viruses were isolated in MDCK or MDCK-SIAT1 cells from RT-PCR positive samples as previously described [8]. Virus isolates were characterised antigenically using post-infection ferret antisera in haemagglutination inhibition (HI) assays, with guinea pig red blood cells [9]

Statistical methods

Persons were defined as vaccinated if date of vaccination with the 2011/12 TIV was 14 or more days before onset of illness. Those in whom the period between vaccination and onset of illness was less than 14 days were excluded, as their immune status was unclear. If the date of vaccination was missing, as the 2011/12 campaign occurred before influenza circulation, it was assumed that TIV vaccination was more than14 days before onset date. If date of onset of symptoms was missing then the date was assumed to have been four days before the swab was taken (the median interval based on the observed data). Respiratory samples with a delay greater than 29 days between onset of illness and sample collection were excluded as the sensitivity of the PCR test decreases for long intervals between onset and sampling. A sensitivity analysis was also undertaken, censoring at seven days between onset of illness and sample collection.

VE was estimated as 1-(odds ratio) using multivariable logistic regression models with influenza A(H3N2) or influenza B PCR results as outcomes and seasonal vaccination status as the linear predictor. In the analyses evaluating VE in preventing influenza A(H3N2) infection, samples positive for influenza B were excluded, and vice versa. Age (coded into five standard age groups, <5 years, 5–14 years, 15–44 years, 45–64 years and ≥65 years), sex, clinical risk group, surveillance

Table 1

Inclusion and exclusion criteria of participants for specimens submitted, United Kingdom, October 2011–April 2012

Criteria	N Excluded	N Included
1. Original participants		**3,869**
Excluded as interval from onset to sampling >29 days	81	
Remaining participants		3,788
2. Analysis of TIV 2010/11		
Excluded as missing vaccination history	166	
Excluded as vaccinated 0–14 before onset	62	
Final remaining study participants		*3,560*
Final for assessment of influenza A(H3N2)		*3,517*
Final for assessment of influenza B		*3,184*

TIV: trivalent seasonal influenza vaccine.

Table 2

Details for influenza A(H3N2) and B cases and controls, United Kingdom, October 2011–April 2012 (n=3,869)

	Controls (N=3,428) n (%)	Influenza B cases (N=45) n (%)	Influenza A(H3N2) cases (N=396) n (%)
Age group (years)			
<5	257 (7.5)	3 (6.6)	57 (14.4)
5–14	292 (8.5)	10 (22.2)	65 (16.4)
15–44	1,609 (47.0)	18 (40.0)	160 (40.4)
45–64	834 (24.3)	12 (26.7)	86 (21.7)
65+	423 (12.3)	2 (4.4)	26 (6.6))
Missing	13 (0.4)	0 (0.0)	2 (0.5)
Sex			
Male	1,350 (39.4)	18 (40.0)	190 (48.0)
Female	2,052 (59.9)	27 (60.0)	201 (50.8)
Missing	26 (0.8)	0 (0.0)	5 (1.3)
Month of sample collection			
October	477 (13.9)	0 (0.0)	3 (0.8)
November	735 (21.4)	1 (2.2)	4 (1.0)
December	731 (21.3)	3 (6.7)	14 (3.5)
January	578 (16.9)	6 (13.3)	56 (14.1)
February	470 (13.7)	20 (44.4)	173 (43.7)
March	365 (10.7)	13 (28.9)	137 (34.6)
April	72 (2.1)	2 (4.4)	9 (2.3)
Surveillance scheme			
RCGP	1,748 (51.0)	23 (51.1)	267 (67.4)
SMN	305 (8.9)	12 (26.7)	31 (7.8)
HPS	1,198 (35.0)	9 (20.0)	89 (22.5)
Wales	61 (1.8)	0 (0.0)	0 (0.0)
Northern Ireland	116 (3.4)	1 (2.2)	9 (2.3)
Risk group			
No	2,365 (69.0)	33 (73.3)	301 (76.0)
Yes	709 (20.7)	6 (13.3)	60 (15.2)
Missing	354 (10.3)	6 (13.3)	35 (8.8)
Interval onset to sampling (days)			
0–1	338 (9.9)	4 (8.9)	62 (15.7)
2–4	1,223 (35.7)	22 (48.9)	193 (48.7)
5–7	812 (23.7)	11 (24.4)	80 (20.2)
8–14	506 (14.8)	5 (11.1)	22 (5.6)
15–29	236 (6.9)	1 (2.2)	10 (2.5)
≥29	74 (2.2)	0 (0.0)	7 (1.8)
Missing onset date	239 (7.0)	2 (4.4)	22 (5.6)
Vaccination status (only considering TIV)			
Unvaccinated	2,586 (75.4)	43 (95.6)	325 (82.1)
Vaccinated (0–13 days ago)	62 (1.8)	1 (2.2)	0 (0.0)
Vaccinated (14–91 days ago[a])	402 (11.7)	0 (0.0)	8 (2.0)
Vaccinated (>91 days ago[a])	221 (6.5)	1 (2.2)	50 (12.6)
Missing	157 (4.6)	0 (0.0)	13 (3.3)

HPS: Health Protection Scotland; RCGP: Royal College of General Practitioners' surveillance scheme; SMN: Health Protection Agency (HPA) Specialist Microbiology Network.

Note: Differences between cases and controls for all variables in this table were statistically significant.

[a] Where a date of vaccination was missing this was estimated by assuming vaccination was on 19 October 2011, the median time of vaccination in controls with onset in 2012.

scheme (RCGP, SMN, HPS, Wales, Northern Ireland) and date of sample collection (month) were investigated as potential confounding variables. To investigate whether the VE changed in relation to time since vaccination analyses stratifying influenza A(H3N2) VE by time since vaccination (<3 months, ≥3 months) and by period (October to January, February to April) were undertaken. To test for the significance of changes in VE with the time since vaccination, the multivariable logistic regression was performed in vaccinated individuals with days since vaccination (between vaccination and onset date) included as a continuous variable. As testing for evidence of waning was one of the primary study objectives of the study, multiple testing adjustments were not made.

All statistical analyses were carried out in Stata version 12 (StataCorp, College Station, Texas).

Results

A total of 3,893 individuals were swabbed in primary care during the study period. Six were excluded because they were positive for influenza A(H1N1) pdm09, two because the swab result was inconclusive and 16 because no laboratory result was available. This left 3,869 persons in the analysis. Table 1 summarises which of those individuals were excluded from the analysis of effectiveness.

Of these 3,869, 2,038 (52%) were collected from the RCGP scheme, 1,296 (33%) from the HPS scheme, 348 (9%) from the SMN scheme, 61 (2%) from the Public Health Wales Scheme and 126 (3%) from the Northern Ireland Scheme. The demographic and epidemiological characteristics of cases and controls are summarised in Table 2. There were statistically significant differences between cases and controls for all variables in Table 2. Vaccine date was unknown for 148 individuals who had received TIV. Although date of onset was missing for 263 (7%) individuals, these were included with onset date defined as swab date minus four days.

Model fitting for vaccine effectiveness estimation

When estimating vaccine effects, age group, sex, time period (defined by month of sample collection) and surveillance scheme were adjusted for in a multivariable logistic regression model. Although all these variables were significantly associated with having a positive swab, only age group and month of sample collection were confounders for the vaccine effects. Tables 3, 4 and 5 show vaccine effectiveness estimates against influenza A(H3N2) and B according to vaccination status and time since vaccination and period.

Vaccine effectiveness against influenza A(H3N2) infection

The adjusted VE estimate for TIV 2011/12 against influenza A(H3N2) was 23% (95% confidence interval (CI): -10 to 47). Stratifying by time period resulted in an adjusted VE for TIV 2011/12 of 43% (95% CI: -34 to 75) for the period October 2011 to January 2012, compared with 17% (95% CI: -24 to 45) for the period February 2012 to April 2012 (Table 3).

Table 3

Samples positive (cases) and negative (controls) for influenza A(H3N2) according to vaccination status and vaccine effectiveness estimates, United Kingdom, October 2011–April 2012 (n=3,517 for crude, n=3,474 for adjusted analysis)

Period	Vaccination status	Number of cases: controls	Crude VE % (95% CI)	Adjusted VE[a] % (95% CI)
Oct 2011–Apr 2012	Unvaccinated	320:2,531	26 (1 to 45)	23 (-10 to 47)
	Vaccinated	57:609		
Oct 2011–Jan 2012	Unvaccinated	60:1,861	42 (-22 to 73)	43 (-34 to 75)
	Vaccinated	8:430		
Feb 2012–Apr 2012	Unvaccinated	260:670	29 (1 to 50)	17 (-24 to 45)
	Vaccinated	49:179		

CI: confidence interval; VE: vaccine effectiveness.

[a] Adjusted for age group, sex, month and surveillance scheme.

Table 4

Samples positive (cases) and negative (controls) for influenza B according to vaccination status and vaccine effectiveness estimates, United Kingdom, October 2011–April 2012 (n=3,184 for crude, n=3,148 for adjusted analysis)

Period	Vaccination status	Number of cases: controls	Crude VE % (95% CI)	Adjusted VE[a] % (95% CI)
October 2011–April 2012	Unvaccinated	43:2,531	90 (30 to 99)	92 (38 to 99)
	Vaccinated	1:609		

CI: confidence interval; VE: vaccine effectiveness.

[a] Adjusted for age group, sex, month and surveillance scheme.

The adjusted age-specific estimates suggested protection was lower in the middle age groups (15 to 64 years), although the observed differences were not significant. There were significant differences in VE in relation to the interval since vaccination, with an adjusted VE of 53% (95% CI: 0 to 78) if the time from onset to vaccination was less than three months, compared with 12% (95% CI: -31 to 41) if the time was three months or more (test for trend: p=0.02).

The adjusted VE for TIV 2011/12 against influenza A(H3N2) with time since vaccination and interval from onset to swab included in the model is shown in Table 5. There was no significant difference in adjusted VE by scheme or by time from onset to swab (Table 5). Information on risk group was missing for 395 of 3,869 samples (10.2%) and was therefore not included in the final model. If risk group was included, the VE estimates remained unchanged.

Vaccine effectiveness against influenza B infection

The adjusted VE of TIV against influenza B was 92% (95% CI: 38 to 99) adjusted for age group, sex, time period and surveillance scheme. There no evidence that the VE varied by age group, although the numbers were small (with only a single vaccinated influenza B case with a B/Yamagata lineage infection). It was therefore not possible to stratify by time since vaccination, or by time period, to determine if there was reduction in protection.

Antigenic characterisation of circulating A(H3N2) viruses

The majority of the 160 A(H3N2) 2011/12 viruses analysed (79.4%) were antigenically similar to the A/Perth/16/2009 2011/12 H3N2 vaccine component, with some (20.6%) A(H3N2) viruses showing reduced reactivity in antigenic characterisation assays with antiserum

Table 5

Adjusted vaccine effectiveness estimates for influenza A(H3N2) by age, surveillance scheme and by time since vaccination, United Kingdom, October 2011–April 2012 (n=3,478)

Factor	Level	Adjusted VE[a] % (95% CI)	p value for VE varying across factor
Age	<5	52 (-446 to 96)	0.83
	5–14	69 (-172 to 97)	
	15–44	7 (-67 to 48)	
	45–64	11 (-56 to 49)	
	All <65	19 (-19 to 45)	
	≥65	48 (-50 to 82)	
Scheme	RCGP	36 (0 to 60)	0.37
	SMN[b]	-46 (-600 to 45)	
	HPS[b]	-4 (-107 to 48)	
	Wales	N too low	
	Northern Ireland	N too low	
Time since vaccination	<3 months	53 (0 to 78)	0.02[c]
	≥3 months	12 (-31 to 41)	
Interval onset to swab	<7 days	23 (-15 to 50)	0.69
	7 to 29 days or not known	29 (-72 to 70)	

CI: confidence interval; HPS: Health Protection Scotland; RCGP: Royal College of General Practitioners' surveillance scheme; RMN: Health Protection Agency (HPA) Specialist Microbiology Network, VE: vaccine effectiveness.

[a] Adjusted for age group, sex, month and surveillance scheme.

[b] Note that positive swabs from SMN and HPS were mainly taken after January 2012 with only four and six positive samples by January, respectively. RCGP had 65 positive swabs by January and gave a VE estimate for samples up to January of 50% (95% CI: -25 to 80), and one of 59% (95% CI: 1 to 83) for those vaccinated within three months before symptom onset.

[c] Test for trend using time since vaccination as continuous.

Table 6

Proportion of influenza A/H3N2 isolates with difference in haemagglutination inhibition assay titres compared to the A/Perth/16/2009 2011/12 H3N2 vaccine component, United Kingdom, October 2011–April 2012 (n=160)

Period	<4-fold difference in HI	4-fold difference in HI	>4-fold difference in HI
October 2011–January 2012	86.9% (20/23)	13.0% (3/23)	0% (0/23)
February 2012–April 2012	78.1% (107/137)	21.9% (30/137)	0% (0/137)
October 2011–April 2012	79.4% (127/160)	20.6% (33/160)	0% (0/160)

HI: haemagglutination inhibition.

raised against influenza A/Perth/16/2009 (fourfold difference in HI assays; Table 6). A more than fourfold difference in HI assay titres with reference antiserum is considered to be significant antigenic drift [10]. The proportion with a fourfold difference increased but did not change significantly over the duration of the 2011/12 season (from 13% in the period October 2011 to January 2012 to 21.9% in the period February 2012 to April 2012). Antigenic analysis of A(H3N2) virus isolates from combined sentinel and non-sentinel sources, confirmed the change in proportion over the two time periods to be non-significant (data not shown).

Discussion

This observational study of influenza VE for TIV against laboratory-confirmed influenza infection in primary care in the UK 2011/12 winter season, a late, low intensity influenza season with A(H3N2) as the dominant circulating strain, has several key findings: firstly, the 2011/12 seasonal influenza vaccine was overall poorly protective in preventing influenza A(H3N2) infection; secondly, vaccine protection was moderate in the first three months of the season, but reduced in the second three months; thirdly, there was evidence of waning protection against influenza A(H3N2) three months after vaccination; and finally, the 2011/12 TIV was highly protective against the circulating influenza B strain.

The test-negative case–control study design is becoming an increasingly well established approach to measure influenza vaccine effectiveness [11,12]. One criticism of the method relates to the selected control population (test-negatives). In fact, use of this control group of individuals consulting in primary care with a respiratory illness that is not influenza is believed to overcome differences in health-seeking behaviour between cases and controls. Another criticism relates to the inclusion of individuals who were tested up to 29 days after disease onset, rather than those tested within seven days of onset. It is argued that test sensitivity declines with time from onset to swab and that such an approach may result in misclassification of cases as controls. We demonstrated that restricting samples to those taken within seven days of symptom onset did not significantly change the estimated vaccine effectiveness, although it did lead to loss of power as individuals were discarded. We did not adjust for multiple testing because waning was a priori of interest and was an objective of the study. This study based on surveillance data only had access to limited information on confounders. However, observational VE studies based on routine electronic health data in primary care using RCGP data [13] suggest that the most important confounders have been captured in our analysis. Indeed in our paper, we found risk status was not an important confounding variable, and to maximise power it was not included in the final multivariable analysis.

Our study demonstrates that during the 2011/12 influenza season, the 2011/12 TIV was overall poorly effective (with a non-significant adjusted VE of 23%) in protecting against confirmed influenza A(H3N2) infection for persons consulting their general practitioner (GP) with an ILI. Early estimates from the 2011/12 season have been published by several other countries – including a pooled case–control study from several European countries [3] and a study from Spain [4], demonstrating a low to moderate VE (43% and 55% respectively). It has been postulated in these studies that this could be due to a combination of a poor match between the 2011/12 TIV A(H3N2) virus strain (A/Perth/16/2009) and the circulating A(H3N2) virus, and a waning protection. In the UK we found that the majority of characterised A(H3N2) viruses were antigenically similar to the vaccine component, with a notable proportion of A(H3N2) viruses showing some reduced reactivity in antigenic characterisation assays, but no significant change in that proportion over the duration of the 2011/12 season. Thus a certain degree of mismatch may explain the initial moderate protection, but does not seem to provide a complete explanation for the observed reduction in vaccine effectiveness over the course of the season and with increasing time since vaccination. These observations could challenge our current view on how mismatch is to be defined – an issue highlighted by Skowronski et al. [14]

An alternative explanation may be waning immunity. Our study demonstrates that influenza A(H3N2) vaccine effectiveness was higher in the first three months of the 2011/12 season compared to the last three months. In addition, TIV VE was moderate and significantly higher when disease onset was within three months of vaccination compared to three months or more. The UK, indeed, experienced an extremely late and mild influenza season in 2011/12, with influenza A(H3N2) activity not peaking until week 8 in 2012, such as has rarely been observed in previous GP weekly consultation data from RCGP (for example activity peaked in week 11 in 1993 when the dominant circulating strains were A(H1N1) and B, with both strains included in the vaccine). This present observation was accompanied by reports of outbreaks of influenza A(H3N2) in nursing home settings, which frequently had a high proportion of vaccinated persons [1]. Waning intraseasonal vaccine protection would provide an explanation for these observations. At least two published studies have demonstrated intraseasonal waning in antibody titre following seasonal influenza vaccination [15,16]. Bothshowed a significant reduction in antibody titre in elderly populations 20 to 22 weeks after vaccination. This would provide a biological explanation for our observed reduction in vaccine effectiveness over this particularly late season, where the median time from vaccination to disease onset was approximately three months. There are few reports of this in the literature: a large summertime outbreak due to circulation of a drifted A/Sydney/05/97-like (H3N2) virus reported in elderly tourists in Alaska was reported to have been

due to a combination of drift and waning immunity [17]. Our study was not adequately powered to be able to examine age-specific differences in waning and to determine if the effect was particularly marked in the elderly.

The 2011/12 TIV VE estimate against influenza B demonstrates high protection. This corresponds only partially with the virological data, which shows that in 2011/12, both B/Yamagata-lineage and B/Victoria-lineage influenza B viruses co-circulated in the UK. Furthermore the majority of influenza B circulated late in the season, like the A(H3N2) virus [1]. Thus although we were not able to formally examine if there had been a reduction in protection connected to either time in the season or time since vaccination, effectiveness against influenza B was still high at the end of the season, with single vaccine failure occurring in a person infected with the B/Yamagata-lineage non-vaccine strain.

In conclusion, this end of season study provides important evidence that the 2011/12 season's TIV provided good protection against influenza B, but overall poor protection against the dominant circulating influenza A(H3N2) virus. This observation seems to be at least partially related to waning protection. The relative contributions of waning immunity and vaccine mismatch are unclear. This highlights the importance of future work to examine this phenomenon further. The study, however, reinforces the recommendation that annual re-immunisation of target groups is required regardless of TIV vaccination the previous season. The concept that vaccine protection can be so short-lived provides a challenge for public health policy. Influenza immunisations are given before the start of the influenza season when vaccine becomes available. In many winters, protection will therefore be optimal when the peak period of activity occurs in the first half of the winter. Influenza activity, however, can occur in the second half of the winter season, when protection may be waning. This highlights the pressing need for the development of influenza vaccines which provide better and longer-lasting protection, whether in terms of antigen content or formulation, e.g. through the use of adjuvants. In the interval, until such vaccines become available, this poses a policy question about whether there is a role for a second dose of seasonal influenza vaccine in certain circumstances: for example, when faced with late season outbreaks particularly in the groups most at risk of complications.

Our findings reinforce the need for annual revaccination and for early intraseasonal estimates of vaccine effectiveness to provide information for public health action, in particular to inform the annual WHO recommendation for composition of the vaccine for the following season. The identification of low or moderate vaccine effectiveness may allow communication of public health messages to clinicians to suspect influenza infection even in their highly vaccinated populations and have a lower threshold for prescribing of antiviral drugs to prevent the worst complications of influenza.

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Conflict of interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that DM Fleming has received funding to attend influenza related meetings and has received consultancy fees from influenza vaccine manufacturers who might have an interest in the submitted work in the previous three years. In addition, The Virus Reference Department of the Health Protection Agency receives funding from a variety of vaccine manufacturers who might have an interest in the submitted work. All other authors declare they have no conflicts of interest.

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Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case–control study

E Kissling (e.kissling@epiconcept.fr)[1], M Valenciano[1], A Larrauri[2], B Oroszi[3], J M Cohen[4], B Nunes[5], D Pitigoi[6,7], C Rizzo[8], J Rebolledo[9,10], I Paradowska-Stankiewicz[11], S Jiménez-Jorge[2], J K Horváth[3], I Daviaud[4], R Guiomar[5], G Necula[6], A Bella[8], J O'Donnell[9], M Głuchowska[11], B C Ciancio[12], A Nicoll[12], A Moren[1]

1. EpiConcept, Paris, France
2. National Centre for Epidemiology, Instituto de Salud Carlos III, Madrid, Spain
3. Office of the Chief Medical Officer, Budapest, Hungary
4. GROG/Open Rome, Paris, France
5. Instituto Nacional de Saude Dr Ricardo Jorge, Lisbon, Portugal
6. Cantacuzino Institute, National Institute of Research – Development for Microbiology and Immunology, Bucharest, Romania
7. Universitatea de Medicina si Farmacie Carol Davila, Bucharest, Romania
8. National Centre for Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità, Roma, Italy
9. Health Protection Surveillance Centre, Dublin, Ireland
10. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
11. National Institute for Public Health, Warsaw, Poland
12. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

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Within the Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) project we conducted a multicentre case–control study in eight European Union (EU) Member States to estimate the 2011/12 influenza vaccine effectiveness against medically attended influenza-like illness (ILI) laboratory-confirmed as influenza A(H3) among the vaccination target groups. Practitioners systematically selected ILI / acute respiratory infection patients to swab within seven days of symptom onset. We restricted the study population to those meeting the EU ILI case definition and compared influenza A(H3) positive to influenza laboratory-negative patients. We used logistic regression with study site as fixed effect and calculated adjusted influenza vaccine effectiveness (IVE), controlling for potential confounders (age group, sex, month of symptom onset, chronic diseases and related hospitalisations, number of practitioner visits in the previous year). Adjusted IVE was 25% (95% confidence intervals (CI): -6 to 47) among all ages (n=1,014), 63% (95% CI: 26 to 82) in adults aged between 15 and 59 years and 15% (95% CI: -33 to 46) among those aged 60 years and above. Adjusted IVE was 38% (95%CI: -8 to 65) in the early influenza season (up to week 6 of 2012) and -1% (95% CI: -60 to 37) in the late phase. The results suggested a low adjusted IVE in 2011/12. The lower IVE in the late season could be due to virus changes through the season or waning immunity. Virological surveillance should be enhanced to quantify change over time and understand its relation with duration of immunological protection. Seasonal influenza vaccines should be improved to achieve acceptable levels of protection.

Introduction

Unlike the formulation of other vaccines, the formulation of seasonal influenza vaccines is reviewed annually by the World Health Organization (WHO) and frequently adapted to the constantly evolving nature of influenza viruses.

How the vaccine performs in target group populations cannot be anticipated by pre-authorisation efficacy trials in healthy young adults, immunogenicity studies or the relatedness of vaccine and circulating viruses. Field influenza vaccine effectiveness (IVE) studies provide essential additional information to advise stakeholders on the performance of the vaccine, to contribute to vaccine strain selection process and to inform when additional measures, such as antivirals, are needed given a low observed effectiveness early in the season.

In the European Union (EU) countries, the seasonal influenza vaccine is recommended annually for specific target groups, including those at risk of severe disease, the largest groups being older individuals (generally 60 or 65 years and above, depending on the country) and all those over six months of age with

underlying medical conditions in the following categories: chronic respiratory and cardiovascular diseases, chronic metabolic disorders, chronic renal and hepatic diseases and immune system dysfunctions (congenital or acquired) [1].

In 2007, the European Centre for Disease Prevention and Control (ECDC) and a network of 18 public health institutes established the Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) project which monitors IVE each season in the EU and the European Economic Area (EEA) [2]. Currently 20 public health institutes from the EU and EEA are part of the I-MOVE network, which is coordinated by EpiConcept under the umbrella of ECDC [3]. One component of I-MOVE is a multicentre case–control study, which has provided IVE estimates each season since the pilot season in 2008/09 [4–8]. All study sites follow a generic protocol [9].

During the pilot phase in the 2008/09 season, the pooled adjusted IVE estimates from the multicentre case–control study, restricted to individuals aged 65 years and above , suggested an overall IVE of 59.1% (95% confidence intervals (CI): 15.3 to 80.3%). In the subsequent season 2009/10, an adjusted pandemic IVE of 71.9% (95% CI: 45.6 to 85.5) among all age groups was estimated and in the 2010/11 season an adjusted IVE of 56.2% (95% CI: 34.3 to 70.7) was calculated among the target group for vaccination [4,5,7].

The aim was to provide overall and age-specific IVE estimates among what is defined as the target group for vaccination in these countries [10–17]. We restricted the analysis to influenza A(H3), as this was the predominant strain during the season [18]. The 2011/12 seasonal influenza A(H3) vaccine virus for the northern hemisphere was A/Perth/16/2009 (H3N2)-like virus.

Methods

The eight study sites included in the 2011/12 I-MOVE multicentre case–control study were based in France, Hungary, Ireland, Italy, Poland, Portugal, Romania and Spain. At each study site, practitioners already participating in the European Influenza Surveillance Network (EISN) were invited to take part in the study [19]. In addition, study sites in Hungary and Portugal invited practitioners outside the EISN network.

The study population consisted of non-institutionalised influenza-like-illness (ILI) patients without contraindications for vaccination who were swabbed within less than eight days after symptom onset. Practitioners carried out naso-pharyngeal swabbing and collected information from patients consulting for ILI or, for France only, for acute respiratory infection (ARI). Only patients adhering to the EU ILI case definition were included (sudden onset of symptoms and at least one of the following four systemic symptoms: fever or feverishness, malaise, headache, myalgia; and at least one of the following three respiratory symptoms: cough, sore throat, shortness of breath) [20]. In all study sites, practitioners swabbed all elderly (60 or 65 years old and older) consulting for ILI, except for France where a proportion of elderly consulting for ARI were systematically selected for swabbing. Practitioners systematically selected patients from other age groups to swab using statistical sampling, except for Romania, where all patients consulting for ILI were swabbed. Hungary restricted their study population to those aged 18 years and over.

All participants in the study gave oral or written consent, in adherence with country requirements for ethical approval at each study site. The study period began 15 days after the start of the respective 2011/12 seasonal influenza vaccination campaign in each country.

Practitioners used standardised country-specific questionnaires to collect information on ILI signs and symptoms, sex, age, seasonal influenza vaccination in the 2011/12 and 2010/11 seasons, pregnancy, chronic conditions (including obesity, as defined in the participating countries), number of hospitalisations for chronic conditions in the past 12 months, receipt of antivirals (Spain and France excluded), and number of general practitioner (GP) visits in the past 12 months. Study sites included a question on belonging to the target group for vaccination, apart from France and Portugal, where this information was gathered using information on age, chronic conditions, and pregnancy. In addition, information related to target groups for vaccination was gathered in Portugal on whether the patient was a health professional or carer and a co-habitant or carer of a patient at-risk aged less than six months.

Among ILI patients fulfilling the inclusion criteria, we defined a case of influenza as a study participant whose swab tested positive for influenza virus by reverse-transcription polymerase chain reaction (RT-PCR) or culture. We classified patients with swabs testing negative for influenza virus as controls.

Swabs were tested for influenza at the respective country's National Influenza Reference Laboratory. In France, Italy, and Spain, tests were also conducted in other laboratories participating in the National Influenza Sentinel Surveillance System. At all study sites a subset of isolates were genetically and/or antigenically characterised. Details of laboratory viral detection, typing, subtyping and variant analysis performed are described elsewhere [21].

We defined a person as vaccinated if they had received at least one dose of 2011/12 seasonal influenza vaccine more than 14 days prior to ILI/ARI symptom onset. All the others were classified as unvaccinated.

The eight study teams sent their data to EpiConcept, where they were pooled and analysed. We carried out an analysis restricted to the A(H3) influenza type. We excluded controls presenting to the practitioner before the week of symptom onset of the first case and after

the last case of influenza A(H3) in each country respectively. We restricted the study population to the target groups for vaccination. We compared the characteristics of cases and controls using chi-square tests, t-tests, Fisher's exact test or the Mann-Whitney test depending on the nature of the variable.

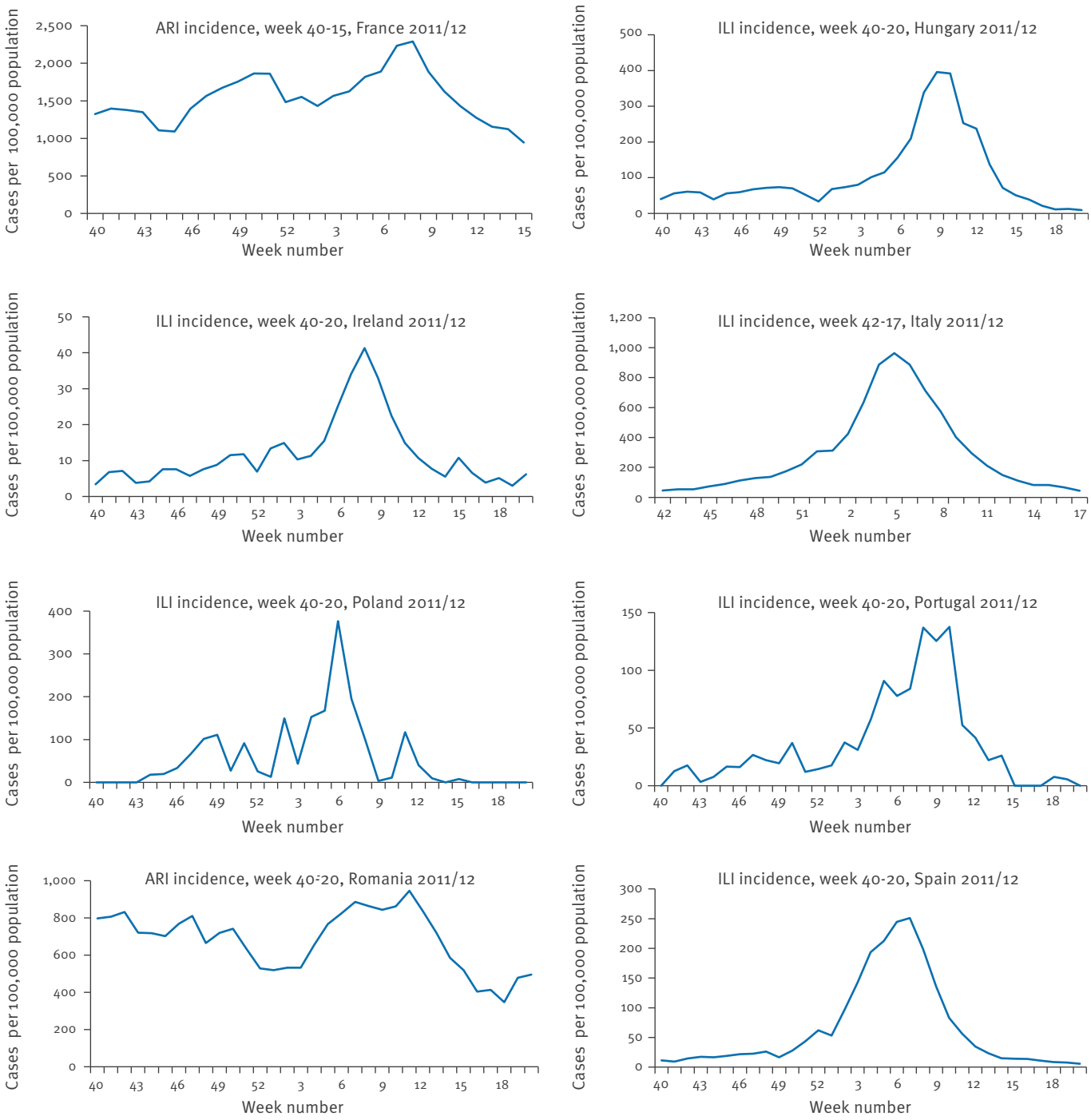
We used Cochran's Q-test and the I2 index to test the heterogeneity between study sites [22].

We estimated the pooled IVE as 1 minus the odds ratio (OR) of being vaccinated in cases versus controls, using a one-stage method with study site as fixed effect in the model.

To estimate adjusted IVE, we used a logistic regression model including potential confounding factors: age (10-year age bands), sex, presence of at least one chronic condition (including pregnancy and obesity), at least

Figure 1

Influenza-like illness / acute respiratory infection rates by week of symptom onset as reported by the national sentinel systems, I-MOVE multicentre case–control study, study sites in eight European Union countries, influenza season 2011/12



ARI: acute respiratory infection; ILI: influenza-like illness.

one hospitalisation in the previous 12 months for the chronic condition, number of practitioner visits in the previous 12 months (0-1, 2-4 and ≥5 visits) and month of symptom onset.

We stratified IVE into three age groups (0–14, 15–59 and 60 years and above). Since the influenza season started unusually late in Europe, we studied IVE in the early and late phase of the season and by time since vaccination [23]. The early and late phases of the influenza season were defined as up to and including week 6 of 2012 and from week 7 respectively, categories which allow for a similar sample size. In each of the two phases, we also calculated IVE by time since vaccination, with IVE estimates for symptom onset less than 93 days (around three months) since vaccination and 93 days or more since vaccination.

We conducted all statistical analysis using Stata version 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

Results

In the eight participating countries, influenza peaked at different times – from week 5 in Italy and Poland to week 10 in Portugal (Figure 1).

A total of 16 vaccines were used at the country study sites, of which four were adjuvanted. The start of the country-specific vaccination campaigns ranged between 12 September 2011 (Poland) and 15 December 2011 (certain regions in Romania). A total of 1,057 practitioners agreed to participate in the study of which 747 (71%) recruited at least one patient, giving a total of 4,746 patients recruited (Table 1).

After exclusion of one individual who had received antivirals prior to swabbing, 21 individuals for whom laboratory results were missing, 10 individuals who received vaccination prior to the begin of the country's national vaccination campaign, 170 individuals who did not adhere to the EU ILI case definition, 19 individuals who were swabbed more than seven days after symptom onset and 163 individuals who presented before or after the week of onset of the first and last influenza case respectively, 4,362 individuals met the study inclusion criteria. Among those, 2,084 were cases, of which 1,764 were positive for influenza A(H3) (84.6%), 30 were positive for influenza A(H1N1) (1.4%), 39 were influenza A unsubtypable (1.9%) and 251 were positive for influenza B (12.0%). As the analysis was restricted to the A(H3) subtype, the 320 individuals who had an influenza type other than A(H3) were excluded. As the study site in Poland reported no A(H3) cases, all controls from this study site were excluded from the analysis (a further 112 records). An additional 18 individuals who presented before and after the first and last case of influenza A(H3) respectively were excluded. This gave a total of 3,912 patients, of whom 1,033 (26.4%) were part of the target group for vaccination (Figure 2).

We included 1,016 ILI patients without missing information on seasonal vaccination (12 patients) or other covariates (five patients) in the IVE complete case analysis: 437 cases and 579 controls. A further eight

Table 1

Participating practitioners and recruited influenza-like illness patients, by A(H3) influenza case–control status, vaccination status and study site, multicentre case–control study, study sites in eight European Union countries[a], 2011/12

Study site	Number of practitioners participating in the study	Number of practitioners recruiting at least one ILI patient[b]	Number of ILI patients[b] recruited by practitioners	Inclusion period (ISO weeks)[c]	Number of ILI patients positive for influenza A(H3) and with known vaccination status[d] included in the study		Number of ILI patients included in the study negative for any influenza and with known vaccination status[d]	
					Total	Vaccinated	Total	Vaccinated
France	499	319	1,264	Week 52/2011–week 15/2012	75	30	84	34
Hungary	94	77	923	Week 49/2011–week 17/2012	30	13	219	73
Ireland	29	16	137	Week 48/2011–week 12/2012	12	7	9	6
Italy	10	10	191	Week 48/2011–week 10/2012	21	7	37	17
Poland	35	22	170	Not included in analysis (no influenza A(H3) cases)	0	NA	NA	NA
Portugal	59	35	352	Week 51/2011–week 12/2012	59	15	77	35
Romania	100	71	238	Week 52/2011–week 14/2012	33	2	45	8
Spain	231	197	1,471	Week 52/2011–week 13/2012	210	81	110	39
Total	**1,057**	**747**	**4,746**	-	**440**	**155**	**581**	**212**

ILI: Influenza-like illness; ISO: International Organization for Standardization. NA: not applicable

[a] France, Hungary, Ireland, Italy, Poland, Portugal, Romania, Spain

[b] ILI patients meeting the European Union case definition, swabbed less than eight days after onset of symptoms.

[c] From 15 days after the start of the vaccination campaign; controls with an onset of symptoms in the weeks prior to the first influenza A(H3) case or after the last influenza A(H3) case were excluded.

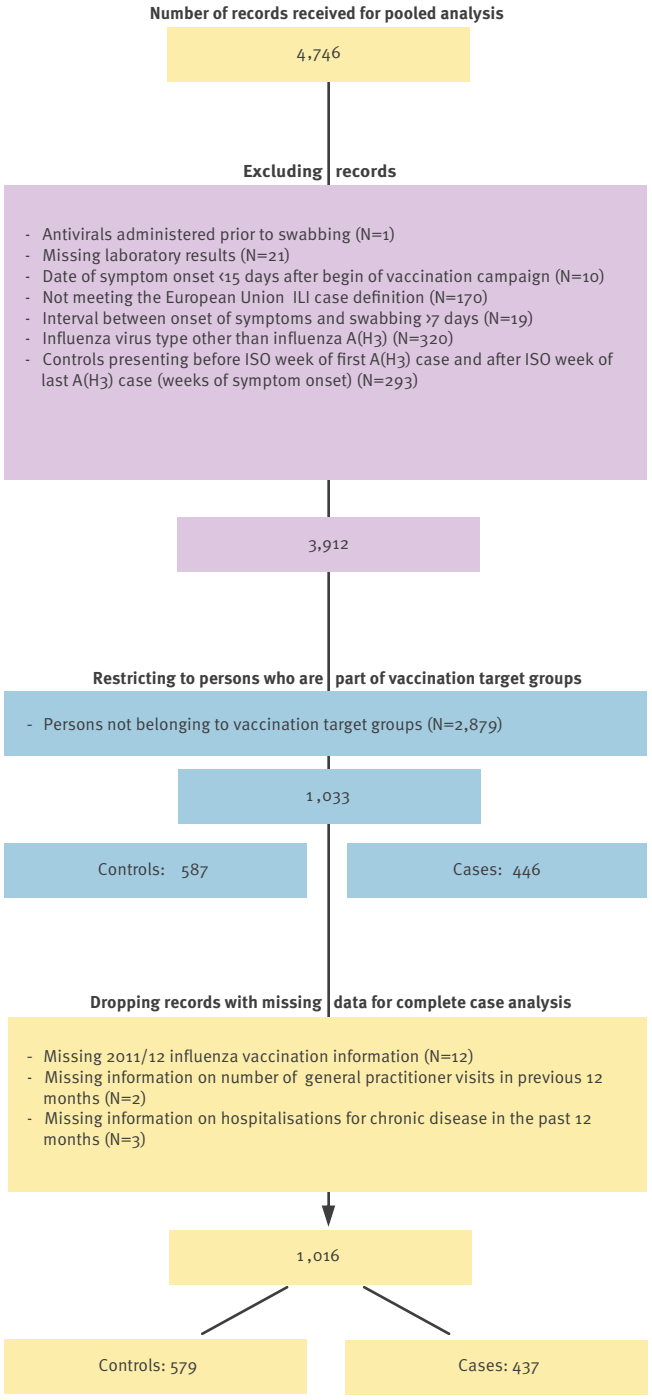
[d] ILI patients in a target group for vaccination included in the study, after excluding those with missing information on laboratory results, vaccination status or date of vaccination.

patients from France with imprecise vaccination dates were excluded in the analysis by time since vaccination.

The vaccination coverage in the studies was 35.9% (n=367) among the target group for vaccination and varied by study site from 12.8% (Romania) to 61.9% (Ireland).

Figure 2

Flowchart of data exclusion for pooled analysis, I-MOVE multicentre case–control study, study sites in eight European Union countries, 2011/12



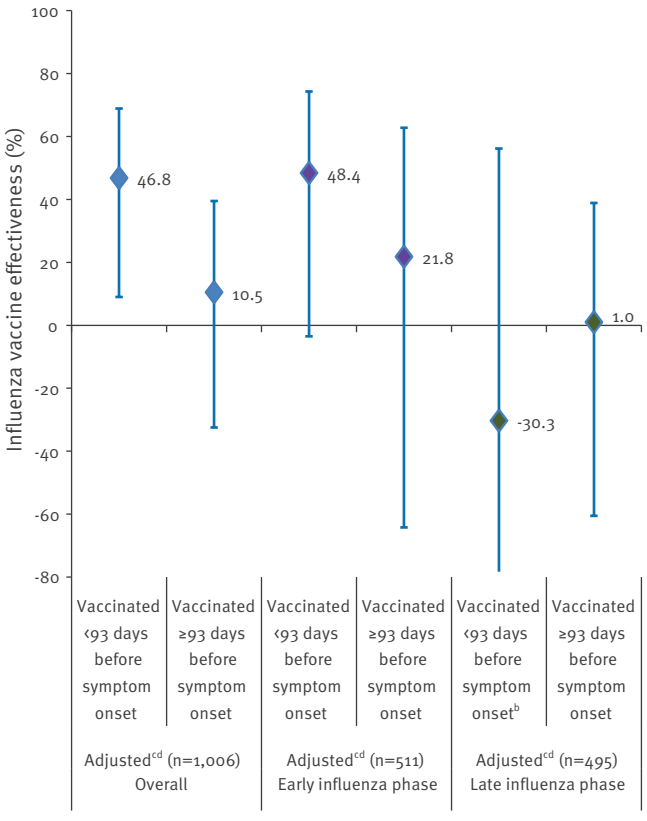
ILI: Influenza-like illness; ISO: International Organization for Standardization.

The median age was higher among cases (62.0 years; interquartile range (IQR): 37–70 years) than among controls (58.0 years; IQR: 41–69 years) (Table 2).

The proportion of cases presenting with any of the following symptoms was higher than controls: fever, malaise, myalgia and cough. A greater proportion of controls than cases had heart disease or at least one chronic condition. A greater proportion of controls visited their practitioner five or more times in the previous 12 months. A greater proportion of cases were swabbed within three days of symptom onset, but this was not statistically significant to the 5% level. The delay between vaccination and symptom onset was shorter for controls (median: 88.5 days, IQR: 64-115 days) than for cases (median: 116.0 days, IQR: 95-131 days).

Figure 3

Pooled adjusted 2011/12 seasonal vaccine effectiveness against laboratory-confirmed influenza A(H3) cases in vaccination target groups, by time since vaccination, at study sites in seven European Union countries[a], week 46/2011–week 17/2012 (n=1,008)



[a] France, Hungary, Ireland, Italy, Portugal, Romania, Spain

[b] Lower boundary 95% CI: -288.

[c] Model adjusted for presence of at least one chronic disease, sex, at least one hospitalisation for chronic disease in the previous 12 months, age group, practitioner visits in the previous 12 months (0-1, 2-4 and ≥5 visits), month of symptom onset and study site.

[d] November dropped due to no cases (two records dropped), eight records omitted from France, due to imprecise vaccination date.

The Q test (p=0.142) and the I2 index (37.6%) testing for heterogeneity between the individual crude IVE estimates of the seven study sites included, suggested low to medium statistical heterogeneity.

Crude IVE against A(H3) was 12.2% (95% CI: -17.2 to 34.2) and the adjusted IVE was 24.8% (95% CI: -5.6 to 46.5) (Table 3). Due to small sample size, an adjusted IVE was not interpretable among the individuals under 15 years of age. Among those aged between 15 and 59 years, the adjusted IVE was 63.3% (95% CI: 25.9 to 81.8) and 15.1% (95% CI: -33.1 to 45.9) among those aged 60 years and over.

In the early phase of the season (week 46/2011 to week 6/2012) the adjusted IVE was 38.1% (95% CI: -7.9 to 64.5) and in the late phase -0.7% (95% CI: -59.8 to 36.5). The adjusted IVE among persons with onset of symptoms less than three months since vaccination was 46.8% (95% CI: 9.0 to 68.9) and the IVE among persons with onset of symptoms three months or more since vaccination was 10.5% (95% CI: –32.5 to 39.5) (Figure 3).

When restricting to the early influenza phase, IVE among persons with onset of symptoms less than 93 days since vaccination was 48.4% (95% CI: -3.5 to 74.3) and the IVE among persons with onset of symptoms

Table 2

Characteristics of A(H3) influenza cases (n=446) and test-negative controls (n=587) in vaccination target groups, multicentre case–control study, seven European Union countries[a], week 46/2011–week 17/2012

Characteristic	Number of A(H3) influenza cases/total n (%)[b]	Number of test-negative controls/total n (%)[b]	p value
Median age	62.0	58.0	0.008[c]
Age group (years)			
0-4	15/446 (3.4)	24/587 (4.1)	0.05[d]
5-14	23/446 (5.2)	19/587 (3.2)	-
15-59	164/446 (36.8)	272/587 (46.3)	-
≥ 60	244/446 (54.7)	272/587 (46.3)	-
Female sex	242/446 (54.3)	357/587 (60.3)	0.056[d]
Symptoms			
Fever	425/444 (95.7)	523/584 (89.6)	<0.001[d]
Malaise	349/365 (95.6)	457/498 (91.8)	0.026[d]
Myalgia	394/444 (88.7)	465/583 (79.8)	<0.001[d]
Cough	425/445 (95.5)	525/587 (89.4)	<0.001[d]
Sore throat	318/441 (72.1)	451/587 (76.8)	0.095[d]
Shortness of breath	96/439 (21.9)	139/580 (24.0)	0.453[d]
Days between onset of symptoms and swabbing			
<4	399/446 (89.5)	508/587 (86.5)	0.179[d]
≥4	47/446 (10.5)	79/587 (13.5)	-
Seasonal vaccination[d] 2011/12	155/440 (35.2)	212/581 (36.5)	0.693[d]
Seasonal vaccination 2010/11	147/441 (33.3)	213/584 (37.0)	0.236[d]
Obesity[f]	56/446 (12.6)	97/587 (16.5)	0.078[d]
Heart diseases	99/446 (22.2)	194/587 (33.0)	<0.001[d]
At least one chronic condition (including pregnancy)	295/446 (66.1)	467/587 (79.6)	<0.001
Smoker			
Current	44/365 (12.1)	75/496 (15.1)	0.176[d]
Former	60/365 (16.4)	96/496 (19.4)	-
Never	261/365 (71.5)	325/496 (65.5)	-
Five or more practitioner visits in the previous 12 months	224/445 (50.3)	347/586 (59.2)	0.005[c]
Any hospitalisation in the previous 12 months for chronic diseases	27/444 (6.1)	50/585 (8.5)	0.152[c]
Median number of days from vaccination[e] to onset of ILI symptoms	116.0	88.5	<0.001[b]

ILI: influenza-like illness.

[a] France, Hungary, Ireland, Italy, Portugal, Romania, Spain

[b] Unless otherwise indicated.

[c] Non parametric test of the median.

[d] Two-sided Fisher's exact test.

[e] Vaccination more than 14 days before onset of influenza-like illness symptoms.

[f] As defined in the respective countries.

93 days or more since vaccination was 21.8% (95% CI: -64.2 to 62.8). When restricting to the late phase, IVE among persons with onset of symptoms less than 93 days since vaccination was -30.3% (95% CI: -287.7 to 56.2) and the IVE among persons with onset of symptoms more than 93 days since vaccination was 1.0% (95% CI: -60.5 to 38.9) (Figure 3).

Discussion

The overall adjusted pooled IVE estimates against influenza A(H3) from the multicentre case–control study in Europe among those targeted for vaccination was 24.8%, ranging between 15.1% in the elderly and 63.3% in persons aged between 15 and 59 years. This suggests a low adjusted IVE against medically attended A(H3) influenza among the target population except among younger adults.

The A(H3) strain was also predominant during the 2008/09 season, the I-MOVE pilot season. In that season, persons aged 65 and above had an IVE of 56.4% (95% CI: -0.2 to 81.0) against A(H3) [5]. We observed a lower IVE in the 2011/12 A(H3) dominated season with an IVE of 15.1% in those aged 60 years and above and an IVE of 12.4% in those aged 65 years and above.

The strength of this study lies in its multicentre nature, enabling recruitment of a large sample size of participants across the EU. It is possible to restrict to the target group for vaccination and to stratify further by influenza type and age. Study sites adhere to a common protocol and carry out systematic sampling. They also collect information on potentially important positive and negative confounders. In addition, data quality is very high with only 1.7% (n=17/1033) of records with missing data.

Due to the observational nature of this study, we cannot exclude biases. We used a test-negative design, which is subject to the usual selection biases particularly for the control group. Study participants are selected according to a systematic sampling procedure by practitioners, who are blinded to the case and control status of the patients. This should minimise selection bias.

As I-MOVE is based on existing sentinel networks, GPs recruited patients according to the case definitions used in their network: the EU ILI case definition or the ARI case definition. As the ARI case definition is a more sensitive case definition than the EU ILI one, we could restrict the analysis to patients meeting the EU ILI case definition for all patients included in the study.

The test-negative design is a commonly used, but not validated study design [24–32]. Using test-negative controls is considered to adjust for healthcare-seeking behaviour more so than if community controls were selected, as vaccination coverage varies by healthcare-seeking behaviour [34,35]. In addition, the covariate 'number of GP visits in the past 12 months' may adjust further for healthcare-seeking behaviour. Despite this adjustment, it is still debatable if test-negative controls properly reflect the vaccine coverage of the source population for cases [33].

While a higher proportion of controls visited their GP more frequently and had a chronic condition than cases,

Table 3

Pooled crude and adjusted 2011/12 seasonal influenza vaccine effectiveness against laboratory-confirmed A(H3) influenza in vaccination target groups, at study sites in seven European Union countries[a], week 46/2011–week 17/2012 (patients with complete information, n=1,016)

		Number	Influenza vaccine effectiveness in %	95% confidence intervals
Overall	Crude[b]	1,016	12.2	-17.2 to 34.2
	Adjusted[c,d]	1,014	24.8	-5.6 to 46.5
<15 years	Crude[b]	78	19.4	-170.1 to 75.9
	Adjusted[c]	-	-	-
15-59 years	Crude[b]	431	59.3	24.4 to 78.1
	Adjusted[c]	431	63.3	25.9 to 81.8
60 years and above	Crude[b]	505	6.4	-40.7 to 37.7
	Adjusted[c,d]	503	15.1	-33.1 to 45.9
First influenza phase (week 46/2011 to week 6/2012)	Crude[b]	515	38.2	2.8 to 60.7
	Adjusted[c,d]	513	38.1	-7.9 to 64.5
Second influenza phase (week 7/2012 to week 17/2012)	Crude[b]	501	-17.6	-75.9 to 21.4
	Adjusted[c,d]	501	-0.7	-59.8 to 36.5

[a] France, Hungary, Ireland, Italy, Portugal, Romania, Spain

[b] Study site included in the model as fixed effect.

[c] Model adjusted for presence of at least one chronic disease, sex, at least one hospitalisation for chronic disease in the previous 12 months, age group, practitioners' visits in the previous 12 months (0-1, 2-4 and ≥5 visits), month of symptom onset and study site.

[d] November dropped due to no cases (two records dropped).

these variables were not strong confounders (-2% and 1% relative difference of IVE between model containing and not containing these confounders respectively). The main confounder was age group, changing the IVE of the adjusted model by 11%.

We cannot exclude residual confounding, either by unmeasured confounders or by use of broad categories within given confounders. However, we used 10-year age bands to reduce residual confounding by age. While we used month of symptom onset as a covariate, the IVE differs only little if using week of symptom onset (24.8% compared to 23.4% for overall IVE).

We included patients who were swabbed within seven days of symptom onset and we observed that a higher proportion of controls were swabbed more than three days after symptom onset than cases, although the difference is not statistically significant. The probability of influenza detection decreases with time between onset and swabbing, although the rate of decrease may vary by patient characteristics [35–38]. It is possible that some misclassification bias is introduced by including false negative controls through including patients with a greater delay between onset of symptoms and swabbing. However the difference is small if we compare our results to an analysis restricting the study population to persons swabbed three days or fewer since symptom onset (24.8% compared to 22.8% for overall IVE).

Our study is limited by a small sample size for the stratified analyses. Therefore precise estimates were not always possible, particularly among the youngest age group, who are often the least numerous target group for vaccination. Estimates by influenza phase and by time since vaccination are also limited by the small sample size and although point estimates differ, confidence intervals overlap.

The majority of countries participating in this study used both adjuvanted and non-adjuvanted influenza vaccines. The different vaccine types were used in different subpopulations. With the data collected for this study, it was not possible to identify the target groups to enable an estimate by vaccine type.

IVE estimates arising from the total population were lower than the estimates from the target group for vaccination, e.g. overall adjusted IVE of 10.9% (95% CI: -16.2 to 31.7) among the total population (data not shown), compared to 24.8% (95% CI: -5.6 to 46.5) among the target group for vaccination. We believe that the target group for vaccination is a more homogeneous study population in relation to vaccination, the main exposure of interest, as study participants belonging to the target group for vaccination are likely to have a more equal access to vaccination than the total population.

One limitation of restricting to this population is that it is identified through the practitioner questionnaires, which did not collect information on target group homogeneously across study sites. In particular information on healthy persons with professions targeted for vaccination may have been omitted from some countries. Despite these limitations, we believe that our study suggests a low adjusted IVE against medically attended A(H3) influenza among the target population except for among young adults in the 2011/12 influenza season.

The lower IVE observed this season compared to the previous A(H3) dominated season (2008/09) may be due to changes in circulating viruses and hence suboptimal antigenic match between the 2011/12 vaccine and circulating strains. WHO and the Community Network of Reference Laboratories (CNRL) report northern hemisphere circulating A(H3N2) viruses being genetically and antigenically distinguishable from the A/Perth/16/2009 vaccine strain and being more related to A/Victoria/361/2011-like reference viruses, differences which may have increased along the season [18,39]. This virological change could have contributed to the lower IVE in the latter part of the season.

As the 2011/12 influenza season was a late season, persons presenting with influenza had a long delay between onset of symptoms and the vaccination, as campaigns were carried out in the autumn of 2011. The observed fall in IVE may also be due in part to waning of the immunity induced by the vaccine, perhaps markedly so in older people [40–43]. Persons vaccinated less than 93 days before symptom onset showed a higher IVE than persons vaccinated 93 days or more before symptom onset. However, persons vaccinated 93 days or more before symptom onset were more likely to present later in the season, co-temporal with the emergence of antigenically drifted influenza viruses. To disentangle the possible effects of waning immunity and antigenic drift, we looked at IVE by early and late influenza phase. In the early influenza phase IVE was higher among persons vaccinated less than 93 days before symptom onset compared to persons vaccinated 93 days or more before symptom onset. This was not the case in the late influenza phase, where we may expect a greater effect of antigenic drift on the IVE estimates. This suggests the waning immunity hypothesis may be plausible.

In conclusion, the I-MOVE multicentre case–control study suggests a low IVE against medically attended A(H3) influenza in the 2011/12 season. The I-MOVE multicentre case control study provides high quality and rapid IVE estimates and should supplement the virological information that informs the WHO recommendations on vaccine strain selection [6,8]. It is difficult to disentangle the respective roles of changes in the circulating viruses, possible waning immunity and otherwise imperfect vaccine. Further virological studies are needed on an annual basis quantifying drift over time as well as large epidemiological studies by time since vaccination with several delay categories to

fully understand these potentially important issues. Production of an improved seasonal influenza vaccine with greater effectiveness should be given a high priority.

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