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

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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).

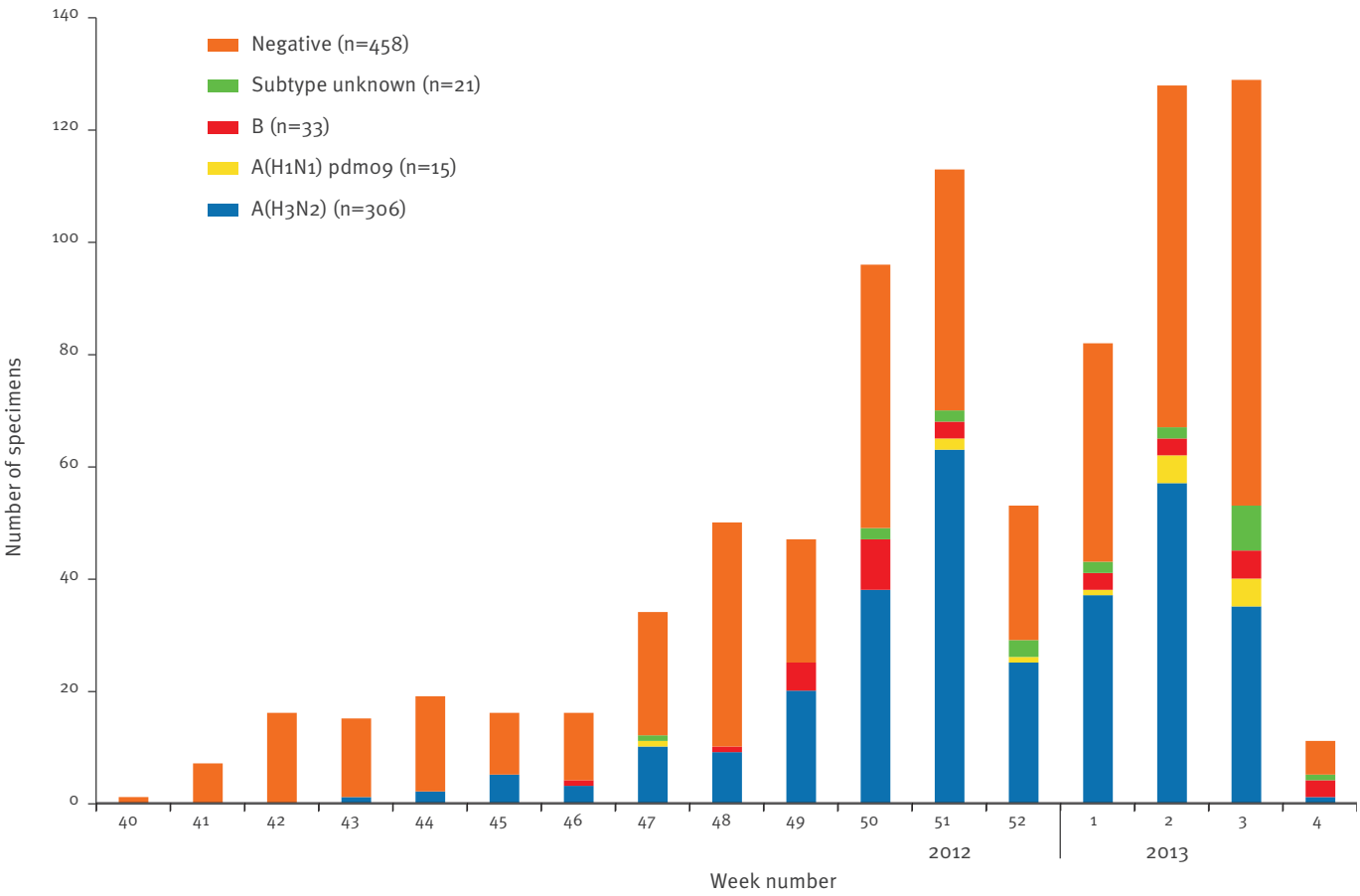
Interim estimates of influenza vaccine effectiveness

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

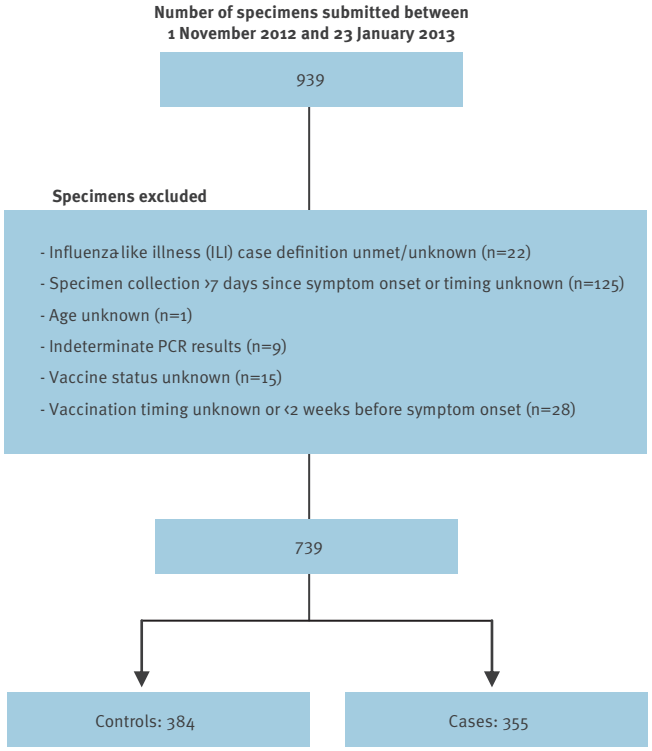
analyses of previous seasons [4,8,9,11]. Those aged 20–49 years contributed most to the analysis (43%) and the median interval between symptom onset and specimen collection was three days (Table 2).

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.

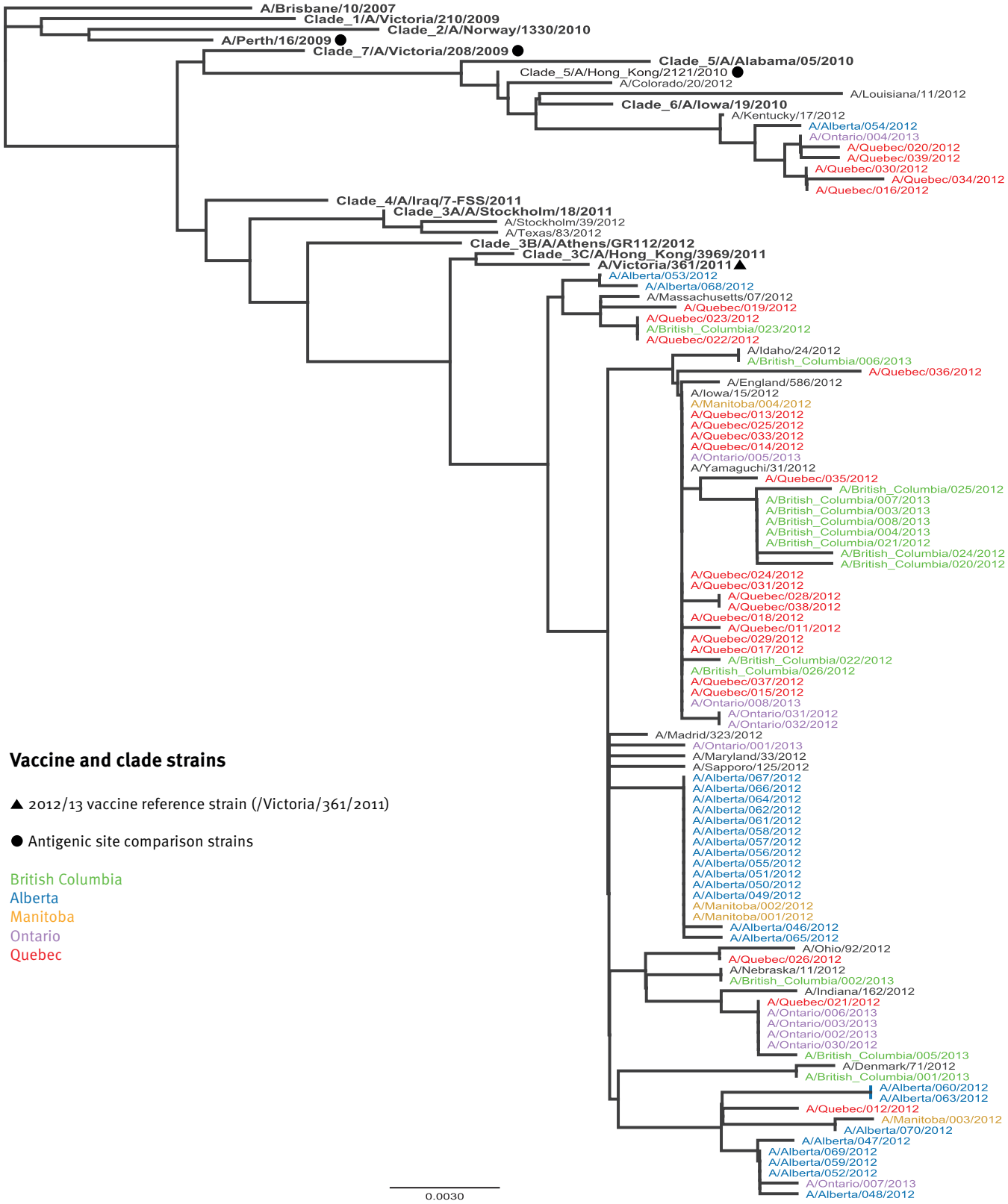
[c] Those immunised <2 weeks before symptom onset or from whom a specimen was collected >7 days since symptom onset (or for whom these were unknown) were excluded but explored in the sensitivity analysis as shown.

[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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