Interim estimates of 2013/14 vaccine effectiveness against influenza A(H1N1)pdm09 from Canada's sentinel surveillance network, January 2014

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The 2013/14 influenza season to date in Canada has been characterised by predominant (90%) A(H1N1) pdm09 activity. Vaccine effectiveness (VE) was assessed in January 2014 by Canada's sentinel surveillance network using a test-negative case–control design. Interim adjusted-VE against medically-attended laboratory-confirmed influenza A(H1N1) pdm09 infection was 74% (95% CI: 58–83). Relative to vaccine, A(H1N1)pdm09 viruses were antigenically similar and genetically well conserved, with most showing just three mutations across the 50 amino acids comprising antigenic sites of the haemagglutinin protein.

Background

Since the 2009 pandemic, influenza A(H1N1)pfdm09 viruses have comprised a small proportion (<20%) of seasonal influenza virus detections each year in Canada [1]. However, A(H1N1)pdm09 activity has recently resurged in North America, comprising more than 90% of detected influenza strains in both Canada and the United States (US) to mid-January of the 2013/14 season [1,2]. This profile is in contrast to that of the same period last season in Canada, when 90% of detected strains instead belonged to the A(H3N2) subtype [3].

The 2013/14 trivalent influenza vaccine (TIV) for the northern hemisphere retains the same A(H1N1)pdm09 (A/California/07/2009-like) strain recommended since 2009 by the World Health Organization (WHO) [4]. In response to substantial A(H1N1)pdm09 resurgence, interim 2013/14 vaccine effectiveness (VE) was assessed in January 2014 using Canada's sentinel surveillance network. VE estimates are discussed in the context of antigenic and genetic characterisation of circulating A(H1N1)pdm09 viruses.

Estimating influenza vaccine effectiveness

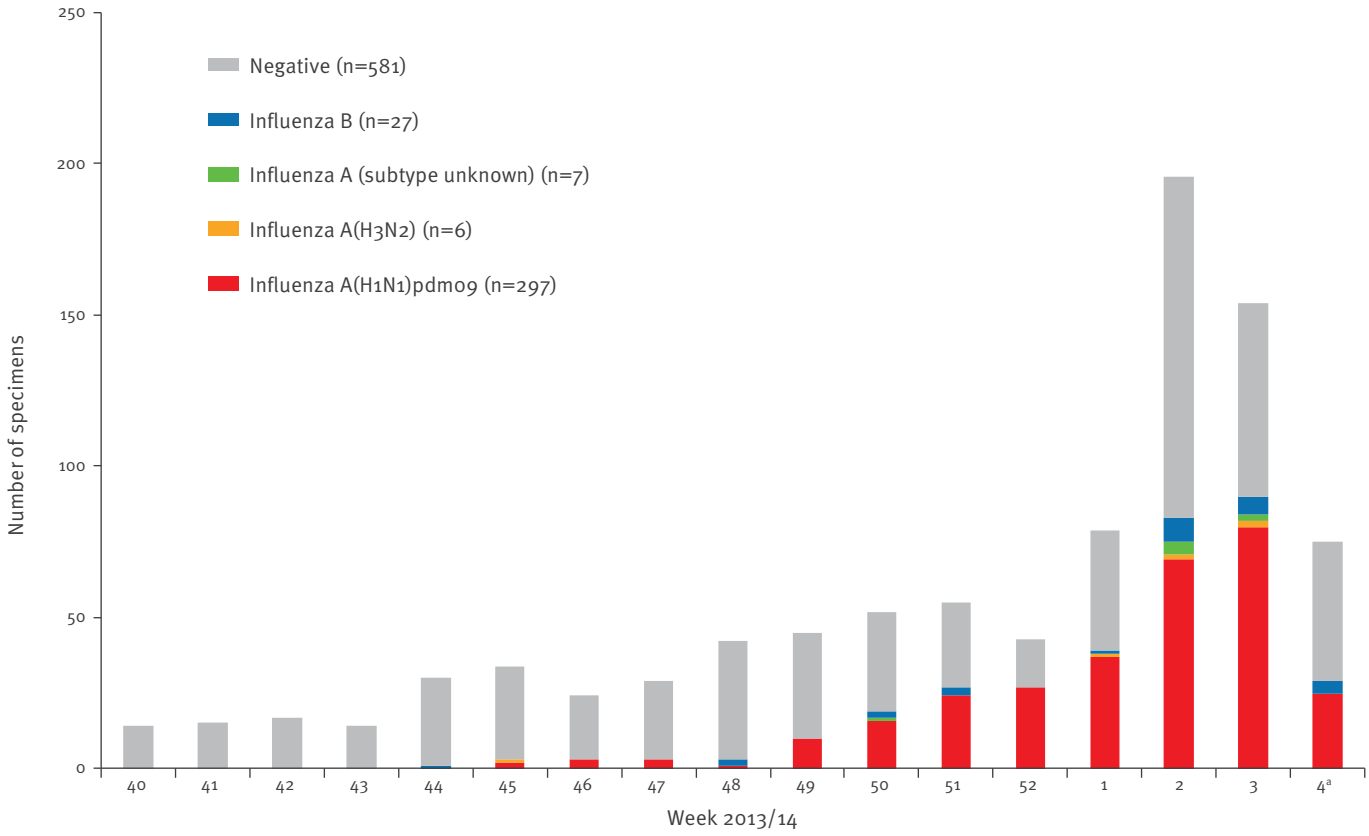
As previously described [3,5-12], a test-negative case–control design was used to estimate VE. Patients presenting with influenza-like illness (ILI) and testing positive for influenza viruses were considered cases, and those testing negative were considered controls.

Community-based practitioners at 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 analysis period included specimens collected from 1 November 2013 (week 44: 27 October 2013–2 November 2013) to 23 January 2014 (week 4: 19–25 January 2014), selected to account for influenza activity beginning in early November (Figure 1) and immunisation campaigns typically commencing in October. Epidemiological information was obtained from consenting patients or their parents/guardians using a standard questionnaire at specimen collection. Ethics review boards in participating provinces approved this study.

Figure 1

Laboratory detection of influenza by week and virus subtype, 2013/14 sentinel surveillance system, Canada, 29 September 2013–23 January 2014 (n=918)[a]



Of 1,200 nasal or nasopharyngeal specimens collected between 29 September 2013 (week 40: 29 September–5 October 2013) and 23 January 2014 (week 4: 19–25 January 2014), 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=50), those whose specimens were collected more than seven days after symptom onset or for whom the interval was unknown (n=169), those whose age was unknown or less than one year (n=10), those with unknown comorbidity status (n=80), and those for whom influenza test results were unavailable or indeterminate (n=10). Specimens were included regardless of the patient's vaccination status or timing of vaccination. Excluded specimens may have more than one exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields.

[a] Week 4 is based on partial week.

Specimens were tested for influenza A (by subtype) and B viruses at provincial reference laboratories using real-time RT-PCR. Odds ratios (OR) for medically-attended, laboratory-confirmed influenza were estimated by multivariable logistic regression. VE was calculated as (1–OR)x100%. Patients for whom comorbidity status was unknown or for whom the timing of vaccination was unknown or less than two weeks before symptom onset were excluded from the primary analysis but explored in sensitivity analyses. Age-stratified analysis and a study period beginning from week 49 (1–7 December 2013) to allow for additional vaccine uptake were also explored.

Genetic characterisation of sentinel influenza A(H1N1)pdm09 viruses

The haemagglutinin (HA) genes (HA1/HA2) from a convenience sample of sentinel influenza A(H1N1) pdm09 viruses from original patient specimens were sequenced for phylogenetic analysis and pair-wise amino acid (aa) identity based on antigenic maps spanning the 50 aa residues across HA1 antigenic sites Sa, Sb, Ca1, Ca2 and Cb [12,13]. Findings were expressed as percentage identity to vaccine, calculated as (1–(number of aa substitutions in antigenic sites)/(total antigenic site aa residues))x100%. After removal of the signal peptide (residues 1–17), the approximate likelihood method was used to generate the phylogenetic tree of aligned nucleotide sequences in FastTree [14], visualised in FigTree [15], including reference HA sequences shown in Table 1.

Interim estimates of influenza vaccine effectiveness

A total of 1,091 specimens were submitted between 1 November 2013 and 23 January 2014. After exclusion

Table 1a

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network, Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI499574	Netherlands	2013-Oct-14	A/Netherlands/2248/2013	Erasmus University of Rotterdam	National Institute for Medical Research	
EPI499572	France	2013-Dec-02	A/Lyon/2899/2013	CRR virus Influenza region Sud	National Institute for Medical Research	
EPI498900	United States	2013-Dec-16	A/Kansas/13/2013	Kansas Department of Health and Environment	Centers for Disease Control and Prevention	
EPI498897	United States	2013-Dec-17	A/Wisconsin/35/2013	Marshfield Clinic Research Foundation	Centers for Disease Control and Prevention	
EPI498865	United States	2013-Dec-01	A/Georgia/14/2013	Georgia Public Health Laboratory	Centers for Disease Control and Prevention	
EPI498639	China	2013-Nov-15	A/Chongqing-Yuzhong/SWL11676/2013	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Yu Lan,Xiyan Li,Xiang Zhao,Yanhui Cheng,minju Tan,Weijuan Huang,Dayan Wang,Dexin Li,Yuelong Shu
EPI498543	China	2013-Nov-21	A/Jiangsu-Qinhuai/SWL11396/2013	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Yu Lan,Xiyan Li,Xiang Zhao,Yanhui Cheng,minju Tan,Weijuan Huang,Dayan Wang,Dexin Li,Yuelong Shu
EPI498415	Spain	2013-Nov-14	A/Galicia/1484/2013	Instituto de Salud Carlos III	National Institute for Medical Research	
EPI498294	United States	2013-Dec-09	A/Montana/15/2013	Montana Public Health Laboratory	Centers for Disease Control and Prevention	
EPI498277	United States	2013-Nov-04	A/Arkansas/20/2013	Arkansas Department of Health	Centers for Disease Control and Prevention	
EPI498274	United States	2013-Dec-03	A/Indiana/30/2013	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI498269	United States	2013-Dec-02	A/Delaware/15/2013	Delaware Public Health Lab	Centers for Disease Control and Prevention	
EPI498230	United States	2013-Dec-02	A/Arkansas/23/2013	Arkansas Department of Health	Centers for Disease Control and Prevention	
EPI498214	United States	2013-Nov-22	A/Alaska/30/2013	Alaska State Virology Lab	Centers for Disease Control and Prevention	
EPI498208	United States	2013-Dec-01	A/Idaho/08/2013	State of Idaho Bureau of Laboratories	Centers for Disease Control and Prevention	

ID: identification number; WHO: World Health Organization.

We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database on which this research is based. All submitters of data may be contacted directly via the GISAID website: www.gisaid.org.

Table 1B

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network, Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI499324	United States	2013-Dec-09	A/Nevada/18/2013	Southern Nevada Public Health Lab	Centers for Disease Control and Prevention	
EPI498191	United States	2013-Nov-18	A/Mississippi/29/2013	New York State Department of Health	Centers for Disease Control and Prevention	
EPI497986	Japan	2013-Nov-28	A/TOKYO/32432/2013	Tokyo Metropolitan Institute of Public Health	National Institute of Infectious Diseases (NIID)	Takashita,Emi; Fujisaki,Seiichiro; Itoh,Reiko; Miura,Mai; Ejima,Miho; Tashiro,Masato; Odagiri,Takato
EPI497984	Japan	2013-Nov-20	A/TOKYO/32417/2013	Tokyo Metropolitan Institute of Public Health	National Institute of Infectious Diseases (NIID)	Takashita,Emi; Fujisaki,Seiichiro; Itoh,Reiko; Miura,Mai; Ejima,Miho; Tashiro,Masato; Odagiri,Takato
EPI497756	Sweden	2013-Dec-06	A/Gothenburg/5/2013		Swedish Institute for Infectious Disease Control	
EPI497694	Norway	2013-Nov-28	A/Norway/3230/2013	Ostfold Hospital - Fredrikstad, Dept. of Microbiology	Norwegian Institute of Public Health	Dudman, SG;Waalen, K; Hungnes, O
EPI497692	Norway	2013-Dec-04	A/Norway/3234/2013	Oslo University Hospital, Ulleval Hospital, Dept. of Microbiology	Norwegian Institute of Public Health	Dudman, SD;Waalen, K; Hungnes, O
EPI497634	United States	2013-Oct-22	A/Texas/42/2013	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
EPI492859	United States	2013-Nov-01	A/Maine/01/2013	Maine Health and Environmental Testing Laboratory	Centers for Disease Control and Prevention	
EPI492816	United States	2013-Oct-23	A/New York/09/2013	New York State Department of Health	Centers for Disease Control and Prevention	
EPI492782	United States	2013-Nov-18	A/Texas/36/2013	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
EPI492779	United States	2013-Nov-21	A/Wyoming/09/2013	Wyoming Public Health Laboratory	Centers for Disease Control and Prevention	
EPI492861	United States	2013-Nov-06	A/Florida/61/2013	Florida Department of Health-Jacksonville	Centers for Disease Control and Prevention	
EPI492856	United States	2013-Oct-30	A/Arizona/06/2013	Arizona Department of Health Services	Centers for Disease Control and Prevention	

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Table 1c

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI492852	United States	2013-Nov-06	A/Iowa/07/2013	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI492244	Norway	2013-Nov-12	A/Norway/3073/2013	Oslo University Hospital, Ulleval Hospital, Dept. of Microbiology	Norwegian Institute of Public Health	Dudman SG, Waalen K, Hungnes O
EPI489358	United States	2013-Oct-23	A/California/25/2013	California Department of Health Services	Centers for Disease Control and Prevention	
EPI489328	United States	2013-Oct-12	A/Mississippi/09/2013	Mississippi Public Health Laboratory	Centers for Disease Control and Prevention	
EPI489322	United States	2013-Nov-04	A/Indiana/23/2013	Indiana State Department of Health Laboratories	Centers for Disease Control and Prevention	
EPI486613	United States	2013-Oct-25	A/Colorado/04/2013	Colorado Department of Health Lab	Centers for Disease Control and Prevention	
EPI486607	United States	2013-Oct-10	A/South Carolina/04/2013	South Carolina Department of Health	Centers for Disease Control and Prevention	
EPI486601	United States	2013-Oct-15	A/North Dakota/04/2013	North Dakota Department of Health	Centers for Disease Control and Prevention	
EPI486407	United States	2013-Oct-07	A/Maryland/08/2013	Maryland Department of Health and Mental Hygiene	Centers for Disease Control and Prevention	
EPI486401	United States	2013-Oct-06	A/Utah/09/2013	Utah Department of Health	Centers for Disease Control and Prevention	
EPI486389	United States	2013-Oct-10	A/Arizona/03/2013	Arizona Department of Health Services	Centers for Disease Control and Prevention	
EPI486379	United States	2013-Oct-07	A/Washington/09/2013	Washington State Public Health Laboratory	Centers for Disease Control and Prevention	
EPI485754	United States	2013-Oct-02	A/Pennsylvania/07/2013	Pennsylvania Department of Health	Centers for Disease Control and Prevention	
EPI485751	United States	2013-Oct-02	A/Mississippi/08/2013	Mississippi Public Health Laboratory	Centers for Disease Control and Prevention	
EPI326206	Hong Kong (SAR)	2011-Mar-29	A/Hong Kong/3934/2011	Government Virus Unit	National Institute for Medical Research	
EPI468476	Norway	2013-May-03	A/Norway/2417/2013	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health	Dudman, SG; Waalen, K; Hungnes, O
EPI466545	Estonia	2013-Mar-13	A/Estonia/76677/2013	Health Protection Inspectorate	National Institute for Medical Research	

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Table 1D

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) and used in phylogenetic analysis, 2013/14 sentinel surveillance network, Canada

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI417158	Ukraine	2012-Dec-02	A/Ukraine/523/2012	Institute of Epidemiology and Infectious Diseases AMS of Ukraine	National Institute for Medical Research	
EPI407291	United Kingdom	2012-Oct-29	A/Scotland/124660532/2012	Centre for Infections, Health Protection Agency	Health Protection Agency	Ellis, J
EPI382424	Hong Kong (SAR)	2012-May-21	A/Hong Kong/5659/2012	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak,G.C.;Lo,J.Y.C.
EPI417552	Norway	2012-Nov-26	A/Norway/2362/2012	Stavanger Universitetssykehus, Avd. for Medisinsk Mikrobiologi	Norwegian Institute of Public Health	Kilander, A.;Khider, M.;Waalen, K.;Dudman, S.;Hungnes, O.
EPI406039	United States	2012-Oct-22	A/South Carolina/19/2012	South Carolina Department of Health	Centers for Disease Control and Prevention	
EPI466588	Norway	2013-Mar-06	A/Norway/1675/2013	WHO National Influenza Centre	National Institute for Medical Research	
EPI418082	France	2012-Nov-29	A/Paris/1878/2012	Institut Pasteur	Institut Pasteur	Enouf, V; Briand, D; Benassaya, M; Garbarg-Chenon, A;
EPI454436	Kenya	2013-Feb-22	A/Kenya/104/2013	CDC-Kenya	Centers for Disease Control and Prevention	
EPI331061	Ghana	2011-May-13	A/Ghana/763/2011	University of Ghana	National Institute for Medical Research	
EPI319590	Russian Federation	2011-Feb-28	A/Astrakhan/1/2011	WHO National Influenza Centre	National Institute for Medical Research	
EPI278607	New Zealand	2010-Jul-12	A/Christchurch/16/2010	Canterbury Health Services	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M; Iannello,P; Caldwell,N; Leang,S-K; Komadina,N
EPI319447	Czech Republic	2011-Jan-18	A/Czech Republic/32/2011	National Institute of Public Health	National Institute for Medical Research	
EPI239901	United States	2009-Apr-09	A/California/07/2009 X-181		Centers for Disease Control and Prevention	
EPI257201	United States	2009-May-01	A/California/07/2009 X-179A		Centers for Disease Control and Prevention	
EPI176470	United States	2009-Apr-01	A/California/04/2009		Centers for Disease Control and Prevention	

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criteria were applied (Figure 2), 792 specimens were included in the primary analysis.

As in previous seasons, adults 20–49 years old contributed the largest proportion of specimens (50%) (Table 2) [3,6-12]. However, compared with the 2012/13 mid-season publication [3], a greater proportion of cases in 2013/14 were adults aged 20–49 years (53% versus 42%; p<0.01) or 50–64 years (22% versus 17%; p=0.13) (p<0.01 combined); proportions were more comparable among controls (48% versus 43%; p=0.17 and 20% versus 21%; p=0.86, respectively) (Table 2). Conversely, individuals younger than 20 years (21% versus 32%; p<0.01) and those 65 years and older (4% versus 9%; p<0.01) comprised a smaller proportion of cases compared with 2012/13 (Table 2) [3]. Adults aged 20–49 years and 50–64 years also comprised a greater proportion of cases in 2013/14 compared with the 2009 monovalent influenza A(H1N1)pdm09 VE analysis (53% versus 46%; p=0.14 and 22% versus 10%; p<0.01, respectively) [10].

Of the 792 specimens tested to date and included in primary VE analysis, 325 (41%) were positive for influenza, and 287 of 318 typed/subtyped viruses (90%) were A(H1N1)pdm09 (Table 3; Figure 1). Overall, 155 of 487 controls (32%) and 41 of 332 cases (12%) reported receipt of 2013/14 TIV (p<0.01). After applying exclusions related to immunisation timing, 29% of controls and 10% of cases were considered immunised (p<0.01) (Table 2). The proportion of controls reporting TIV receipt in 2013/14 and earlier seasons was comparable to that reported in previous VE analyses and other community-based surveys in Canada (ca 30%) [3,7-9,11,12,16]. Proportions comparable to previous community surveys were also observed in 2013/14 for receipt of the 2009 monovalent A(H1N1)pdm09 vaccine (43% versus 41%) [17]. The proportion of participants with co-morbidity was comparable to previous Canadian estimates (15–20%) [3,6-12,18] (Table 2).

The majority of participants immunised in 2013/14 also reported prior immunisation: 30 of 31 cases (97%) and 103 of 119 controls (87%) were immunised in 2012/13 (p=0.11); 26 of 29 cases (90%) and 89 of 116 controls (77%) were immunised in both 2012/13 and 2011/12 (p=0.12); and 21 of 26 cases (81%) and 83 of 108 controls (77%) received the 2009 monovalent A(H1N1) pdm09 vaccine (p=0.67).

The adjusted VE estimate for any influenza, driven predominately by A(H1N1)pdm09, was 71% (95% CI: 54–81), and for A(H1N1)pdm09 alone was 74% (95% CI: 58–83) (Table 4). In sensitivity analyses, VE estimates remained within 1–7% of primary analysis.

Virus characterisation

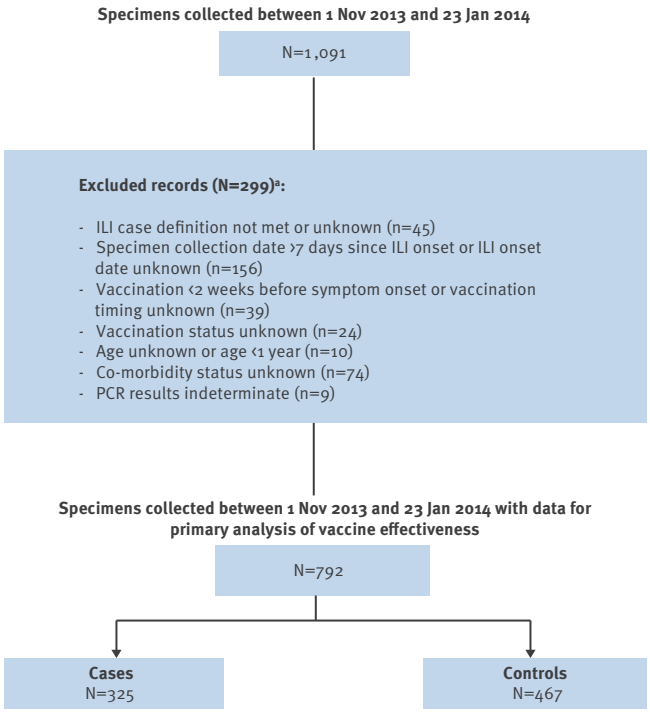
All A(H1N1)pdm09 isolates from Canada this season through week 4 (n=473, including 84 sentinel submissions) were identified by haemagglutination inhibition (HI) assay as antigenically similar to the A/California/07/2009 reference virus [1]. Only two A(H1N1)pdm09 isolates and none of the tested sentinel viruses, showed eightfold or higher reduction in HI titres against the reference strain, signalling sporadic antigenic change in only a very small proportion (<0.5%) [1,19].

HA1/HA2 sequences of a subset of 76 of 287 (26%) sentinel A(H1N1)pdm09 viruses were also assessed, including four collected in November, 45 in December and 27 in January (Figure 3; Table 5). All 76 sequences clustered within the European Centre for Disease Prevention and Control (ECDC)-described clade 6B (Figure 3) [20], representing a switch from clade 6C viruses that predominated among A(H1N1)pdm09 viruses during the 2012/13 season, albeit at substantially lower levels than A(H3N2) viruses [21].

Figure 3. Phylogenetic tree of influenza A(H1N1) pdm09 viruses, 2013/14 sentinel surveillance network, Canada, 1 November 2013–23 January 2014 (n=76)

Figure 2

Specimen exclusion, interim 2013/14 influenza vaccine effectiveness evaluation, Canada, 1 November 2013–23 January 2014 (n=1,091)



ILI: influenza-like illness.

[a] Excluded specimens may have more than one exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields.

Table 2

Profile of participants included in primary analysis, interim 2013/14 influenza vaccine effectiveness evaluation, Canada, 1 November 2013–23 January 2014 (n=792)

Characteristics	Test-positive: cases (n=325)	Test-negative: controls (n=467)	**Total (n=792)**	p value[a]
	n (%)	n (%)	**n (%)**	
Age group in years				<0.01
1–8	39 (12)	44 (9)	**83 (10)**	
9–19	30 (9)	50 (11)	**80 (10)**	
20–49	172 (53)	224 (48)	**396 (50)**	
50–64	71 (22)	95 (20)	**166 (21)**	
≥65	13 (4)	54 (12)	**67 (8)**	
Median age in years (range)	37 (1–81)	38 (1–93)	**37 (1–93)**	0.09
Female sex[b]	197 (61)	296 (64)	**493 (63)**	0.39
Co-morbidity[c]				0.09
No	263 (81)	354 (76)	**617 (78)**	
Yes	62 (19)	113 (24)	**175 (22)**	
Received 2013/14 TIV[d,e,f,g]				
≥2 weeks before symptom onset	34 (10)	135 (29)	**169 (21)**	<0.01
Among those				
without co-morbidity	19 (7)	84 (24)	**103 (17)**	<0.01
with co-morbidity	15 (24)	51 (45)	**66 (38)**	<0.01
Among those				
aged 1–8 years	3 (8)	7 (16)	**10 (12)**	0.32
aged 9–19 years	0 (0)	8 (16)	**8 (10)**	0.02
aged 20–49 years	16 (9)	58 (26)	**74 (19)**	<0.01
aged 50–64 years	10 (14)	34 (36)	**44 (27)**	<0.01
aged ≥65 years	5 (38)	28 (52)	**33 (49)**	0.39
Received prior influenza vaccine				
2012/13 TIV[h]	60/302 (20)	165/425 (39)	**225/727 (31)**	<0.01
2011/12 TIV[i]	60/284 (21)	159/414 (38)	**219/698 (31)**	<0.01
2009 A(H1N1)pdm09 vaccine[j]	91/265 (34)	156/366 (43)	**247/631 (39)**	0.04
Collection interval (days)				<0.01
≤4	264 (81)	329 (70)	**593 (75)**	
5–7	61 (19)	138 (30)	**199 (25)**	
Median interval in days (range)	3 (0–7)	3 (0–7)	**3 (0–7)**	0.04

TIV: trivalent inactivated vaccine.

[a] Differences between cases and controls were compared using the chi-squared test, Fisher's Exact test, or Wilcoxon rank-sum test.

[b] Patient's sex was missing for four specimens.

[c] Chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization (NACI) [43], including heart, pulmonary (including asthma), renal, metabolic (such as diabetes), blood, cancer, immune comprising 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 of these conditions without specifying.

[d] Vaccination status was based on self/parent/guardian report. Detail related to special paediatric dosing requirements was not sought.

[e] Immunised participants were predominantly offered split (non-adjuvanted) 2013/14 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 [43]. Others are encouraged to receive vaccine but must purchase it. In Ontario, Alberta and Manitoba, the vaccine is provided free of charge to all residents aged six months or older.

[f] In Canada, live-attenuated vaccine for nasal administration is approved for those aged two to 59 years [43] but its use remains infrequent. For the 2013/14 season (as of 23 January 2014), of 169 participants reporting vaccine receipt at least two weeks before symptom onset in this study, 149 reported this was given through muscular injection and five through nasal spray (of whom four were individuals younger than 20 years); route of administration was unspecified for 15 participants.

[g] In Canada, MF59-adjuvanted vaccine is approved for people aged 65 years and older [43]. For the 2013/14 season (as of 23 January 2014), of the 33 people aged 65 years and older who were immunised at least 2 weeks before symptom onset in this study, eight reported they had received the adjuvanted vaccine and 13 did not know, while 12 received the non-adjuvanted formulation.

[h] Participants with unknown 2012/13 vaccine receipt and children younger than two years in 2013/14 were excluded from 2012/13 vaccine uptake analysis. Children younger than two years may not have been eligible for vaccination during the fall 2012/13 immunisation campaign on the basis of age under six months.

[i] Participants with unknown 2011/12 vaccine receipt and children younger than three years in 2013/14 were excluded from 2011/12 vaccine uptake analysis. Children younger than three years may not have been eligible for vaccination during the fall 2011/12 immunisation campaign on the basis of age under six months.

[j] Participants with unknown 2009 vaccine receipt and children younger than five years in 2013/14 were excluded from monovalent A(H1N1)pdm09 vaccine uptake analysis. Children younger than five years may not have been eligible for vaccination during the fall 2009 immunisation campaign on the basis of age under six months. More than 95% of the monovalent A(H1N1)pdm09 vaccine administered in Canada during the 2009 campaign was AS03-adjuvanted product [10].

Table 3

Laboratory profile of specimens included in primary analysis, interim 2013/14 influenza vaccine effectiveness evaluation, Canada, 1 November 2013–23 January 2014 (n=792)

Specimen included	Alberta (n=256)	British Columbia (n=149)	Manitoba (n=38)	Ontario (n=187)	Quebec (n=162)	**Total (n=792)**
	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Influenza-negative	166 (65)	95 (64)	25 (66)	113 (60)	68 (42)	**467 (59)**
Influenza-positive	90 (35)	54 (36)	13 (34)	74 (40)	94 (58)	**325 (41)**
A-positive	89 (99)	53 (98)	12 (92)	73 (99)	73 (78)	**300 (92)**
B-positive	1 (1)	1 (2)	1 (8)	1 (1)	21 (22)	**25 (8)**
Influenza A-positive						
(H1N1)pdm09	88 (99)	48 (91)	9 (75)	69 (95)	73 (100)	**287 (96)**
H3N2	1 (1)	4 (8)	0 (0)	1 (1)	0 (0)	**6 (2)**
Subtype unknown	0 (0)	1 (2)	3 (25)	3 (4)	0 (0)	**7 (2)**

Two egg-adapted A/California/07/2009 seed strains, NYMC X-179A and X-181, have been available to manufacturers for vaccine production since 2009, both identical in their antigenic site aa sequence to the WHO-recommended A/California/07/2009 reference strain (with a single substitution in a non-antigenic site (N129D) in X-181). Of the publicly supplied TIV in Canada, 70% was derived from X-179A and 30% from X-181. Sentinel viruses shared 90%-94% aa identity with the vaccine across antigenic sites, the majority showing 94% identity with the vaccine. All 76 sentinel sequences had the same three antigenic site mutations: K163Q (site Sa), a clade 6B marker, as well as S185T (site Sb) and S203T (site Ca1), both of which were also identified among dominant circulating A(H1N1) pdm09 viruses of the past two seasons [12,21]. Five of 76 sequences bore a fourth aa substitution unique to each virus, and one Quebec sequence bore five substitutions (Table 5). Other than S185T, present in all 76 sequences, A186T, present in the single Quebec sequence, and possibly N156K and S157L [22], each present in a single and different Alberta sequence, none of the other substitutions were located within or adjacent to the receptor-binding site. With the exception of the single Quebec sequence, antigenic site mutations R205K, A141T, and A186T, which are located close to the receptor-binding site [22-25] and which occurred in 37%, 30% and 14%, respectively, of sentinel sequences during the 2012/13 season [21], were not evident in 2013/14.

Discussion

To date, the 2013/14 influenza season in North America has been characterised by substantial A(H1N1)pdm09 activity. This dramatic resurgence after only low-level circulation in the years since the 2009 pandemic has raised questions about possible virus evolution (i.e. antigenic drift) and reduced VE (i.e. vaccine failure). Our interim 2013/14 virological and VE analysis provides timely reassurance against both of these concerns. We show that circulating A(H1N1)pdm09 viruses are well-conserved based on genotypic and phenotypic characterisation, and that vaccine protection is substantial, reducing the risk of medically-attended laboratory-confirmed A(H1N1)pdm09 illness by about three quarters.

Our point estimate of ca 75% VE for the 2013/14 non-adjuvanted TIV against influenza A(H1N1)pdm09 is comparable, if not exceeding, 2009 estimates for non-adjuvanted formulations of the monovalent pandemic vaccine used in the US (ca 60%) [26,27], albeit lower than the 93% VE estimated by our sentinel system for the 2009 AS03-adjuvanted pandemic vaccine used in Canada [10]. The 2013/14 mid-season VE estimate against influenza A(H1N1)pdm09 of ca 75% is in the upper range of recent seasons' VE estimates for non-adjuvanted TIV against A(H1N1)pdm09 reported since 2010 from Canada [11,12,21], Europe [28-32] and the US [33-35], which span ca 60–80%. With several times more influenza A(H1N1)pdm09 cases already contributing thus far in 2013/14 than in previous seasons in Canada, we are likely to converge upon a more stable and accurate estimate of TIV protection against A(H1N1) pdm09 infection this season.

Although a switch from clade 6C to clade 6B* occurred between the 2012/13 and 2013/14 seasons [21], A(H1N1) pdm09 viruses remain genetically and antigenically similar to the A/California/07/2009 vaccine strain, a somewhat surprising finding given that this virus has circulated globally since 2009. Historically, however, H1N1 compared with H3N2 subtype viruses generally have shown a slower pace of HA antigenic change, judging at least by the recommended updates to vaccine composition made by the WHO between 1990/91 and 2008/09 (five H1N1 versus 11 H3N2 vaccine strain switches), with two H1N1 (but no H3N2) strains retained as TIV components for at least seven consecutive years during that period [4,36]. Genetic conservation of A(H1N1)pdm09 viruses may also be surprising in the context of population-level immune pressure. A

Table 4

Interim 2013/14 influenza vaccine effectiveness evaluation, influenza A(H1N1)pdm09 and influenza (any), Canada, 1 November 2013–23 January 2014 (n=792)

Analysis scenarios	A(H1N1)pdm09[a]		Influenza (any)	
	VE (95% CI)	Number Total (Cases; Vac) [Controls; Vac]	VE (95% CI)	Number Total (Cases; Vac) [Controls; Vac]
Primary analysis[b]				
Crude (unadjusted)	73 (59–83)	754 (287; 28) [467; 135]	71 (57–81)	792 (325; 34) [467; 135]
Age (1–8, 9–19, 20–49, 50–64, ≥65 years)	71 (55–82)		69 (53–80)	
Comorbidity (yes/no)	73 (58–83)		71 (56–81)	
Province (AB, BC, MB, ON, QC)	72 (56–82)		68 (52–79)	
Specimen collection interval (≤4/5–7 days)	73 (58–82)		71 (56–80)	
Week of illness onset	76 (62–85)		74 (61–83)	
Age, comorbidity, province, interval, week	74 (58–83)		71 (54–81)	
Sensitivity analysis[c]				
Restricted to specimens collected from 1 Dec 2013 to 23 Jan 2014 (week 49, 2013 to week 4, 2014)				
Crude	78 (65–86)	639 (279; 28) [360; 120]	76 (63–84)	674 (314; 34) [360; 120]
Adjusted	76 (60–85)		73 (57–83)	
Vaccination defined without regard to vaccination timing (i.e. any immunisation)				
Crude	72 (58–81)	780 (293; 34) [487; 155]	70 (56–79)	819 (332; 41) [487; 155]
Adjusted	71 (56–81)		68 (52–79)	
Restricted to patients with no comorbidities				
Crude	79 (63–89)	587 (233; 14) [354; 84]	75 (58–85)	617 (263; 19) [354; 84]
Adjusted[d]	81 (64–90)		76 (58–86)	
Restricted to participants with specimen collection interval ≤4 days				
Unadjusted	75 (58–85)	566 (237; 21) [329; 92]	74 (58–84)	593 (264; 24) [329; 92]
Adjusted[e]	76 (58–86)		74 (57–85)	
Restricted to participants aged 20–49 years				
Unadjusted	74 (50–86)	378 (154; 13) [224; 58]	71 (47–84)	396 (172; 16) [224; 58]
Adjusted[f]	75 (51–88)		71 (46–85)	
Restricted to participants aged 50–64 years				
Unadjusted	73 (36–89)	156 (61; 8) [95; 34]	71 (35–87)	166 (71; 10) [95; 34]
Adjusted[f]	80 (49–92)		77 (45–90)	
Restricted to participants aged 20–64 years				
Unadjusted	73 (56–84)	534 (215; 21) [319; 92]	70 (53–82)	562 (243; 26) [319; 92]
Adjusted[f]	76 (59–86)		73 (55–84)	

AB: Alberta; BC: British Columbia; CI: confidence interval; 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 H3N2 or unknown subtype or with influenza B were excluded from the A(H1N1)pdm09 analysis.

[b] For primary analysis, those with unknown comorbidity and those immunised less than two weeks before symptom onset or with unknown interval between immunisation and symptom onset were excluded but explored in sensitivity analysis as shown.

[c] Adjusted for age, comorbidity, province, specimen collection interval, and week of illness onset, unless otherwise specified.

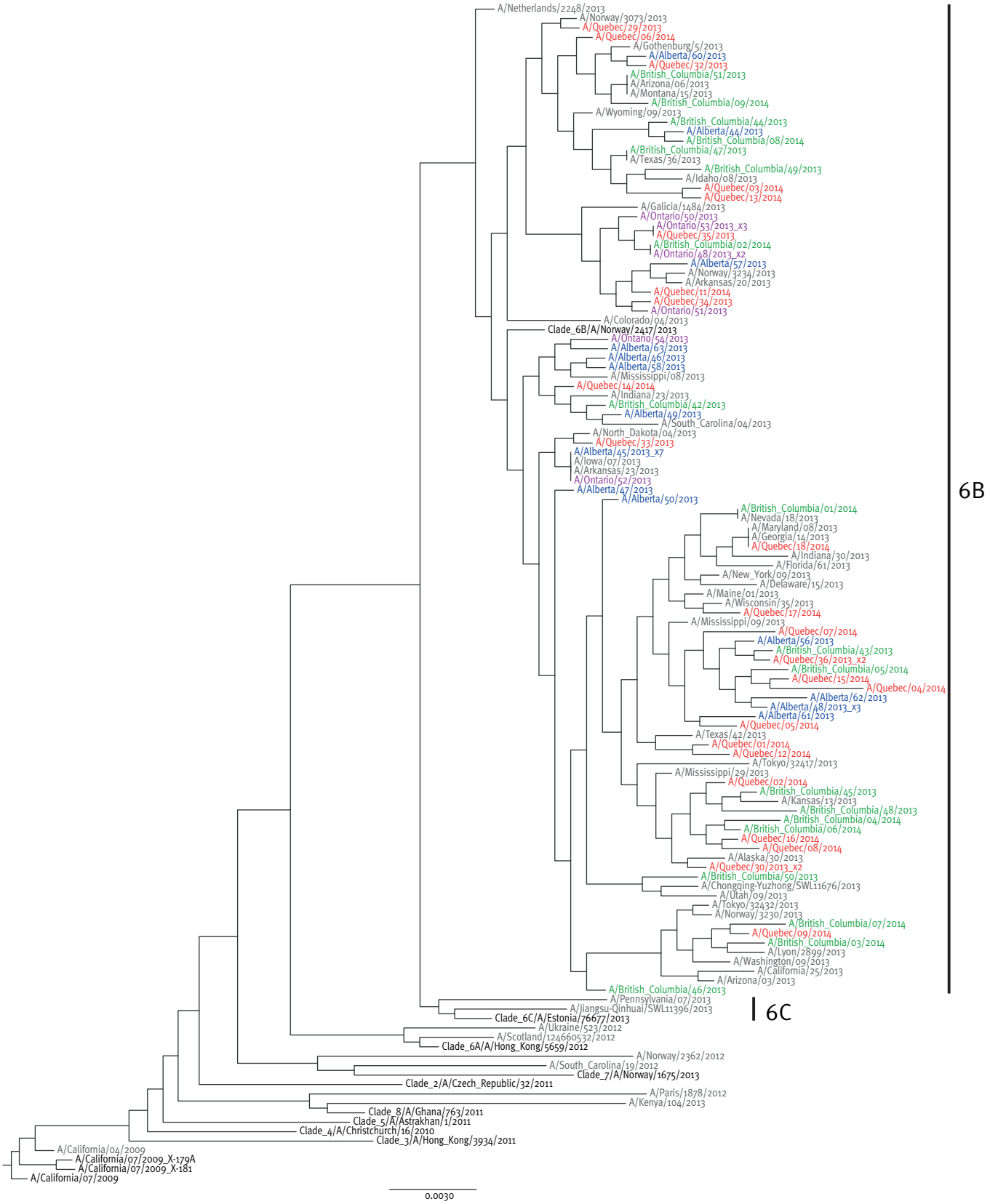
[d] Adjusted for age, province, specimen collection interval, and week of illness onset.

[e] Adjusted for age, comorbidity, province, and week of illness onset.

[f] Adjusted for comorbidity, province, specimen collection interval, and week of illness onset.

Figure 3

Phylogenetic tree of influenza A(H1N1)pdm09 viruses, 2013/14 sentinel surveillance network, Canada, 1 November 2013–23 January 2014 (n=76)



The phylogenetic tree was created by aligning the 76 Canadian sentinel sequences (colour-coded green for British Columbia, blue for Alberta, purple for Ontario and red for Quebec) against sequences representative of emerging viral clades as described by the European Centre for Disease Prevention and Control (ECDC) [20] (n=9), a random selection of A(H1N1)pdm09 sequences collected globally between 1 October 2013 and 21 January 2014 and obtained from the Global Initiative on Sharing Avian Influenza Data (GISAID) (n=43), and recent vaccine reference and egg-adapted seed strains (n=3).

Table 5

Amino acid changes in the haemagglutinin (HA1) genes (antigenic regions)[a] of a subset of 2013/14 Canadian sentinel influenza A(H1N1)pdm09 strains relative to vaccine reference strains[b], Canada, 1 November 2013–23 January 2014 (n=76)

Antigenic site		Cb	Sa				Ca1	Sb		Ca1
Amino acid number HA1		71	156	157	162	163	168	185	186	203
A/California/07/2009		S	N	S	S	K	D	S	A	S
A/California/07/2009 (X-179A)		S	N	S	S	K	D	S	A	S
British Columbia	n									
A/British Columbia/42/2013	16					**Q**		**T**		**T**
A/British Columbia/43/2013	1	**P**				**Q**		**T**		**T**
A/British Columbia/48/2013	1					**Q**	**N**	**T**		**T**
A/British Columbia/05/2014	1				**N**	**Q**		**T**		**T**
Alberta	n									
A/Alberta/44/2013	20					**Q**		**T**		**T**
A/Alberta/49/2013	1		**K**			**Q**		**T**		**T**
A/Alberta/62/2013	1			**L**		**Q**		**T**		**T**
Ontario	n									
A/Ontario/48/2013	9					**Q**		**T**		**T**
Quebec	n									
A/Quebec/29/2013	25					**Q**		**T**		**T**
A/Quebec/17/2014	1				**R**	**Q**		**T**	**T**	**T**

[a] Antigenic regions Sa, Sb, Ca1, Ca2 and Cb comprise 50 amino acid residues [12,13]. Only the nine positions in those 50 residues showing mutations in the present study are displayed.

[b] The northern hemisphere influenza A(H1N1)pdm09 vaccine reference strain since 2009, including the current 2013/14 season, is A/California/07/2009. The two egg-adapted seed strains available to manufacturers for vaccine production (NYMC X-179A and NYMC X-181) are both identical in their antigenic site amino acid sequences to the A/California/07/2009 reference strain recommended by the World Health Organization.

Bold font signifies amino acid substitution compared with the 2013/14 northern hemisphere vaccine reference strain.

All sequences were deposited into GenBank (accession numbers: KJ395993–KJ396037, KJ406381-KJ406387, KJ406507-KJ406528).

recent serosurvey conducted in May 2013 in Canada showed that levels of seroprotective antibody to A/California/07/2009 were high among school-aged children and the elderly; however, seroprotection was lower among very young children and adults between 20 and 69 years of age [37]. These findings may explain why conserved A(H1N1)pdm09 viruses resurged in 2013/14 and why there has been an apparent shift in the age distribution toward 20–64 year-old adults among medically-attended laboratory-confirmed influenza cases identified through the sentinel surveillance network this season. Such a demographic shift in disease burden toward adults following the 2009 pandemic was previously predicted in mathematical models from Canada [38] and warrants further empiric evaluation in additional surveillance datasets.

Limitations of the Canadian sentinel surveillance network for VE estimation have been described previously [3,5-12]. Although the validity of VE estimates derived by the test-negative approach has been demonstrated theoretically and in relation to randomised clinical trial analysis [39,40], the design remains observational, and bias and confounding cannot be ruled out. VE estimates for 2013/14 may vary at the end of the season, particularly since A(H1N1)pdm09 activity is still peaking in some regions of Canada [1]. However, end-of-season estimates for the 2012/13 VE differed by less than 5% from interim results presented in mid-season, even though the number of contributing cases increased by more than one third [3,21]. Ongoing monitoring is nevertheless warranted for changes in virus and/or VE with further time across the season. Variable

efficacy of repeated immunisation has previously been described, with differential effects depending upon the antigenic distance between successive vaccine components and circulating strains [41]. In that context, as in previous years, we emphasise that a substantial proportion of our immunised participants are repeat recipients of unchanged A(H1N1)pdm09 vaccine antigen. Generalisability to regions with a different profile of vaccine uptake may be limited on that basis. In recent analyses, we [12] and others [29,30,42] have noted a trend toward improved VE with recurrent receipt of the A(H1N1)pdm09 antigen, although other studies have reported contrary findings [28,31,35]. Assessment of these effects may benefit from the additional power available in end-of-season analysis.

In summary, our interim findings indicate that the 2013/14 TIV provides substantial protection against resurgent but conserved A(H1N1)pdm09 viruses circulating in Canada during the 2013/14 season, reducing the risk of medically-attended laboratory-confirmed A(H1N1)pdm09 illness by about three quarters. Neither antigenic drift nor homologous vaccine failure can account for resurgent A(H1N1)pdm09 activity this season in Canada. Other factors involved in agent–host interaction, including pre-existing antibody, should be considered in explaining the current epidemiology of this virus.

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Conflict of interest

Within 36 months of manuscript submission, GDS received research grants from GlaxoSmithKline (GSK) and Sanofi Pasteur for unrelated vaccine studies and travel fee reimbursement to attend an ad hoc GSK Advisory Board, without honorarium. JG 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. SMM has received research grants from GSK, Sanofi Pasteur and Pfizer. SMM is a Canada Research Chair in Pharmaco-epidemiology and Vaccine Evaluation. SS and TLK are funded by the Canadian Institutes of Health Research Grant (TPA-90193). The other authors declare that they have no competing interests to report.

Authors' contributions

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

*Authors' correction:

On request of the authors, this passage was changed on 7 February 2014 from "a switch from clade 6B to clade 6C occurred" to "a switch from clade 6C to clade 6B occurred".

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