Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015

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

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. University of Alberta, Edmonton, Canada
9. Alberta Provincial Laboratory, Edmonton, Canada
10. Alberta Provincial Laboratory, Calgary, Canada
11. University of Toronto, Toronto, Canada
12. National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
13. University of Manitoba, Winnipeg, Canada

Citation style for this article:
Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson JA, Winter AL, Drews SJ, Fonseca K, Charest H, Gubbay JB, Petric M, Krajden M, Kwindt TL, Martineau C, Eshaghi A, Bastien N, Li Y. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. Euro Surveill. 2015;20(4):pii=21022. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21022

Article submitted on 21 January 2015 / published on 29 January 2015

The 2014/15 influenza season to date in Canada has been characterised by predominant influenza A(H3N2) activity. Canada's Sentinel Physician Surveillance Network (SPSN) assessed interim vaccine effectiveness (VE) against medically attended, laboratory-confirmed influenza A(H3N2) infection in January 2015 using a test-negative case–control design. Of 861 participants, 410 (48%) were test-positive cases (35% vaccinated) and 451 (52%) were test-negative controls (33% vaccinated). Among test-positive cases, the majority (391; 95%) were diagnosed with influenza A, and of those with available subtype information, almost all influenza A viruses (379/381; 99%) were A(H3N2). Among 226 (60%) A(H3N2) viruses that were sequenced, 205 (91%) clustered with phylogenetic clade 3C.2a, considered genetically and antigenically distinct from the 2014/15 A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain, and typically bearing 10 to 11 amino acid differences from the vaccine at key antigenic sites of the haemagglutinin protein. Consistent with substantial vaccine mismatch, little or no vaccine protection was observed overall, with adjusted VE against medically attended influenza A(H3N2) infection of –8% (95% CI: –50 to 23%). Given these findings, other adjunct protective measures should be considered to minimise morbidity and mortality, particularly among high-risk individuals. Virus and/or host factors influencing this reduced vaccine protection warrant further in-depth investigation.

Background

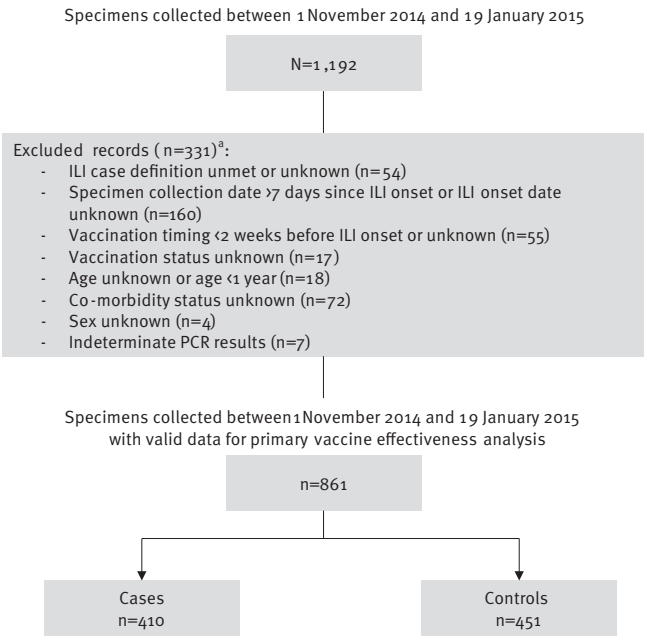
In Canada, the 2014/15 influenza season has been distinguished by an early and intense epidemic due almost exclusively (›90%) to influenza A(H3N2) subtype viruses. Virtually all (›99%) of these A(H3N2) viruses have been characterised as genetically and/or antigenically distinct from the A/Texas/50/2012(H3N2)-like (clade 3C.1) vaccine reference strain used for both the current 2014/15 and prior 2013/14 northern hemisphere influenza vaccines [1].

This profile of dominant influenza A(H3N2) activity is in sharp contrast to the 2013/14 season, when an early epidemic peak also occurred, but was instead due to predominant but antigenically well-conserved A(H1N1) pdm09 viruses [2]. The 2014/15 season more closely resembles that of 2012/13, although the predominant vaccine-mismatched influenza A(H3N2) activity in that season in Canada was related to a different combination of vaccine-virus divergence, notably mutations in that season's egg-adapted vaccine strain used for manufacturing, rather than antigenic drift in circulating viruses [3,4]. In some parts of Canada, an unprecedented number of influenza outbreaks in long-term care facilities (LTCF) were reported in association with vaccine mismatch in 2012/13 [4,5], but the mid-season tally for 2014/15 has already exceeded even that of 2012/13 in some jurisdictions [5].

In response to surveillance signals suggesting suboptimal vaccine performance, Canada's Sentinel Physician

Figure 1

Specimen inclusion and exclusion criteria, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)



ILI: influenza-like illness.

[a] Exclusions are not mutually exclusive; specimens may have >1 exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded.

Surveillance Network (SPSN) assessed interim influenza vaccine effectiveness (VE) in January 2015. VE findings are presented in the context of in-depth genetic and antigenic characterisation of contributing sentinel influenza A(H3N2) viruses, relevant to the upcoming selection of vaccine strains in February 2015 by the World Health Organization (WHO) for the 2015/16 northern hemisphere influenza vaccine. Findings are also considered in relation to virus-host interactions, notably the effects of influenza vaccination in the previous season on protection by the current season's vaccine.

Methods

Epidemiological estimation of influenza vaccine effectiveness

As previously described [2-4,6,7], a test-negative case–control design was used to estimate VE. Inclusion and exclusion criteria applied to the current dataset are shown in Figure 1. Patients presenting to community-based practitioners at sentinel sites across participating provinces (British Columbia, Alberta, Ontario and Quebec) within seven days of onset of influenza-like illness (ILI) and testing positive for influenza were considered cases; those testing negative were considered controls. ILI was defined as acute onset of respiratory illness with fever and cough and one or more of the following symptoms: sore throat, arthralgia, myalgia, or prostration. Fever was not an eligibility requirement for elderly adults 65 years and older.

As annual influenza immunisation campaigns typically commence in October across Canada, and increased influenza virus circulation (exceeding 10% test-positivity) typically begins in early November, nasal or nasopharyngeal specimens collected from 1 November 2014 (week 44) were eligible for inclusion in the primary VE analysis. Epidemiological information was obtained from consenting patients or their parent/guardian using a standard questionnaire at specimen collection. Ethics review boards in participating provinces approved this study.

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 by self-reported vaccination status were estimated by multivariable logistic regression. VE was calculated as $(1 - OR) \times 100\%$. Vaccine was administered to participants during the seasonal immunisation campaign. Non-adjuvanted, inactivated, split trivalent influenza vaccine (TIV) is primarily used in Canada. Live attenuated influenza vaccine (LAIV) is approved for individuals two to 59 years-old, including the trivalent but for the first time in Canada also the quadrivalent formulation, and was publicly funded in the SPSN provinces of British Columbia, Alberta and Quebec. An adjuvanted subunit TIV is approved for elderly Canadians and publicly funded in British Columbia and Ontario. Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated. Those for whom vaccination timing was unknown or less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analyses, as were participants whose comorbidity status was unknown. The effects of prior 2013/14 influenza vaccine receipt on current vaccine protection were explored through indicator variable analysis.

Influenza vaccine manufacturers require an egg-adapted, high-growth reassortant (HGR) version of the reference strain recommended by WHO for further high-yield propagation in embryonated hens' eggs. The HGR version of the WHO-recommended A/Texas/50/2012(H3N2) reference strain [8] used by manufacturers for both the 2014/15 and 2013/14 northern hemisphere influenza vaccines is called X-223A and differs from the A/Texas/50/2012(H3N2) prototype by three amino acids (aa) in antigenic sites of the haemagglutinin (HA) protein.

Laboratory characterisation of contributing sentinel viruses

The HA1 and HA2 regions of the HA gene from a convenience sample of sentinel influenza A(H3N2) viruses

Table 1

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing All Influenza Data and used in phylogenetic analysis, 2014/15 Canadian Sentinel Physician Surveillance Network (n = 13)

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI539806	Hong Kong (SAR)	30 Apr 2014	A/Hong Kong/5738/2014	Government Virus Unit	National Institute for Medical Research	
EPI539576	Hong Kong (SAR)	26 Feb 2014	A/Hong Kong/4801/2014	Government Virus Unit	National Institute for Medical Research	
EPI426061	Hong Kong (SAR)	11 Jan 2013	A/Hong Kong/146/2013	Government Virus Unit	National Institute for Medical Research	
EPI530647	Norway	3 Feb 2014	A/Norway/466/2014	WHO National Influenza Centre	National Institute for Medical Research	
EPI460558	Russian Federation	12 Mar 2013	A/Samara/73/2013	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
EPI360950	Germany	3 Jul 2011	A/Berlin/93/2011	National Institute for Medical Research	Centers for Disease Control and Prevention	
EPI530687	Switzerland	6 Dec 2013	A/Switzerland/9715293/2013	Hopital Cantonal Universitaire de Geneves	National Institute for Medical Research	
EPI543062	Switzerland	1 Jan 2013	A/Switzerland/9715293/2013 X-247	New York Medical College	Centers for Disease Control and Prevention	
EPI551814	Australia	1 Jan 2014	IVR-176(A/ Switzerland/9715293/2013)	CSL Ltd	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M.; Iannello,P.; Spirason,N.; Jelley,L.; Lau,H.; Komadina,N.
EPI377499	United States	15 Apr 2012	A/Texas/50/2012	Texas Department of State Health Services -Laboratory Services	Centers for Disease Control and Prevention	
EPI407126	United States	1 Jan 2012	A/Texas/50/2012 X-223A	New York Medical College	Centers for Disease Control and Prevention	
EPI349103	Australia	24 Oct 2011	A/Victoria/361/2011	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M; Caldwell,N; Iannello,P; Komadina,N
EPI358038	Australia	1 Jan 2011	IVR-165(A/Victoria/361/2011)	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	

WHO: World Health Organization.

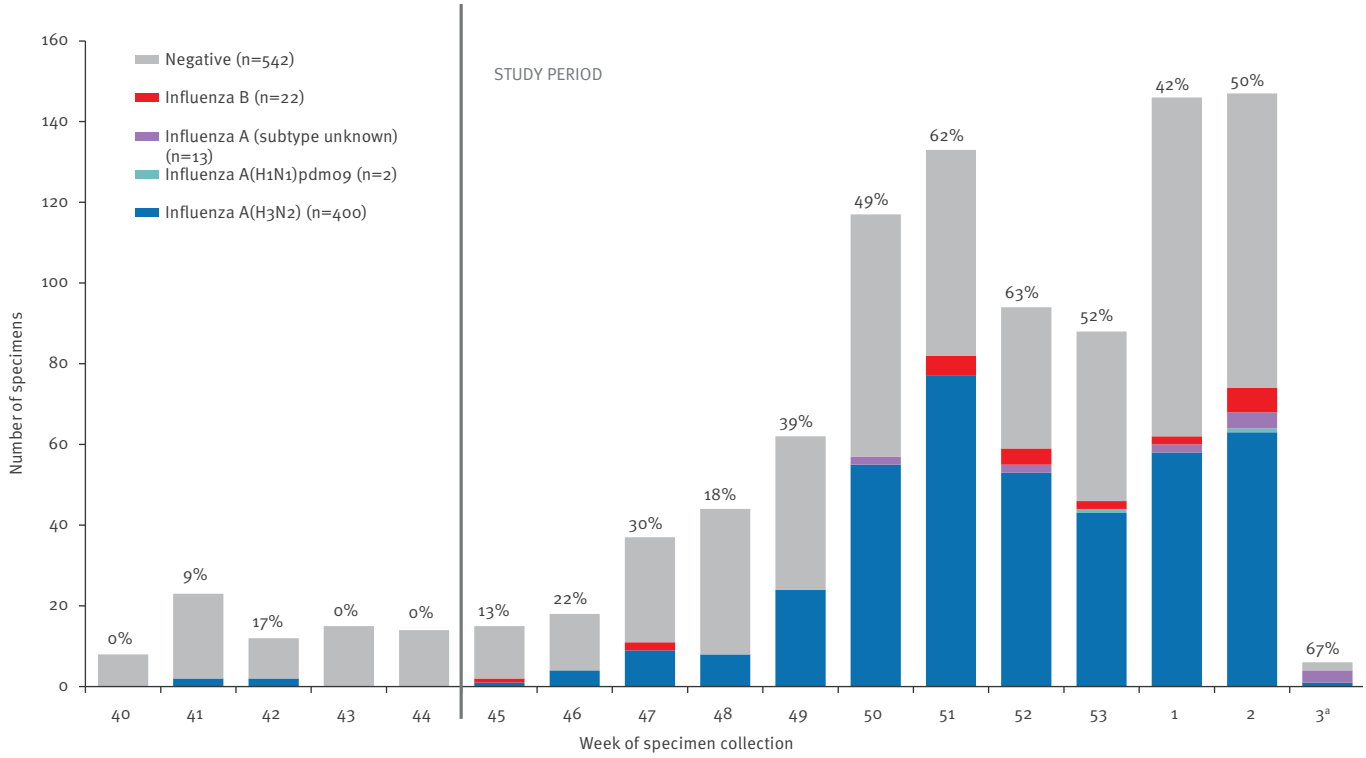
from original patient specimens contributing to VE analysis were sequenced for phylogenetic and pairwise aa identity analysis based on antigenic maps spanning the 131 aa residues across HA1 antigenic sites A–E [4,6,7,9]. The approximate likelihood method was used to generate the phylogenetic tree of aligned translated sequences in FastTree [10], visualised in FigTree [11], including representative vaccine reference, HGR and clade-specific HA sequences shown in Table 1, kindly made available by the Global Initiative on Sharing All Influenza Data (GISAID), and using clade nomenclature specified by the European Centre for Disease Prevention and Control (ECDC) [12].

Historically, each new significant antigenic drift variant has, in general, had at least four aa substitutions located in at least two antigenic sites [13]. However, substitutions at antigenic sites A, B and D of the H3 globular head located closest to the receptor-binding site (RBS) are typically considered most influential [14], with site B being emphasised as particularly immunodominant among more recent influenza A(H3N2) strains [15]. Substitutions at just seven antigenic site positions, located in antigenic site A (position 145) and B (positions 155, 156, 158, 159, 189 and 193) have been emphasised in relation to all major A(H3N2) antigenic cluster transitions since 1968 [16]. Substitutions associated with gain or loss of glycosylation may also influence antibody binding [17]. Sequencing findings among sentinel influenza A(H3N2) viruses are thus interpreted within these key antigenic considerations.

A convenience sample of influenza-positive specimens was also inoculated into Madin Darby Canine Kidney (MDCK) (British Columbia, Alberta, Quebec) or Rhesus Monkey Kidney (Ontario) cell culture for virus isolation.

Figure 2

Laboratory detections of influenza by week and type/subtype, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 28 September 2014–19 January 2015 (n = 978)



[a] Based on partial week.

Influenza percent positivity by week is shown above bars.

One participant in week 1 had co-infection with influenza A(H3N2) and influenza B; subtotals for influenza A and B will add to more than the total number of influenza positives.

Of the 1,286 nasal or nasopharyngeal specimens collected between week 40 (starting 28 September 2014) and week 3 (starting 18 January 2015), we excluded 308 specimens from the epidemic curve: those failing to meet the influenza-like illness (ILI) case definition or for whom it was unknown (n=58), those whose specimens were collected more than seven days after ILI onset or for whom the interval was unknown (n=173), those whose age was unknown or who were younger than one year (n=20), those with unknown comorbidity status (n=80), those with unknown sex (n=4) 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. 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.

Note that the epidemic curve displays specimen collection and influenza detections from week 40 and regardless of the patient's vaccination status or timing; as such, tallies do not match those in the text.

Aliquots of virus isolates were submitted to the National Microbiology Laboratory (NML), Canada's influenza reference laboratory, for antigenic characterisation by haemagglutination inhibition (HI) assay using guinea pig erythrocytes [4,18] in relation to the cell-passaged A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain and the A/Switzerland/9715293/2013(H3N2)-like clade 3C.3a reference strain recommended for the 2015 southern hemisphere vaccine [8]. To address potential neuraminidase-mediated binding of influenza A(H3N2) viruses to erythrocytes, the HI assay was conducted in the presence of 20 nM oseltamivir carboxylate following re-growth of viruses in MDCK-SIAT1 cells [19]. HI titres were recorded as the reciprocal of the highest ferret serum dilution at which inhibition of haemagglutination was detected. Previously, a ≥ 4-fold reduction in post-infection ferret HI-antibody titre was considered a signal of antigenic distinction between the field isolate and vaccine reference strain, but this has more recently been revised to a ≥ 8-fold titre reduction [18]. Due to difficulties this season in growing influenza A(H3N2) viruses to sufficient titres for antigenic characterisation by HI assay in the presence of oseltamivir carboxylate, genetic characterisation by sequencing at the NML and provincial public health laboratories was performed to infer antigenic properties of sentinel viruses, as also reported in national laboratory-based surveillance summaries in the United States [20] and Canada [1] for the current 2014/15 season.

Results

Epidemiological findings

A total of 1,192 specimens were submitted within the VE study period, of which 861 (72%) were included in primary VE analyses with collection dates between 3

Table 2

Influenza virus characterisation by type and subtype, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Specimen	Alberta n (%)	British Columbia n (%)	Ontario n (%)	Quebec n (%)	Overall n (%)
Total	**262**	**156**	**228**	**215**	**861**
Influenza-negative	128 (49)	89 (57)	130 (57)	104 (48)	451 (52)
Influenza-positive	134 (51)	67 (43)	98 (43)	111 (52)	410 (48)
Influenza A[a]	131 (98)	63 (94)	96 (98)	101 (91)	391 (95)
A(H3N2)	130 (99)	57 (90)	95 (99)	97 (96)	379 (97)
A(H1N1)pdm09	0 (0)	0 (0)	1 (1)	1 (1)	2 (1)
Subtype unknown	1 (1)	6 (10)	0 (0)	3 (3)	10 (3)
Influenza B[a]	3 (2)	4 (6)	2 (2)	11 (10)	20 (5)
Antigenic characterisation of A(H3N2) sentinel viruses by HI assay[b]					
Total	**6**	**1**	**0**	**0**	**7**
A/Texas/50/2012-like[c]	**0**	**0**	**0**	**0**	**0**
< 4-fold reduced titre	0	0	0	0	0
≥ 4-fold reduced titre	5	0	0	0	5
≥ 8-fold reduced titre	5	0	0	0	5
Insufficient volume for HI assay	1	1	0	0	2
A/Switzerland/9715293/2013-like[c]	**6**	**1**	**0**	**0**	**7**
< 4-fold reduced titre	3	1	0	0	4
≥ 4-fold reduced titre	3	0	0	0	3
≥ 8-fold reduced titre	0	0	0	0	0
Genetic characterisation of A(H3N2) sentinel viruses by sequencing					
Total	**104**	**30**	**28**	**64**	**226**
Clade 3C.2a	98 (94)	17 (57)	27 (96)	63 (98)	205 (91)
Clade 3C.3x	5 (5)	13 (43)	0 (0)	1 (2)	19 (8)
Clade 3C.3	1 (1)	0 (0)	1 (4)	0 (0)	2 (1)

HI: haemagglutination inhibition.

[a] One participant in Quebec had co-infection with influenza A(H3N2) and influenza B; subtotals for influenza A and B will add to more than the total number of influenza positives.

[b] 37 additional specimens (34 Alberta, 3 Quebec) submitted to the National Microbiology Laboratory for antigenic characterisation had insufficient titre to characterise by HI assay.

[c] In two-way HI assay, anti-sera raised to the cell-passaged A/Switzerland/9715293/2013(H3N2) referent virus inhibited the homologous antigen at a titre of 320, equivalent to the titre in inhibiting the heterologous cell-passaged A/Texas/50/2012(H3N2) antigen. Conversely, anti-sera raised to the A/Texas/50/2012(H3N2) referent strain inhibited homologous antigen at an HI titre of 1280 and the heterologous A/Switzerland/9715293/2013(H3N2) antigen at a titre of 80, a 16-fold titre reduction. These referent strains are antigenically distinct.

November 2014 (week 45: 2–8 November 2014) and 19 January 2015 (week 3: 18–24 January 2015) (Figure 1, Figure 2). Of these, 410 (48%) were test-positive cases and 451 (52%) were test-negative controls. Among test-positive cases, the majority (n = 391; 95%) were influenza A, and of those with subtype information available, almost all (379/381; 99%) were A(H3N2) (Figure 2, Table 2).

As in previous SPSN publications, adults 20–49 years-old contributed the largest proportion of specimens (40%) (Table 3) [2-4,6,7]. However, compared with the 2013/14 mid-season analysis [2], a significantly lower proportion of participants in 2014/15 were 20–49 years-old (40% vs 50%; $p < 0.01$), more notable among cases (36% vs 53%; $p < 0.01$) than controls (44% vs 48%; $p > 0.05$). Conversely, a greater proportion of participants were elderly adults ≥ 65 years and older (16% vs 8%; $p < 0.01$), again more notable among cases (16% vs 4%; $p < 0.01$) than controls (15% vs 12%; $p > 0.05$) [2]. The proportion of female participants (62%) and those with chronic comorbidity (24%) were comparable to observations in the 2013/14 mid-season analysis (63% and 22%, respectively) [2].

When vaccination status was assessed without regard to timing of ILI onset, 166 of 470 (35%) controls self-reported receipt of the 2014/15 influenza vaccine, comparable to the 2013/14 mid-season analysis (32%) [2] and the most recent influenza immunisation coverage survey for the general adult population in Canada (37%) [21]. Overall, 291 (34%) participants self-reported

Table 3A

Profile of participants included in interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

	Distribution by case status n (%)				Vaccination coverage within strata n (%) vaccinated[a]			
	Overall	Cases	Controls	p value[b]	Overall	p value[b]	Cases	Controls
N (%)	861	410 (48)	451 (52)		291 (34)		144 (35)	147 (33)
Age group (years)				0.08		< 0.01		
1–8	102 (12)	48 (12)	54 (12)		18 (18)		12 (25)	6 (11)
9–19	109 (13)	62 (15)	47 (10)		19 (17)		13 (21)	6 (13)
20–49	344 (40)	146 (36)	198 (44)		93 (27)		36 (25)	57 (29)
50–64	172 (20)	87 (21)	85 (19)		64 (37)		36 (41)	28 (33)
≥ 65	134 (16)	67 (16)	67 (15)		97 (72)		47 (70)	50 (75)
Median (range)	39 (1–103)	39 (1–103)	39 (1–94)	0.98	NA		NA	NA
Sex				< 0.01		< 0.01		
Female	533 (62)	228 (56)	305 (68)		201 (38)		90 (39)	111 (36)
Male	328 (38)	182 (44)	146 (32)		90 (27)		54 (30)	36 (25)
Co-morbidity[c]				0.43		< 0.01		
No	655 (76)	307 (75)	348 (77)		180 (27)		86 (28)	94 (27)
Yes	206 (24)	103 (25)	103 (23)		111 (54)		58 (56)	53 (51)
Province				0.11		< 0.01		
Alberta	262 (30)	134 (33)	128 (28)		107 (41)		58 (43)	49 (38)
British Columbia	156 (18)	67 (16)	89 (20)		39 (25)		14 (21)	25 (28)
Ontario	228 (26)	98 (24)	130 (29)		87 (38)		42 (43)	45 (35)
Quebec	215 (25)	111 (27)	104 (23)		58 (27)		30 (27)	28 (27)

ILI: influenza-like illness; LAIV: live attenuated influenza vaccine; NA: not applicable.

[a] Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated; participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analysis. Vaccination status was based on self/parent/guardian report. Details related to special paediatric dosing requirements was not sought.

[b] Differences between cases and controls or vaccinated and unvaccinated participants (based on overall sample to explore potential confounding) were compared using the chi-squared test or Wilcoxon rank-sum test.

[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, including heart, pulmonary, renal, metabolic, blood, cancer and immunocomprising conditions or those that compromise management of respiratory secretions, or morbid obesity. Questionnaire answers were 'yes,' 'no,' or 'unknown' without specifying the co-morbidity.

receipt of the 2014/15 vaccine at least two weeks before ILI onset and were considered vaccinated for the purpose of VE analysis. Among vaccinated participants reporting vaccine type, the proportion that received LAIV was 10% (16/165) in those two to 59 years-old and 47% (16/34) in those two to 19 years-old (i.e. all LAIV recipients were two to 19 years-old) (Table 3). The proportion of vaccinated participants overall did not differ significantly between cases and controls (35% vs 33%; p = 0.43). As observed in previous publications of the SPSN [2-4,6,7], the vast majority of vaccinated participants in 2014/15 were repeat recipients, including 251 of 283 (89%) who had also been vaccinated in 2013/14 and 237 of 269 (88%) also vaccinated in 2012/13.

Crude VE against influenza A was −17% (95% CI: −55 to 12%), and −21% (95% CI: −61 to 9%) against the dominant circulating A(H3N2) viruses (Table 4). With full adjustment for covariates, VE estimates increased to −4% (95% CI: −45 to 25%) and −8% (95% CI: −50 to 23%) for influenza A and A(H3N2), respectively. Calendar time was the covariate most influential on adjusted VE. In sensitivity analyses, adjusted VE estimates remained within 10% of the primary analysis with confidence intervals slightly wider but consistently overlapping zero (Table 4). Among participants immunised in 2014/15 only, crude and adjusted VE estimates were higher at ca 40–50% (vs unvaccinated participants) compared with those immunised in 2013/14 only or in 2013/14 and 2014/15 (<10%); however, confidence intervals were wide and overlapping with the further reduced sample size (Table 4).

Laboratory findings

In total, 44 of 379 (12%) influenza A(H3N2)-positive specimens were submitted to Canada's NML, of which just seven of 44 (16%), collected between 17 November and 18 December 2014, had sufficient titre for antigenic characterisation by HI assay when tested in the presence of oseltamivir carboxylate. All viruses were considered antigenically distinct from the cell-passaged A/Texas/50/2012-like vaccine reference strain and

Table 3B

Profile of participants included in interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

	Distribution by case status n (%)				Vaccination coverage within strata n (%) vaccinated[a]			
	Overall	Cases	Controls	p value[b]	Overall	p value[b]	Cases	Controls
N (%)	861	410 (48)	451 (52)		291 (34)		144 (35)	147 (33)
Collection interval				<0.01		0.51		
≤4 days	642 (76)	337 (82)	305 (68)		213 (33)		118 (35)	95 (31)
5–7 days	219 (25)	73 (18)	146 (32)		78 (36)		26 (36)	52 (36)
Median (range)	3 (0–7)	3 (0–7)	3 (0–7)	<0.01	NA		NA	NA
Calendar time[d]				<0.01		0.06		
Week 45–46	31 (4)	5 (1)	26 (6)		5 (16)		1 (20)	4 (15)
Week 47–48	72 (8)	16 (4)	56 (12)		17 (24)		3 (19)	14 (25)
Week 49–50	173 (20)	78 (19)	95 (21)		57 (33)		31 (40)	26 (27)
Week 51–52	217 (25)	135 (33)	82 (18)		84 (39)		51 (38)	33 (40)
Week 53–1	221 (26)	102 (25)	119 (26)		74 (33)		32 (31)	42 (35)
Week 2–3	147 (17)	74 (18)	73 (16)		54 (37)		26 (35)	28 (38)
Received 2014/15 influenza vaccine[a]								
Any vaccination[e]	326/896 (36)	160/426 (38)	166/470 (35)	0.49	NA		NA	NA
≥2 weeks before ILI onset	291 (34)	144 (35)	147 (33)	0.43	NA		NA	NA
Received LAIV[f]	16/165 (10)	11/85 (13)	5/80 (6)	0.15	NA		NA	NA
Received adjuvanted vaccine[g]	27/51 (53)	11/21 (52)	16/30 (53)	0.95	NA		NA	NA
Prior vaccination history								
Received 2013/14 vaccine[h]	358/804 (45)	177/388 (46)	181/416 (44)	0.55	251/358 (70)	<0.01	131/177 (74)	120/181 (66)
Received 2012/13 vaccine[i]	343/761 (45)	178/377 (47)	165/384 (43)	0.24	237/343 (69)	<0.01	127/178 (71)	110/165 (67)

[a] Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated; participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analysis. Vaccination status was based on self/parent/guardian report. Details related to special paediatric dosing requirements was not sought.

[b] Differences between cases and controls or vaccinated and unvaccinated participants (based on overall sample to explore potential confounding) were compared using the chi-squared test or Wilcoxon rank-sum test.

[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, including heart, pulmonary, renal, metabolic, blood, cancer and immunocomprising conditions or those that compromise management of respiratory secretions, or morbid obesity. Questionnaire answers were 'yes,' 'no,' or 'unknown' without specifying the co-morbidity.

[d] Based on week of specimen collection. 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. Week 3 of 2015 based on partial week.

[e] Participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset or for whom vaccination timing was unknown were excluded from the primary analysis. They were included for assessing 'any' immunisation, regardless of timing, for comparison with other sources of vaccination coverage. The denominator is shown for 'any' immunisation.

[f] Among participants 2–59 years-old who received 2014/15 influenza vaccine at least two weeks before ILI onset and had valid data for type of vaccine. All 16 participants who received LAIV were 2–19 years of age. Among vaccinated participants 2–19 years-old, 16 of 34 (47%) overall received LAIV including 11 of 24 cases (46%) and five of 10 controls (50%).

[g] Among participants 65 years and older who received 2014/15 influenza vaccine at least two weeks before ILI onset and had valid data for receipt of adjuvanted vaccine.

[h] Children younger than two years in 2014/15 were excluded from 2013/14 vaccine uptake analysis as they may not have been eligible for vaccination during the immunisation campaign in autumn 2013 on the basis of age under six months.

[i] Children younger than three years in 2014/15 were excluded from 2012/13 vaccine uptake analysis as they may not have been eligible for vaccination during the immunisation campaign in autumn 2012 on the basis of age under six months.

were instead antigenically similar to the cell-passaged A/Switzerland/9715293/2013-like reference strain (Table 2). Based on phylogenetic analysis, five of these viruses clustered with clade 3C.2a and two with an emerging clade of viruses awaiting official ECDC clade-level designation and thus temporarily labelled in the current analysis as 3C.3*x*. Both clade 3C.3*x* viruses had an L157S substitution in antigenic site B and an N122D substitution in antigenic site A, as discussed below.

Of the 379 sentinel A(H3N2) viruses collected between 11 November 2014 and 10 January 2015, 226 (60%) were sequenced; 205 (91%) belonged to clade 3C.2a, 19 (8%) to our provisionally named clade 3C.3*x*, and two (1%) to clade 3C.3 (Table 2, Figure 3, Figure 4).

Table 4A

Interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Model	Influenza (any)	Influenza A	Influenza A(H3N2)
	VE (95% CI)	VE (95% CI)	VE (95% CI)
Primary analysis			
N [n case (% vac); n control (% vac)]	861 [410 (35); 451 (33)]	842 [391 (36); 451 (33)]	830 [379 (37); 451 (33)]
Unadjusted	−12 (−49 to 16)	−17 (−55 to 12)	−21 (−61 to 9)
Age group (1–8, 9–19, 20–49, 50–64, ≥ 65 years)	−11 (−51 to 18)	−17 (−60 to 14)	−22 (−67 to 10)
Sex (female/male)	−19 (−58 to 11)	−24 (−65 to 7)	−29 (−73 to 4)
Comorbidity (no/yes)	−10 (−47 to 18)	−15 (−54 to 14)	−19 (−60 to 12)
Province (Alberta, British Columbia, Ontario, Quebec)	−12 (−49 to 16)	−15 (−54 to 14)	−19 (−59 to 11)
Collection interval (≤ 4/5–7 days)	−14 (−52 to 14)	−19 (−59 to 11)	−23 (−65 to 8)
Calendar time (2-week interval)	0 (−34 to 25)	−4 (−39 to 23)	−8 (−45 to 20)
Age, sex, comorbidity, province, interval, time	−1 (−40 to 28)	−4 (−45 to 25)	−8 (−50 to 23)
Sensitivity analysis – vaccination timing			
Vaccination defined without regard to vaccination timing (i.e. any vaccination)			
N [n case (% vac); n control (% vac)]	896 [426 (38); 470 (35)]	876 [406 (38); 470 (35)]	861 [391 (39); 470 (35)]
Unadjusted	−10 (−45 to 16)	−14 (−51 to 13)	−16 (−54 to 12)
Fully adjusted[a]	0 (−37 to 27)	−2 (−41 to 26)	−5 (−44 to 24)
Participants vaccinated < 2 weeks before ILI onset recoded as 'unvaccinated'			
N [n case (% vac); n control (% vac)]	887 [422 (34); 465 (32)]	867 [402 (35); 465 (32)]	853 [388 (36); 465 (32)]
Unadjusted	−12 (−48 to 15)	−17 (−55 to 12)	−22 (−62 to 8)
Fully adjusted[a]	1 (−38 to 28)	−3 (−43 to 26)	−8 (−51 to 22)
Participants vaccinated < 2 weeks before ILI onset recoded as 'vaccinated'			
N [n case (% vac); n control (% vac)]	887 [422 (37); 465 (35)]	867 [402 (38); 465 (35)]	853 [388 (38); 465 (35)]
Unadjusted	−11 (−46 to 16)	−15 (−52 to 13)	−18 (−56 to 11)
Fully adjusted[a]	−2 (−41 to 26)	−4 (−44 to 24)	−7 (−48 to 23)
Sensitivity analysis – comorbidity			
N [n case (% vac); n control (% vac)]	910 [433 (35); 477 (31)]	890 [413 (36); 477 (31)]	878 [401 (37); 477 (31)]
Includes participants with unknown comorbidity			
Unadjusted	−17 (−54 to 11)	−22 (−61 to 8)	−26 (−67 to 5)
Fully adjusted[b]	−7 (−47 to 23)	−10 (−52 to 20)	−14 (−58 to 18)
Participants with unknown comorbidity recoded as 'no'			
Unadjusted	−17 (−54 to 11)	−22 (−61 to 8)	−26 (−67 to 5)
Fully adjusted[a]	−5 (−46 to 24)	−9 (-51 to 21)	−13 (−56 to 19)
Participants with unknown comorbidity recoded as 'yes'			
Unadjusted	−17 (−54 to 11)	−22 (−61 to 8)	−26 (−67 to 5)
Fully adjusted[a]	−6 (−46 to 23)	−9 (−51 to 21)	−13 (−57 to 18)

CI: confidence interval; ILI: influenza-like illness; VE: vaccine effectiveness; % vac: percentage vaccinated.

[a] Adjusted for age group, sex, comorbidity, province, collection interval, and calendar time.

Clade 3C.2a viruses comprised the majority (> 90%) of viruses in all contributing SPSN provinces, with the exception of British Columbia, where there was more equal contribution of clade 3C.2a (17/30; 57%) and clade 3C.3*x* (13/30; 43%). None of the 226 sentinel A(H3N2) viruses contributing to the VE analysis that were sequenced belonged to the northern hemisphere 2014/15 A/Texas/50/2012(H3N2) vaccine clade 3C.1, nor to the 2015 southern hemisphere A/Switzerland/9715293/2013(H3N2) vaccine clade 3C.3a. However, as described above, all seven viruses that could be characterised by HI assay were considered antigenically similar to the A/Switzerland/9715293/2013(H3N2) strain, even though none of those seven viruses clustered within clade 3C.3a.

Relative to the X-223A HGR, sentinel clade 3C.2a viruses typically differed by 10 or 11 antigenic site aa substitutions as itemised in Figure 3. In addition to the

Table 4B

Interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Model	Influenza (any)	Influenza A	Influenza A(H3N2)
	VE (95% CI)	VE (95% CI)	VE (95% CI)
Stratified analysis – restricted to non-elderly adult participants 20–64 years old			
N [n case (% vac); n control (% vac)]	516 [233 (31); 283 (30)]	506 [223 (32); 283 (30)]	496 [213 (33); 283 (30)]
Unadjusted	−4 (−52 to 29)	−11 (−62 to 24)	−16 (−71 to 20)
Fully adjusted[a]	11 (−35 to 41)	6 (−43 to 38)	2 (−49 to 36)
Stratified analysis – restricted to specimens collected from week 50 onward			
N [n case (% vac); n control (% vac)]	699 [365 (36); 334 (36)]	682 [348 (37); 334 (36)]	670 [336 (38); 334 (36)]
Unadjusted	1 (−34 to 28)	−4 (−42 to 24)	−8 (−48 to 21)
Fully adjusted[c]	−3 (−47 to 28)	−9 (−55 to 24)	−13 (−61 to 21)
Indicator variable analysis – effect of prior 2013/14 influenza vaccine receipt on 2014/15 VE[d]			
Unvaccinated both seasons			
N [n case (%); n control (%)]	414 [201 (52); 213 (51)]	400 [187 (51); 213 (51)]	392 [179 (50); 213 (51)]
Unadjusted/fully adjusted	Reference	Reference	Reference
Current 2014/15 influenza vaccine only			
N [n case (%); n control (%)]	32 [10 (3); 22 (5)]	32 [10 (3); 22 (5)]	32 [10 (3); 22 (5)]
Unadjusted	52 (−4 to 78)	48 (−12 to 76)	46 (−17 to 75)
Fully adjusted[a]	49 (−15 to 78)	46 (−24 to 76)	43 (−29 to 75)
Prior 2013/14 influenza vaccine only			
N [n case (%); n control (%)]	107 [46 (12); 61 (15)]	105 [44 (12); 61 (15)]	105 [44 (12); 61 (15)]
Unadjusted	20 (−23 to 48)	18 (−27 to 47)	14 (−33 to 44)
Fully adjusted[a]	8 (−47 to 42)	8 (−47 to 43)	4 (−54 to 40)
Both 2013/14 and 2014/15 influenza vaccine			
N [n case (%); n control (%)]	251 [131 (34); 120 (29)]	248 [128 (35); 120 (29)]	247 [127 (35); 120 (29)]
Unadjusted	−16 (−58 to 15)	−21 (−67 to 12)	−26 (−73 to 8)
Fully adjusted[a]	−8 (−56 to 26)	−11 (−62 to 23)	−15 (−67 to 21)

CI: confidence interval; ILI: influenza-like illness; VE: vaccine effectiveness; % vac: percentage vaccinated.

[a] Adjusted for age group, sex, comorbidity, province, collection interval, and calendar time.

[b] Adjusted for age group, sex, province, collection interval, and calendar time; not adjusted for comorbidity.

[c] Adjusted for age group, sex, comorbidity, province, and collection interval; not adjusted for calendar time.

[d] Based on same exclusion criteria as primary analysis, with further restriction to participants aged ≥ 2 years in 2014/15 and those with data for 2013/14 and 2014/15 influenza vaccine receipt.

N145S site A cluster-transition substitution distinguishing all clade 3C.2 (and 3C.3) viruses generally, differences between clade 3C.2 viruses and X-223A include N128T (gain of glycosylation) and P198S site B substitutions. The latter two substitutions are the result of having switched the vaccine prototype strain from A/Victoria/361/2011(H3N2) (a clade 3C virus) in 2012/13 to A/Texas/50/2012(H3N2) (a clade 3C.1 virus) since the 2013/14 season. Clade 3C.2 viruses also differ from X-223A at positions 186 (site B), 219 (site D) and 226 (site D) due to mutations in the egg-adapted HGR. Sentinel viruses within the dominant 3C.2a subgroup were further distinguished through an N144S (site A) substitution associated with loss of glycosylation, an additional F159Y (site B) cluster-transition mutation and an adjacent K160T (site B) substitution associated with the gain of a potential glycosylation site, as well as Q311H (site C) and N225D substitutions, the latter within the RBS (but not within defined antigenic sites A–E [4,6,9]). Other substitutions relative to X-223A were scattered through antigenic sites A, C and E.

The provisionally named clade 3C.3*x* sentinel viruses typically differed from X-223A by 12 antigenic site aa substitutions, as also shown in Figure 3. Of note, in addition to the L157S substitution at antigenic site B that distinguishes this emerging subgroup, 18 of 19 clade 3C.3*x* viruses also bore an N122D antigenic site A substitution associated with loss of glycosylation.

Discussion

Interim VE estimates from the Canadian SPSN show little or no protection from the 2014/15 influenza vaccine against the A(H3N2) epidemic strain. The disappointing 2014/15 mid-season VE of −8%, with 95% confidence intervals (CI) overlapping zero and extending to just

Figure 3A

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B				A	D	B			D							E	C					Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
British Columbia	n																																									
A/British Columbia/81/2014	7											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/British Columbia/94/2014	4											T					S	S			Y	T			G		S					S	I	L				H		3C.2a	11 (91.6%)
A/British Columbia/89/2014	1											T					S	S			Y				G		S					S	I	L				H		3C.2a	10 (92.4%)
A/British Columbia/93/2014	2											T					S	S			Y	T			G		S					S	I					H	N	3C.2a	11 (91.6%)
A/British Columbia/97/2014	2											T					S	S			Y	T			G		S		G			S	I					H		3C.2a	11 (91.6%)
A/British Columbia/10/2015	1							R				T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/British Columbia/67/2014	11			K			R				D	A				G		S		S					G		S					S	I	Q						3C.3x	12 (90.8%)
A/British Columbia/83/2014	1			K			R				D	A				G		S		S					G		S					Y	I	Q						3C.3x	12 (90.8%)
A/British Columbia/100/2014	1		N									A				G		S		S					G		S				T	S	I							3C.3x	10 (92.4%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 3B

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B				A	D	B			D						E	C					Clade	# aa
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		(% identity)[a,b]
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
Alberta	n																																								
A/Alberta/57/2014	48											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/Alberta/56/2014	11											T					S	S			Y	T			G		S		G			S	I					H		3C.2a	11 (91.6%)
A/Alberta/77/2014	16							I				T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/113/2014	1							I				T					S	S			Y				G		S					S	I					H		3C.2a	10 (92.4%)
A/Alberta/55/2014	1											T					S	S			Y	T	I		G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/60/2014	1				K							T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/67/2014	1									H		T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/100/2014	1					D						T					S	S			Y	T			G		S					S	I	L				H		3C.2a	12 (90.8%)
A/Alberta/119/2014	1		N									T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/120/2014	3											T	A				S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/127/2014	1											T					S	S			Y	T			G		S					S	I	P				H		3C.2a	11 (91.6%)
A/Alberta/133/2014	3										S	T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/138/2014	1											T					S	S			Y	T			G		S					S	I			F		H		3C.2a	11 (91.6%)
A/Alberta/159/2014	1							I				T					S	S			Y	T			G		S			I		S	I					H		3C.2a	12 (90.8%)
A/Alberta/166/2014	2	T										T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Alberta/124/2014	1											A				G		S							G		S					S	I	Q						3C.3	8 (93.9%)
A/Alberta/88/2014	4			K			R				D	A				G		S		S					G		S					S	I	Q						3C.3x	12 (90.8%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 3C

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B					A	D	B			D								E	C				Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
Ontario	n																																									
A/Ontario/44/2014	14											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/Ontario/42/2014	8											T					S	S			Y	T			G		S					S	I	L				H		3C.2a	11 (91.6%)
A/Ontario/41/2014	1	T								H		T					S	S			Y	T			G		S					S	I					H		3C.2a	12 (90.8%)
A/Ontario/58/2014	1											T					S	S			Y	T			G		S					S	I				I	H		3C.2a	11 (91.6%)
A/Ontario/68/2014	1											T					S				Y	T			G		S					S	I					H		3C.2a	9 (93.1%)
A/Ontario/71/2014	1											T					S	S			Y	T		K	G		S					S	I	L				H		3C.2a	12 (90.8%)
A/Ontario/74/2014	1	T										T					S	S			Y	T			G		S					Y	I					H		3C.2a	11 (91.6%)
A/Ontario/61/2014	1											A				G		S							G		S					S	I							3C.3	7 (94.7%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 3D

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C		E							A	B	A						B				A	D	B			D							E	C				Clade	# aa (% identity)[a,b]
Amino acid number HA1		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312		
A/Victoria/361/2011 (MDCK)[c]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)
2012-13 HGR: A/Victoria/361/2011 (IVR-165)[d]		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)
A/Texas/50/2012 (MDCK)[e]		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)
HGR: A/Texas/50/2012 (X-223A)[f]		**I**	**D**	**E**	**N**	**G**	**K**	**V**	**S**	**Y**	**N**	**N**	**S**	**A**	**I**	**R**	**N**	**N**	**H**	**L**	**F**	**K**	**M**	**N**	**V**	**I**	**P**	**K**	**R**	**V**	**I**	**F**	**N**	**R**	**K**	**S**	**V**	**Q**	**S**	**3C.1**	-
A/Switzerland/9715293/2013 (MDCK)[g]		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)
2015 HGR: A/Switzerland/9715293/2013 (IVR-176)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)
2015 HGR: A/Switzerland/9715293/2013 (X-247)[h]		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)
Quebec	n																																									
A/Quebec/55/2014	15											T					S	S			Y	T			G		S					S	I					H		3C.2a	10 (92.4%)
A/Quebec/36/2014	26											T					S	S			Y	T			G		S					S	I	L				H		3C.2a	11 (91.6%)
A/Quebec/110/2014	1											T					S	S			Y				G		S					S	I	L				H		3C.2a	10 (92.4%)
A/Quebec/35/2014	13	T										T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Quebec/112/2014	1	T										T					S	S			Y	T			G							S	I	Q				H		3C.2a	11 (91.6%)
A/Quebec/59/2014	1								N			T					S	S			Y	T			G		S					S	I					H		3C.2a	11 (91.6%)
A/Quebec/76/2014	2											T					S	S			Y	T			G		S	R				S	I					H		3C.2a	11 (91.6%)
A/Quebec/109/2014	1											T					S	S			Y	T			G	A	S					S	I					H		3C.2a	11 (91.6%)
A/Quebec/117/2014	1					S						T					S	S			Y	T			G		S					S	I	L				H		3C.2a	12 (90.8%)
A/Quebec/44/2014	1			K							D	A				G		S		S					G		S					S	I							3C.3x	10 (92.4%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/– N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

[a] #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

[b] % identity calculated as [1 – (number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

[c] A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

[d] IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

[e] A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

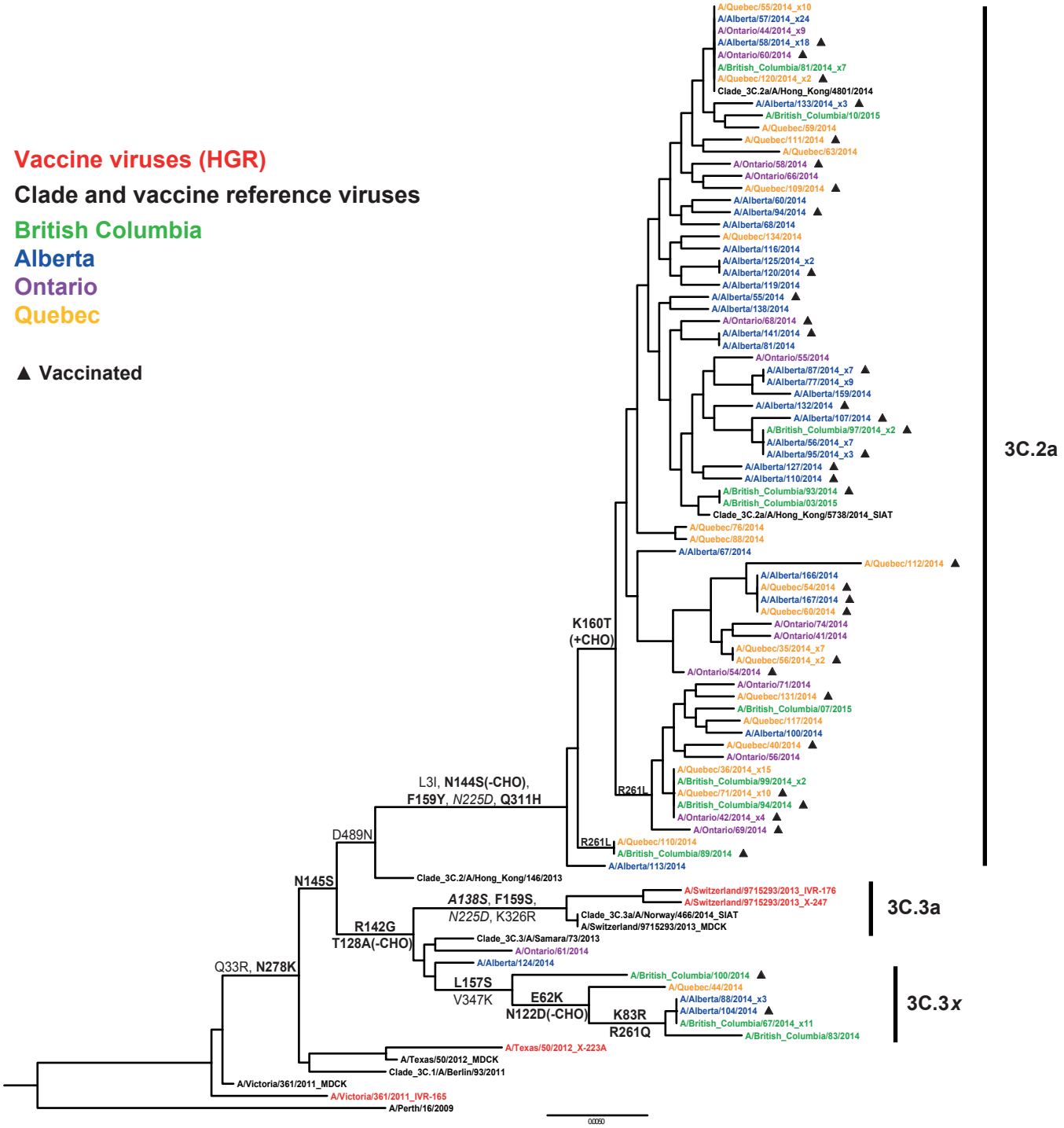
[f] X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

[g] A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

[h] IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Figure 4

Phylogenetic tree of influenza A(H3N2) viruses 2014/15, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 215)



The phylogenetic tree was constructed by alignment of 215 Canadian sentinel translated sequences covering the 514 residues of the extracellular domain against sequences representative of emerging viral clades as described by the European Centre for Disease Prevention and Control (n=6) [12], and recent vaccine A(H3N2) prototype and high-growth reassortant strains (n=8) (Table 1). Substitutions in bold are in antigenic sites and italicised substitutions are in the receptor-binding site.

23%, is in striking contrast to the 2013/14 mid-season VE analysis. During that season's interim analysis with comparable sample size, we measured substantial and statistically significant VE of 74% (95% CI: 58–83%) against the dominant but antigenically well-conserved A(H1N1)pdm09 epidemic strain [2]. The VE point estimate reported here for the 2014/15 seasonal vaccine is the lowest component-specific estimate reported by the Canadian SPSN against any seasonal strain of the past 10 years, including other recent influenza A(H3N2) vaccine-mismatched seasons in 2012/13 (VE=45% mid-season [3], 41% end-of-season [4]) or 2010/11 (VE=39%) [7].

Consistent with the low VE we report for 2014/15, virtually all (99%) of the sentinel influenza A(H3N2) viruses contributing to VE analysis showed genetic and/or antigenic evidence of vaccine mismatch. Although only seven SPSN viruses contributing to VE analysis grew to sufficient titre for antigenic characterisation by HI assay, the high proportion of vaccine-mismatched viruses reported here is similar to reports from national laboratory-based surveillance summaries for Canada [1]. Of the 62 A(H3N2) viruses HI-characterised in the presence of oseltamivir carboxylate and reported to date nationally by Canada's NML (including non-SPSN viruses), 61 (98%) have shown reduced titres to the A/Texas/50/2012(H3N2) vaccine strain [1]. The majority of these viruses have clustered with clade 3C.2a, and the remainder with what we have provisionally labelled here as clade 3C.3*x*. Nationally, based on genetic characterisation of viruses unable to grow to sufficient titre for HI assay, 393 of 395 (99%) viruses to date have been found to belong to one of these two genetic groups (foremost clade 3C.2a) and are considered antigenically distinct from the vaccine strain [1]. The approach used this season to impute vaccine mismatch based on phylogenetic findings follows that established by the United States Centers for Disease Control and Prevention (US CDC) where only 64% of circulating A(H3N2) viruses so far this season have been considered antigenically distinct from the vaccine strain [20]. This substantial difference between Canada and the US in the proportion of A(H3N2) viruses that are considered vaccine-mismatched may explain the higher (albeit still suboptimal) VE estimate reported in mid-season analysis by the US CDC (22%) [22]; however, other methodological, demographic or immunological differences should also be considered.

As in previous seasons, non-elderly adults contributed most (60%) to our VE analyses, although elderly participants were slightly more represented (16%) this season compared to previous years (10% or less) [2-4,6,7]. The adult predominance in our sample may be relevant to consider when comparing our 2014/15 mid-season VE estimates to those from the US CDC, where there was a greater paediatric contribution (43% of the overall sample) [22]. Children are less likely to have had prior influenza vaccine or virus exposure history and are more likely to have received LAIV. LAIV has been associated with better efficacy than inactivated vaccine in the very young [23-27], although the opposite was observed against influenza A(H1N1)pdm09 in the US during the 2013/14 season [28] and relative effectiveness in the context of substantial vaccine mismatch or with history of prior repeat immunisation is uncertain. Our VE estimate against influenza A(H3N2) in non-elderly adults of 2% is comparable to (within 10% of) the US mid-season VE estimate for adults 18–49 years-old (12%), although neither country's estimate in adults is statistically significant and confidence intervals overlap. More nuanced evaluation of age and other influences on VE will be important to explore with larger sample size in end-of-season analyses.

At the genetic level, vaccine-virus divergence in 2014/15 was defined among Canadian SPSN viruses by a substantial number of aa differences (10–11) in the dominant (>90%) clade 3C.2a viruses relative to the vaccine component, including substitutions at pivotal antigenic, cluster-transition and receptor-binding sites and/or in association with potential gain or loss of glycosylation, each of which may influence antibody recognition. Substitutions evident in the vaccine strain, notably associated with egg-adaptation and HGR generation, may also have compounded the effects of antigenic drift in circulating viruses [4]. The emerging but as yet minor subgroup of viruses bearing the L157S +/- N122D mutation (here labelled clade 3C.3*x*) also warrants close monitoring. Although position 157 has not been identified historically as a cluster-transition residue, it is within the same pocket as other key residues (i.e. 155, 156, 158, 159) and may be of emerging significance [16]. The loss of glycosylation associated with the N122D substitution may also be influential [17]. Clade 3C.3 viruses with this particular combination of aa substitutions have not previously been identified by the Canadian SPSN, but were detected in Spain during the 2013/14 season, cited in association with the low VE (13%) against A(H3N2) viruses in mid-season analysis from that country [29]. Compared with Spanish sequences from 2013/14, clade 3C.3*x* viruses characterised by the Canadian SPSN in 2014/15 have acquired an additional three aa mutations in antigenic site E, an antigenic site distant from the RBS and not typically considered immuno-dominant but possibly relevant to overall virus fitness.

As published previously by the Canadian SPSN [4,6] and US CDC and other investigators [30-33], we observed variability in VE by prior vaccination history. In particular, VE against influenza A(H3N2) among those who received the 2014/15 influenza vaccine without prior vaccination in 2013/14 was higher (43%) than among participants who were vaccinated with the same A(H3N2) vaccine component in both 2013/14 and 2014/15 (–15%). Although none are statistically significant, these substantial differences in VE based on prior immunisation are consistent with the antigenic distance hypothesis articulated by Smith et al. [34]. That hypothesis suggests that negative interference from

prior immunisation may be more pronounced when the antigenic distance is small between successive vaccine components but large between vaccine and circulating strains. Such is the scenario for the current 2014/15 season for which the identical A(H3N2) vaccine component was used as during the 2013/14 season, poorly matched to the 2014/15 epidemic strain. However, limited sample size precludes definitive conclusions, particularly since a large proportion (nearly 90%) of vaccinated SPSN participants are repeat vaccine recipients [2-4,6,7]. There may also be other unrecognised differences across subgroups of participants with differing immunisation histories. Further evaluation is required across additional study settings and seasons and with greater sample size to confirm these findings, assess possible underlying immunological interactions, and inform implications for vaccine reformulation and policy recommendation.

There are limitations to this study, notably related to sample size, in particular in subgroup analyses. Mid-season analysis was undertaken with the recognition that sample size was sufficient to provide 80% statistical power to detect a VE of at least 40%, given vaccine coverage typically spanning 30 to 40% in our setting. The absence of statistical significance with much lower VE is not unexpected given that in order to measure a VE of 10% in either direction from zero with the same statistical power would require more than 10,000 participants and more than 1 million participants would be required to show a significant VE of 1%. Our findings are thus consistent with a VE close to zero, where a precise estimate may never be resolved statistically. Higher VE may be observed in final end-of-season analyses, particularly if other influenza types or subtypes for which the trivalent vaccine is a better match circulate through the remainder of the 2014/15 season. Vaccine status in this study was based on self-reporting which may introduce some misclassification bias. However, this information was collected at the time of specimen collection, before the test result was known, minimising differential misclassification. As in prior seasons' analyses by the SPSN, the predominance of adults and repeat influenza vaccine recipients among our study participants is relevant to consider in the generalisation of our findings to other settings where the population profile may differ. Although we uniquely characterised more than half of our sentinel A(H3N2) viruses to the level of clade specification, and our virological profile reflected that of national surveillance summaries for Canada [1], we cannot rule out systematic differences in viruses available for genetic or antigenic characterisation, a problem for all laboratory-based surveillance. The validity of VE estimates derived by the test-negative approach has been previously demonstrated [35,36] but the design remains observational and bias and confounding cannot be ruled out.

In summary, interim VE findings from the Canadian SPSN indicate that the 2014/15 influenza vaccine has provided little or no protection against medically attended illness due to predominant and substantially mismatched A(H3N2) viruses this season. Given limited vaccine protection, other adjunct protective measures should be considered to minimise associated morbidity and mortality, particularly among high-risk individuals. The virological and/or host factors influencing reduced vaccine protection against influenza A(H3N2) during the 2014/15 season warrant further in-depth investigation.

GenBank Accession Numbers

Viruses from original specimens with complete or partial sequences of the haemagglutinin (HA) gene (HA1 and HA2) provided by provincial laboratories and contributing to the 2014/15 interim influenza vaccine effectiveness analysis by the Canadian Sentinel Physician Surveillance Network were deposited in GenBank with accession numbers KP701523 –KP701743.

Acknowledgements

The authors gratefully acknowledge the contribution of sentinel sites whose regular submission of specimens and data provide the basis of our analyses. We wish to acknowledge the coordination and technical support provided by epidemiologic and laboratory staff in all participating provinces. We wish to thank the following for network coordination and data entry activities in each province including: Elaine Douglas and Kinza Rizvi for TARRANT in Alberta; Romy Olsha for Public Health Ontario; and Sophie Auger for the Institut national de santé publique du Québec. We thank those who provided laboratory support in each of the British Columbia Public Health Microbiology and Reference, the Alberta Provincial, and Public Health Ontario Laboratories; and the Laboratoire de santé publique du Québec. We further acknowledge the virus detection and gene sequencing support provided by Kanti Pabbaraju, Sallene Wong and Danielle Zarra of the Alberta Provincial Laboratory; Aimin Li and Stephen Perusini of Public Health Ontario; and Joel Ménard and Lyne Désautels of the Québec Provincial Laboratory. Finally, we acknowledge the authors, originating and submitting laboratories of the reference virus sequences from GISAID's EpiFlu Database (www.gisaid.org) (see Table 1).

Funding was provided by the Canadian Institutes of Health Research (CIHR grant # TPA-90193), the British Columbia Centre for Disease Control, Alberta Health and Wellness, Public Health Ontario, Ministère de la santé et des services sociaux du Québec, Institut national de santé publique du Québec, and the Public Health Agency of Canada.

Conflict of interest

Within 36 months of manuscript submission, GDS received research grants from GlaxoSmithKline (GSK) for unrelated vaccine studies. JG has received a research grant from Pfizer. MK has received research grants from Roche, Merck, Gen-Probe and Siemens. 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). Investigators (laboratory): JBG (Ontario); HC and CM (Québec); MP and MK (British Columbia); SD and KF (Alberta); 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.

References

1. Public Health Agency of Canada (PHAC). FluWatch. Weekly reports 2014-2015 season. January 11 to January 17, 2015 (week 2). Ottawa: PHAC. [Accessed: 25 Jan 2015]. Available from: http://www.phac-aspc.gc.ca/fluwatch/14-15/w02_15/index-eng.php
2. Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson J, Winter A, et al. Interim estimates of 2013/14 vaccine effectiveness against influenza A(H1N1)pdm09 from Canada s sentinel surveillance network, January 2014. Euro Surveill. 2014;19(5):20690. http://dx.doi.org/10.2807/1560-7917.ES2014.19.5.20690 PMID:24524234
3. Skowronski DM, Janjua NZ, De Serres G, Dickinson JA, Winter AL, Mahmud SM, et al. Interim estimates of influenza vaccine effectiveness in 2012/13 from Canada's sentinel surveillance network, January 2013. Euro Surveill. 2013;18(5):20394. PMID:23399422
4. Skowronski DM, Janjua NZ, De Serres G, Sabaiduc S, Eshaghi A, Dickinson JA, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS ONE. 2014;9(3):e92153. http://dx.doi.org/10.1371/journal.pone.0092153 PMID:24667168
5. BC Centre for Disease Control (BCCDC). Influenza surveillance reports: BC influenza surveillance bulletin: Influenza season 2014-15. Report no. 14. Week 2. January 11 to January 17, 2015. Vancouver, BCCDC. [Accessed: 25 Jan 2015]. Available from: http://www.bccdc.ca/NR/rdonlyres/27E73D7B-55D4-47C0-B2EB-68B94D2618DD/0/InfluBulletin_Number14_Week2_201415.pdf
6. Skowronski DM, Janjua NZ, Sabaiduc S, De Serres G, Winter AL, Gubbay JB, et al. Influenza A/subtype and B/lineage effectiveness estimates for the 2011-2012 trivalent vaccine: cross-season and cross-lineage protection with unchanged vaccine. J Infect Dis. 2014;210(1):126-37. http://dx.doi.org/10.1093/infdis/jiu048 PMID:24446529
7. Skowronski DM, Janjua NZ, De Serres G, Winter AL, Dickinson JA, Gardy JL, et al. A sentinel platform to evaluate influenza vaccine effectiveness and new variant circulation, Canada 2010-2011 season. Clin Infect Dis. 2012;55(3):332-42. http://dx.doi.org/10.1093/cid/cis431 PMID:22539661
8. World Health Organization (WHO). WHO recommendations on the composition of influenza virus vaccines. Geneva: WHO. [Accessed: 19 Jan 2015]. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/en/index.html
9. Bush RM, Bender CA, Subbarao K, Cox NJ, Fitch WM. Predicting the evolution of human influenza A. Science. 1999;286(5446):1921-5. http://dx.doi.org/10.1126/science.286.5446.1921 PMID:10583948
10. Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for large alignments. PLoS ONE. 2010;5(3):e9490. http://dx.doi.org/10.1371/journal.pone.0009490 PMID:20224823
11. Rambaut A. FigTree v1.4.0, a graphical viewer of phylogenetic trees. Edinburgh: University of Edinburgh. Accessed 19 Jan 2015. Available from: http://tree.bio.ed.ac.uk/software/figtree
12. European Centre for Disease Prevention and Control (ECDC). Influenza virus characterisation. Summary Europe, November 2014. Surveillance report. Stockholm: ECDC. 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/ERLI-Net%20report%20November%202014.pdf
13. Cox NJ, Bender CA. The molecular epidemiology of influenza viruses. Semin Virol. 1995;6(6):359-70. http://dx.doi.org/10.1016/S1044-5773(05)80013-7
14. Ndifon W, Wingreen NS, Levin SA. Differential neutralization efficiency of hemagglutinin epitopes, antibody interference, and the design of influenza vaccines. Proc Natl Acad Sci USA. 2009;106(21):8701-6. http://dx.doi.org/10.1073/pnas.0903427106 PMID:19439657
15. Popova L, Smith K, West AH, Wilson PC, James JA, Thompson LF, et al. Immunodominance of antigenic site B over site A of hemagglutinin of recent H3N2 influenza viruses. PLoS ONE. 2012;7(7):e41895. http://dx.doi.org/10.1371/journal.pone.0041895 PMID:22848649
16. Koel BF, Burke DF, Bestebroer TM, van der Vliet S, Zondag GC, Vervaet G, et al. Substitutions near the receptor binding site determine major antigenic change during influenza virus evolution. Science. 2013;342(6161):976-9. http://dx.doi.org/10.1126/science.1244730 PMID:24264991
17. Tate MD, Job ER, Deng Y-M, Gunalan V, Maurer-Stroh S, Reading PC. Playing hide and seek: how glycosylation of the influenza virus hemagglutinin can modulate the immune response to infection. Viruses. 2014;6(3):1294-316. http://dx.doi.org/10.3390/v6031294 PMID:24638204
18. Katz JM, Hancock K, Xu X. Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. Expert Rev Anti Infect Ther. 2011;9(6):669-83. http://dx.doi.org/10.1586/eri.11.51 PMID:21692672
19. Lin YP, Gregory V, Collins P, Kloess J, Wharton S, Cattle N, et al. Neuraminidase receptor binding variants of human influenza A(H3N2) viruses resulting from substitution of aspartic acid 151 in the catalytic site: a role in virus attachment? J Virol. 2010;84(13):6769-81. http://dx.doi.org/10.1128/JVI.00458-10 PMID:20410266
20. Centers for Disease Control and Prevention (CDC). FluView. 2014-2015 influenza season week 2 ending January 17, 2015. U.S. Virologic Surveillance, Influenza Virus Characterization. Atlanta: CDC. [Accessed: 25 Jan 2015]. Available from: http://www.cdc.gov/flu/weekly
21. Public Health Agency of Canada (PHAC). Vaccine coverage amongst adult Canadians: Results from the 2012 adult National Immunization Coverage (aNIC) survey. Ottawa: PHAC. [Accessed: 19 Jan 2015]. Available from: http://www.phac-aspc.gc.ca/im/nics-enva/vcac-cvac-eng.php
22. Flannery B, Clippard J, Zimmerman RK, Nowalk MP, Jackson ML, Jackson LA, et al. Early estimates of seasonal influenza vaccine effectiveness - United States, January 2015. MMWR Morb Mortal Wkly Rep. 2015;64(1):10-5. PMID:25590680
23. Ashkenazi S, Vertruyen A, Arístegui J, Esposito S, McKeith DD, Klemola T, et al.. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. Pediatr Infect Dis J. 2006;25(10):870-9. http://dx.doi.org/10.1097/01.inf.0000237829.66310.85 PMID:17006279
24. Fleming DM, Crovari P, Wahn U, Klemola T, Schlesinger Y, Langussis A, et al. Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. Pediatr Infect Dis J. 2006;25(10):860-9. http://dx.doi.org/10.1097/01.inf.0000237797.14283.cf PMID:17006278
25. Belshe RB, Edwards KM, Vesikari T, Black SV, Walker RE, Hultquist M, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med. 2007;356(7):685-96. http://dx.doi.org/10.1056/NEJMoa065368 PMID:17301299
26. Advisory Committee on Immunization Practices (ACIP). Grading of recommendations, assessment, development, and evaluation (GRADE) for use of live attenuated influenza vaccine (LAIV) and inactivated influenza vaccine (IIV) in children. Atlanta: Centers for Disease Control and Prevention. [Accessed: 25 Jan 2015]. Available from: http://www.cdc.gov/vaccines/acip/recs/GRADE/LAIV-child.html
27. Public Health Agency of Canada. Revised wording to the National Advisory Committee on Immunization (NACI) recommendation for live attenuated influenza vaccine (LAIV) in healthy children and adolescents 2-17 years of age. Canada Commun Dis Rep. 2013;39(ACS-4). [Accessed: 25 Jan 2015]. Available from: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/13vol39/acs-dcc-4/rev-eng.php
28. Centers for Disease Control and Prevention (CDC). CDC statement on LAIV effectiveness and vaccination of children. Atlanta: CDC. [Accessed: 25 Jan 2015]. Available from: http://www.cdc.gov/flu/news/nasal-spray-effectiveness.htm
29. Castilla J, Martinez-Baz I, Nacascues A, Fernandez-Alonso M, Reina G, Guevara M, et al. Vaccine effectiveness in preventing laboratory-confirmed influenza in Navarre, Spain: 2013/14 mid-season analysis. Euro Surveill. 2014;19(6):pii=20700.
30. Ohmit SE, Petrie JG, Malosh RE, Cowling BJ, Thompson MG, Shay DK, et al. Influenza vaccine effectiveness in the community and the household. Clin Infect Dis. 2013;56(10):1363-9. http://dx.doi.org/10.1093/cid/cit060 PMID:23413420
31. Ohmit SE, Petrie JG, Malosh RE, Fry AM, Thompson MG, Monto AS. Influenza vaccine effectiveness in households with children during the 2012-13 season: assessments of prior vaccination and serologic susceptibility. J Infect Dis. 2014;jiu650. PMID:25416812

32. Ohmit SE, Thompson MG, Petrie JG, Thaker SN, Jackson ML, Belongia EA, et al. Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. Clin Infect Dis. 2014;58(3):319-27. http://dx.doi.org/10.1093/cid/cit736 PMID:24235265

33. McLean HQ, Thompson MG, Sundaram ME, Meece JK, McClure DL, Friedrich TC, et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis. 2014;59(10):1375-85. http://dx.doi.org/10.1093/cid/ciu680 PMID:25270645

34. Smith DJ, Forrest S, Ackley DH, Perelson AS. Variable efficacy of repeated annual influenza vaccination. Proc Natl Acad Sci USA. 1999;96(24):14001-6. http://dx.doi.org/10.1073/pnas.96.24.14001 PMID:10570188

35. Orenstein EW, De Serres G, Haber MJ, Shay DK, Bridges CB, Gargiullo P, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. Int J Epidemiol. 2007;36(3):623-31. http://dx.doi.org/10.1093/ije/dym021 PMID:17403908

36. De Serres G, Skowronski DM, Wu XW, Ambrose CS. The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled trials. Euro Surveill. 2013;18(37):20585. PMID:24079398