Appendix A

Sentinel test-negative case-control study – Additional details

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Appendix A1. Additional methodological detail related to Canada's sentinel vaccine effectiveness monitoring system for 2008-09

The sentinel vaccine effectiveness study is based on a surveillance approach using a network of community-based practitioners provided with kits in order to submit respiratory specimens for influenza testing along with standardized epidemiologic information collected from eligible consenting patients. Both cases and controls are ambulatory patients consulting for influenza-like illness (ILI). Eligible participants in participating provinces (British Columbia (BC), Alberta, Ontario and Quebec) present within 7 days of ILI onset. ILI is defined as the acute onset of fever and cough and one or more of sore throat, myalgia, arthralgia, headache or prostration. Because trivalent split inactivated influenza vaccine (TIV) is only recommended for people ≥6 months of age and because this eligibility varied through the analysis period, infants <1 year of age were excluded from analyses. The National Advisory Committee on Immunization recommends two doses of TIV for previously unimmunized children < 9 years of age but one or two dose receipt was not verified. Children less than 3 years of age are recommended to receive 0.25mL per dose while all others receive 0.5mL per dose. TIV is administered intra-muscularly during the regular fall immunization campaign and immunization status is elicited from participants or parents at the time of medical visit for ILI, prior to specimen submission and testing by the designated provincial laboratory. Information on underlying health status is also collected prior to test result: in BC and Alberta, the questionnaire elicited whether the patient had a "vaccine-eliqible" condition inclusive of heart, lung, renal, metabolic, blood or immune conditions; in Ontario and Quebec this guestion referred to "chronic" conditions inclusive of the same list. Submission of duplicate specimens is discouraged but not further assessed. Cases were participants who tested positive for influenza; controls were participants who tested negative for both influenza A and B. Per usual protocol, specimens positive for any influenza were excluded as controls (i.e. the specimens positive for seasonal influenza during the pH1N1 analysis period were excluded as controls as were those positive for pH1N1 when examining vaccine effectiveness against seasonal influenza during the same period). Because pH1N1 was not considered until after April 17, specimens with ILI onset in early April were not included in sentinel study analyses.

Testing for seasonal influenza in BC, Ontario and Quebec consisted of screening using a real-time reverse transcription PCR (RT-PCR) assay targeting the matrix (M) gene of influenza A virus and the hemagglutinin (HA) [1-3] or NS1 gene of influenza B virus (CDC real time RT-PCR protocol). Sub-typing for seasonal human influenza A (A/H1 or A/H3) was performed by real-time RT-PCR targeting the HA gene. In Alberta, all specimens were tested by Luminex RVP Assay [4] which detects seasonal influenza, pH1N1 [5] and influenza B viruses and directly subtypes seasonal influenza as A/H1 or A/H3.

Testing was appropriately modified in all provinces after April 17 to detect pH1N1. Owing to increased test volume, specimens were prospectively tested for influenza A alone by the screening real-time RT-PCR assay in BC and Ontario and were retrospectively tested for influenza B by Luminex RVP in BC and real-time RT-PCR in Ontario. In Quebec and Alberta, all specimens continued to be tested for both influenza A and B by screening RT-PCR or Luminex RVP respectively as per above. Influenza A positive specimens were subtyped by RT-PCR. The A/H1 and A/H3 subtypes were detected as described above. pH1N1 was detected using a conventional (end point) RT-PCR assay targeting the H1 gene using the forward primer CAGACACTGTAGACACAGTAC, and reverse primer CTAGTAGATGGATGGTGAATGC (NML Endpoint RT-PCR protocol distributed April 24, 2009). The 517 bp product was visualized by agarose gel electrophoresis or by real-time RT-PCR using hydrolysis probes on a Taqman platform to this region. Sequence analysis was also performed on a subset to confirm pH1N1 detection.

References:

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- 3. Fouchier, R.A, Bestebroer, TM, Herfst S, Van Der Kemp L, Rimmelzwaan GF et al. (2000) Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. J. Clin. Microbiol. 38:4096–4101.
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- 5. Mahony JB, Hatchette T, Ojkic D, Drews SJ, Gubbay J, et al. (2009) Multiplex PCR tests sentinel the appearance of pandemic influenza viruses including H1N1 swine influenza. J Clin Virol 45:200-202.

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Appendix A2. Sentinel test-negative case-control study participant inclusion and exclusion – seasonal influenza and pH1N1 analysis periods

	Novembe	sonal er 1, 2008 – 31, 2009	pH1N1 April 17, 2009 – July 22, 2009			
Exclusion Criteria	N excluded	N included	N excluded	N included		
Submitted from consenting patients within study period		1775		936		
Patient did not meet influenza-like illness (ILI) case definition or unknown	71	1704	62	874		
Vaccine status unknown	32	1672	57	817		
Specimen collected > 7 days after ILI onset or interval unknown	77	1595	52	765		
Vaccine-eligible (chronic) conditions unknown	36	1559	39	726		
Age unknown	3	1556	1	725		
Age < 1 year	12	1544	8	717		
Influenza RT-PCR results unavailable or inconclusive on typing	15	1529	6	711		
Specimens positive for seasonal influenza or with subtype unknown or pending (pH1N1 analysis period)	N/A	1529	31	680		
Contributed to analysis		1529		680		

pH1N1= pandemic influenza A (H1N1); ILI= influenza-like illness defined as acute onset of fever and cough and one or more of sore throat, arthralgia, myalgia, headache or prostration; RT-PCR=reverse-transcription polymerase chain reaction; N/A=Not applicable.

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Appendix A3a: Laboratory profile of included sentinel specimens with dates of ILI onset November 1, 2008 - March 31, 2009

Specimens tested	Alberta N=521	BC N=380	Ontario N=292	Quebec N=336	Total N=1529
•	n (%)	n (%)	n (%)	n (%)	n (%)
Influenza negative	375 (72%)	189 (50%)	166 (57%)	127 (38%)	857 (56%)
Influenza positive	146 (28%)	191 (50%)	126 (43%)	209 (62%)	672 (44%)
A positive	94 (64%)	122 (64%)	71 (56%)	136 (65%)	423 (63%)
B positive	52 (36%)	69 (36%) [°]	55 (44%)	73 (35%)	249 (37%)
Influenza A positive	,	,	, ,	,	,
A/H1	43 (46%)	38 (31%)	36 (51%)	43 (32%)	160 (38%)
A/H3	50 (53%)	84 (69%)	29 (41%)	90 (66%)	253 (60%)
Subtype unknown	1 (1%)	0 (0%)	6 (9%)	3 (2%)	10 (2%)
pH1N1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Appendix A3b: Laboratory profile of included sentinel specimens with dates of ILI onset April 17, 2008-July 22, 2009

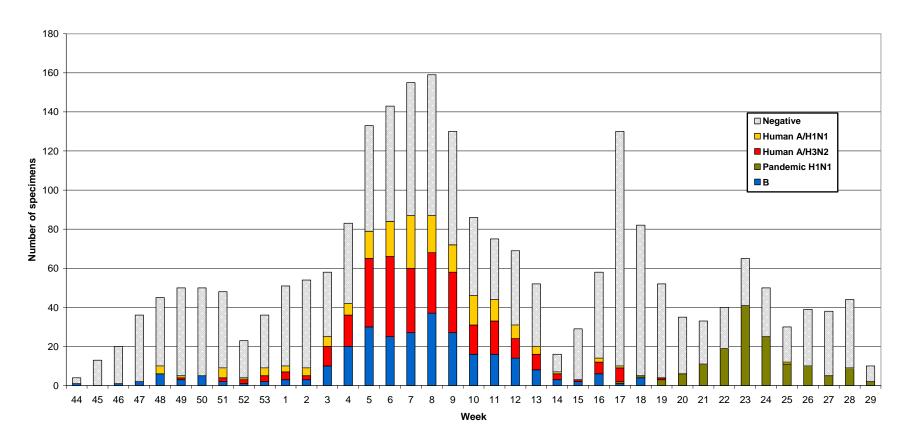
Specimens tested	Alberta N=202	BC N=197	Ontario N=140	Quebec N=172	Total N=711
·	n (%)	n (%)	n (%)	n (%)	n (%)
Influenza negative	167 (83%)	166 (84%)	102 (73%)	101 (59%)	536 (75%)
Influenza positive	35 (17%)	31 (16%)	38 (27%)	71 (41%)	175 (25%)
A positive	31 (89%)	28 (90%)	38 (100%)	67 (94%)	164 (94%)
B positive	4 (11%)	3 (10%)	0 (0%)	4 (6%)	11 (6%)
Influenza A positive	,	, ,	, ,	,	, ,
A/H1	1 (3%)	1 (4%)	1 (3%)	1 (2%)	4 (2%)
A/H3	2 (7%)	8 (29%)	2 (5%)	2 (3%)	14 (9%)
Subtype unknown	1 (3%)	0 (0%)	1 (3%)	0 (0%)	2 (1%)
pH1N1	27 (87%)	19 (68%)	34 (90%)	64 (96%)	144 (88%)

pH1N1= pandemic influenza A (H1N1)

ILI=influenza-like illness

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Appendix A4. Influenza detection by subtype and week (based on date of ILI onset), 2008-09: British Columbia, Alberta, Ontario and Quebec, combined among sentinel study participants meeting inclusion/exclusion criteria (n=2324)*



*Twelve specimens of unknown subtype not represented in this figure. ILI=influenza-like illness

NOTE: Week 44 = 26 October, 2008 – 1 November, 2008; Week 29 = 19 July, 2009 – 25 July, 2009

NOTE: 1 November, 2008 in week 44; 31 March, 2009 in week 13; 17 April, 2009 in week 15; 17 May, 2009 in week 20; 22 July, 2009 in week 29.

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Table A5a: Sentinel test-negative case-control study: participant profile, seasonal influenza

Seasonal influenza test-negative cases (N=672)

Seasonal influenza test-negative controls (N=857)

		Sea	asonal inf			e cases (N	N=672)			Seasonal influenza test-negative controls (N=857)							
-					N (%)					n/N (%)							
	1-8	9-19	20-49	50-64	≥ 65	< 50	≥ 50	Overall	1-8	9-19	20-49	50-64	≥ 65	< 50	≥ 50	Overall	
-	years	years	years	years	years	years	years		years	years	years	years	years	years	years		
N	100	176	311	69	16	587	85	672	93	123	384	172	85	600	257	857	1529
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
2008-09 TIV Receipt	14 (14)	14 (8)	39 (12)	17 (25)	13 (81)	67 (11)	30 (35)	97 (14)	20 (22)	23 (19)	86 (22)	84 (49)	74 (87)	129 (22)	158 (61)	287 (33)	384 (25)
Sex Female	48 (48)	93 (53)	182 (58)	38 (55)	10 (63)	323 (55)	48 (56)	371 (55)	45 (48)	71 (58)	224 (58)	103 (60)	60 (71)	340 (57)	163 (63)	503 (59)	874 (57)
Interval between ILI on	set and sp	ecimen co	llection														
≤ 4 days	80 (80)	153 (87)	266 (86)	58 (84)	12 (75)	499 (85)	70 (82)	569 (85)	77 (83)	99 (80)	297 (77)	119 (69)	59 (69)	473 (79)	178 (69)	651 (76)	1220 (80)
5-7 days	20 (20)	23 (13)	45 (14)	11 (16)	4 (25)	88 (11)	15 (18)	103 (15)	16 (17)	24 (19)	87 (23)	53 (31)	26 (31)	127 (21)	79 (31)	206 (24)	309 (20)
Median (range)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-6)	3 (1-6)	3 (0-7)	3 (0-6)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)
With Chronic conditions																	
N	6 (6)	14 (8)	41 (13)	17 (25)	11 (69)	61 (10)	28 (33)	89 (13)	13 (14)	12 (10)	53 (14)	64 (37)	57 (67)	78 (13)	121 (47)	199 (23)	288 (19)
Vaccinated 2008-09	2 (33)	5 (36)	16 (39)	6 (35)	8 (73)	23 (38)	14 (50)	37 (42)	5 (38)	7 (58)	27 (51)	44 (69)	50 (88)	39 (50)	94 (78)	133 (67)	170 (59)
Without chronic condition	ıs																
N	94 (94)	162 (92)	270 (87)	52 (75)	5 (31)	526 (90)	57 (67)	583 (87)	80 (86)	111 (90)	331 (86)	108 (63)	28 (33)	522 (87)	136 (53)	658 (77)	1241 (81)
Vaccinated 2008-09	12 (13)	9 (6)	23 (8)	11 (21)	5 (100)	44 (8)	16 (28)	60 (10)	15 (19)	16 (14)	59 (18)	40 (37)	24 (86)	90 (17)	64 (47)	154 (23)	214 (17)
Province																	
Alberta	19 (19)	36 (20)	77 (25)	13 (19)	1 (6)	132 (22)	14 (16)	146 (22)	35 (38)	61 (50)	176 (46)	74 (43)	29 (34)	272 (45)	103 (40)	375 (44)	521 (34)
BC	23 (23)	64 (36)	91 (29)	8 (12)	5 (31)	178 (30)	13 (15)	191 (28)	11 (12)	27 (22)	100 (26)	29 (17)	22 (26)	138 (23)	51 (20)	189 (22)	380 (25)
Ontario	18 (18)	27 (15)	60 (19)	15 (22)	6 (38)	105 (18)	21 (25)	126 (19)	21 (23)	25 (20)	58 (15)	42 (24)	20 (23)	104 (17)	62 (24)	166 (19)	292 (19)
Quebec	40 (40)	49 (28)	83 (27)	33 (48)	4 (25)	172 (29)	37 (43)	209 (31)	26 (28)	10 (8)	50 (13)	27 (16)	14 (16)	86 (14)	41 (16)	127 (15)	336 (22)
Chronic conditions by	province																
Alberta	0	5 (14)	12 (16)	2 (15)	0	17 (13)	2 (14)	19 (13)	6 (17)	10 (16)	31 (18)	29 (39)	23 (79)	47 (17)	52 (50)	99 (26)	118 (23)
BC	4 (17)	4 (6)	13 (14)	1 (12)	5 (100)	21 (12)	6 (46)	27 (14)	2 (18)	0	13 (13)	11 (38)	14 (64)	15 (11)	25 (49)	40 (21)	67 (18)
Ontario	1 (6)	2 (7)	11 (18)	3 (20)	3 (50)	14 (13)	6 (29)	20 (16)	1 (5)	2 (8)	3 (5)	15 (36)	12 (60)	6 (6)	27 (44)	33 (20)	53 (18)
Quebec	1 (3)	3 (6)	5 (6)	11 (33)	3 (75)	9 (5)	14 (38)	23 (11)	4 (15)	0	6 (12)	9 (33)	8 (57)	10 (12)	17 (41)	27 (21)	50 (15)
2008-09 TIV immunized	l by provir	ice															
Alberta	2 (10)	4 (11)	7 (9)	1 (8)	1 (100)	13 (10)	2 (14)	15 (10)	8 (23)	11 (18)	37 (21)	34 (46)	23 (79)	56 (21)	57 (55)	113 (30)	128 (25)
BC	1 (4)	4 (6)	11 (12)	0	4 (80)	16 (9)	4 (31)	20 (10)	0 (0)	4 (15)	18 (18)	12 (41)	21 (95)	22 (16)	33 (65)	55 (29)	75 (20)
Ontario	3 (17)	5 (18)	13 (22)	6 (40)	5 (83)	21 (20)	11 (52)	32 (25)	6 (29)	7 (28)	21 (36)	24 (57)	17 (85)	34 (33)	41 (66)	75 (45)	107 (37)
Quebec	8 (20)	1 (2)	8 (10)	10 (30)	3 (75)	17 (10)	13 (35)	30 (14)	6 (23)	1 (10)	10 (20)	14 (52)	13 (93)	17 (20)	27 (66)	44 (35)	74 (22)
2007-08 TIV immunized	l by provir	ıce*															
Alberta	3 (16)	5 (14)	16 (22)	3 (23)	1 (100)	24 (19)	4 (29)	28 (20)	6 (17)	14 (23)	40 (23)	36 (49)	25 (86)	60 (22)	61 (60)	121 (33)	149 (29)
Ontario	5 (29)	8 (32)	21 (36)	7 (50)	6 (0)	34 (34)	13 (65)	47 (39)	7 (35)	8 (33)	24 (44)	25 (61)	16 (84)	39 (40)	41 (68)	80 (51)	127 (46)
Quebec	8 (21)	0	13 (16)	9 (28)	3 (75)	21 (13)	12 (33)	33 (16)	7 (29)	0	7 (15)	14 (52)	12 (92)	14 (17)	26 (65)	40 (33)	73 (22)

ILI=influenza-like illness; TIV=trivalent inactivated influenza vaccine; BC=British Columbia; *BC did not collect information on 2007-08 vaccine status. 2007-08 percentages derived based on participants for whom vaccine status known. Denominators do not include those with missing information (never exceeding 4 missing counts for any age category).

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Table A5b: Sentinel test-negative case-control study: participant profile, pH1N1 influenza pH1N1 test-positive cases (N=144) pH1N1

pH1N1 test-negative controls (N=536)*

			n/N (%)								ALL						
	1-8 9-19 20-49 50-64 \geq 65 < 50 \geq 50 Overall									1-8 9-19 20-49 50-64 \geq 65 < 50 \geq 50 Overall							
	years	years	years	years	years	years	years		years	years	years	years	years	years	years		
N	13	59	59	10	3	131	13	144	41	66	272	109	48	379	157	536	680
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
2008-09 TIV* Receipt	5 (38)	13 (22)	22 (37)	2 (20)	3 (100)	40 (30)	5 (38)	45 (31)	4 (10)	6 (9)	63 (23)	60 (55)	33 (69)	73 (19)	93 (59)	166 (31)	211 (31)
Sex Female	7 (54)	28 (47)	37 (63)	5 (50)	2 (67)	72 (55)	7 (54)	79 (55)	14 (34)	34 (51)	184 (68)	74 (68)	26 (55)	232 (61)	100 (64)	332 (62)	411 (60)
Interval between ILI on	set and sp	ecimen co	llection														
≤ 4 days	10 (77)	54 (91)	52 (88)	10 (100)	2 (67)	116 (89)	12 (92)	128 (89)	34 (83)	56 (85)	222 (82)	71 (65)	35 (73)	312 (82)	106 (67)	418 (78)	546 (80)
5-7 days	3 (23)	5 (8)	7 (12)	0 (0)	1 (33)	15 (11)	1 (8)	16 (11)	7 (17)	10 (15)	50 (18)	38 (35)	13 (27)	67 (18)	51 (32)	118 (22)	134 (20)
Median (range)	2 (0-7)	2 (0-5)	2 (0-7)	2 (1-3)	2 (1-7)	2 (0-7)	2 (1-7)	2 (0-7)	2 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)	3 (0-7)
With Chronic conditions																	
N	4 (31)	4 (7)	9 (15)	3 (30)	3 (100)	17 (13)	6 (46)	23 (16)	1 (2)	1 (1)	39 (14)	34 (31)	27 (56)	41 (11)	61 (39)	102 (19)	125 (18)
Vaccinated 2008-09	2 (50)	3 (75)	5 (56)	1 (33)	3 (100)	10 (59)	4 (67)	14 (61)	1 (100)	1 (100)	19 (49)	20 (74)	20 (74)	21 (51)	40 (66)	61 (60)	75 (60)
Without chronic condition	ns																
N	9 (69)	55 (93)	50 (85)	7 (70)	0	114 (87)	7 (54)	121 (84)	40 (98)	65 (98)	233 (86)	75 (69)	21 (44)	338 (89)	96 (61)	434 (81)	555 (82)
Vaccinated 2008-09	3 (33)	10 (18)	17 (34)	1 (14)	0	30 (26)	1 (14)	31 (26)	3 (8)	5 (8)	44 (19)	40 (53)	13 (62)	52 (15)	53 (55)	105 (24)	136 (24)
Province																	
Alberta	0	9 (15)	13 (22)	3 (30)	2 (67)	22 (17)	5 (38)	27 (19)	10 (24)	23 (35)	87 (32)	33 (30)	14 (29)	120 (32)	47 (30)	167 (31)	194 (29)
BC	1 (8)	6 (10)	10 (17)	2 (20)	0	17 (13)	2 (15)	19 (13)	10 (24)	16 (24)	89 (33)	34 (31)	17 (35)	115 (30)	51 (32)	166 (31)	185 (27)
Ontario	4 (31)	16 (27)	13 (22)	1 (10)	0	33 (25)	1 (8)	34 (24)	11 (27)	11 (17)	46 (17)	22 (20)	12 (25)	68 (18)	34 (22)	102 (19)	136 (20)
Quebec	8 (61)	28 (47)	23 (39)	4 (40)	1 (33)	59 (45)	5 (38)	64 (44)	10 (24)	16 (24)	50 (18)	20 (18)	5 (10)	76 (20)	25 (16)	101 (19)	165 (24)
Chronic conditions by	province																
Alberta	0	0 (0)	3 (23)	1 (33)	2 (100)	3 (14)	3 (60)	6 (22)	0	1 (4)	12 (14)	14 (42)	7 (50)	13 (11)	21 (45)	34 (20)	39 (20)
BC	1 (100)	0 (0)	3 (30)	2 (100)	0	4 (24)	2 (100)	6 (32)	0	0	15 (17)	11 (32)	13 (76)	15 (13)	24 (47)	39 (23)	45 (24)
Ontario	0	2 (12)	2 (15)	0	0	4 (12)	0 (0)	4 (12)	0	0	3 (6)	3 (14)	4 (33)	3 (4)	7 (21)	10 (10)	14 (10)
Quebec	3 (37)	2 (7)	1 (4)	0	1 (100)	6 (10)	1 (20)	7 (11)	1 (10)	0	9 (18)	6 (30)	3 (60)	10 (13)	9 (36)	19 (19)	26 (16)
2008-09 TIV immunized																	
Alberta	0	1 (11)	5 (38)	1 (33)	2 (100)	6 (27)	3 (60)	9 (33)	0	3 (13)	27 (31)	18 (55)	8 (57)	30 (25)	26 (55)	56 (34)	65 (34)
ВС	0	0	3 (30)	1 (50)	0	3 (18)	1 (50)	4 (21)	0	0 (0)	18 (20)	18 (53)	10 (59)	18 (16)	28 (55)	46 (28)	50 (27)
Ontario	2 (50)	5 (31)	4 (31)	0 (0)	0	11 (33)	0	11 (32)	2 (18)	2 (18)	10 (22)	13 (59)	10 (83)	14 (21)	23 (68)	37 (36)	48 (35)
Quebec	3 (37)	7 (25)	10 (43)	0 (0)	1 (100)	20 (34)	1 (20)	21 (33)	2 (20)	1 (6)	8 (16)	11 (55)	5 (100)	11 (14)	16 (64)	27 (27)	48 (29)
2007-08 TIV immunized Alberta	by provin 0		4 (31)	2 (67)	1 (50)	5 (23)	3 (60)	8 (30)	1 (10)	3 (15)	24 (20)	14 (44)	10 (71)	28 (25)	24 (52)	52 (33)	52 (28%)
Ontario	1 (33)	1 (11) 6 (40)	4 (31) 7 (70)	2 (67) 0	1 (50) 0	5 (23) 14 (50)	3 (60) 0	o (30) 14 (48)	1 (10) 1 (9)	3 (13) 7 (64)	24 (29) 14 (31)	14 (44) 11 (52)	10 (71) 8 (67)	28 (25) 22 (33)	24 (52) 19 (58)	52 (55) 41 (41)	41 (32%)
Quebec	3 (38)	6 (23)	8 (40)	0	1 (100)	17 (31)	1 (20)	18 (31)	3 (33)	2 (13)	9 (18)	9 (50)	5 (100)	14 (19)	14 (61)	28 (29)	28 (18)
Quodo	0 (00)	0 (20)	0 (10)	•	. (100)	(5.)	. (20)	10 (01)	0 (00)	- (10)	0 (10)	0 (00)	3 (100)	()	(0.)	20 (20)	_0 (10)

pH1N1= pandemic influenza A (H1N1); ILI=influenza-like illness; TIV=trivalent inactivated influenza vaccine; BC=British Columbia; *BC did not collect information on 2007-08 vaccine status. 2007-08 percentages derived based on participants for whom vaccine status known. Denominators do not include those with missing information (never exceeding 3 missing counts for any age category).

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