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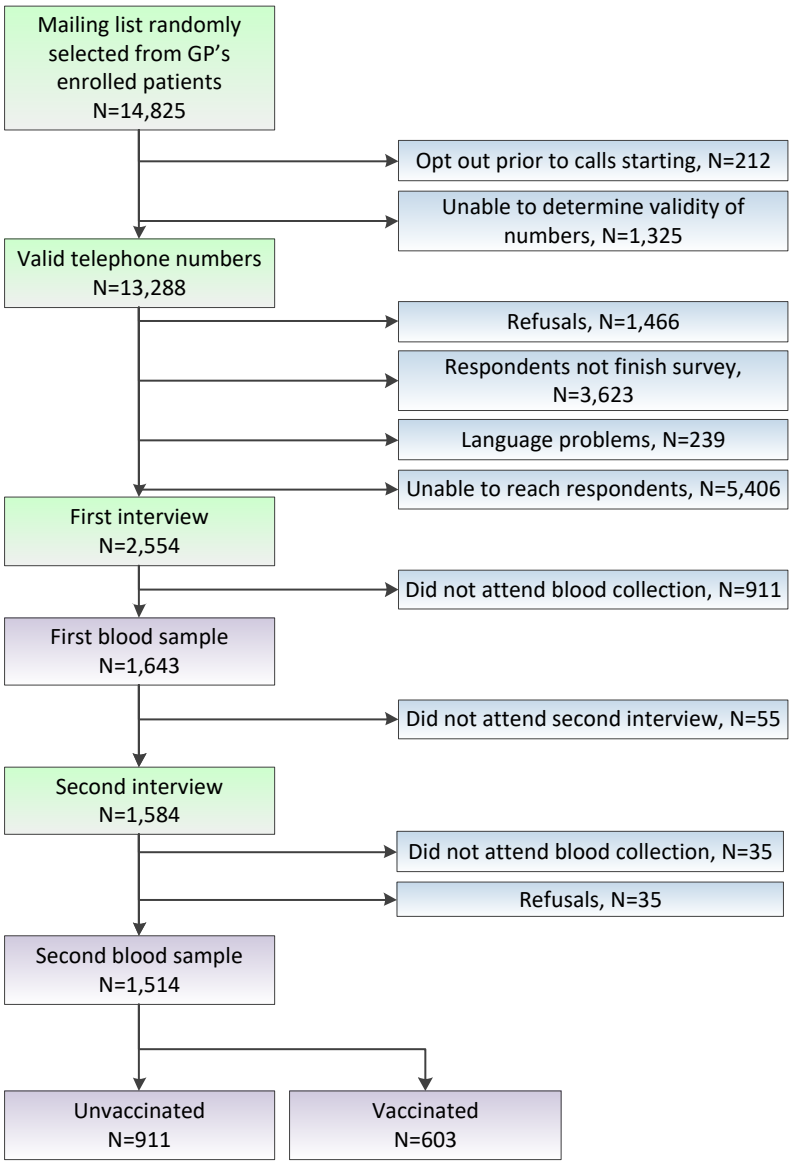
Supplement materials

Huang et al: Risk factors and attack rates of seasonal influenza infection: Results of the SHIVERS sero-epidemiologic cohort study.

Supplement Tables and Graphs

Supplement Figure S1 – inclusion and exclusion and the whole recruitment process



Supplement Figure S1 – inclusion and exclusion and the whole recruitment process. Using a stratified random sampling approach, a total of 14,825 individuals were selected from the 14 general practices' enrolled patient list (88,011) and invitation letters were sent during the February to April 2015. This was then followed by telephone recruitment through computer assisted telephone interview (CATI). Of them, 1643 (11%, 1643/14825) participants provided the first questionnaire information and the first blood sample. During mid-October to December 2015, 1514 (10%, 1514/14825) participants provided the second questionnaire information and the second blood sample. Of them, 911 (60%) were unvaccinated and 603 (40%) reported receipt of the 2015 southern hemisphere influenza vaccines.

Supplement Table S1 – Characteristics of the cohort compared to ADHB and CMDHB population

Characteristics		Cohort		ADHB & CMDHB		Ratio
		n	(%)	n	(%)	
Overall		911		905622		
Age (years)	0-4	160	17.6	66386	7.3	2.4
	5-19	264	29.0	192733	21.3	1.4
	20-64	444	48.7	549988	60.7	0.8
	65+	43	4.7	96515	10.7	0.4
Sex	Female	538	59.1	2529790	51.2	1.2
	Male	373	40.9	2412785	48.8	0.8
Ethnicity	Maori	123	13.5	99471	11	1.2
	Pacific	97	10.7	137995	15.2	0.7
	Asian	197	21.6	210331	23.2	0.9
	Other*	494	54.2	457825	50.6	1.1
NZDep**	1 or 2	206	22.6	178420	19.7	1.1
	3 or 4	195	21.4	166822	18.4	1.2
	5 or 6	170	18.7	145562	16.1	1.2
	7 or 8	164	18.0	165615	18.3	1.0
	9 or 10	176	19.3	249203	27.5	0.7

Note: ADHB refers to Auckland District Health Board that administers healthcare for the Central Auckland population. CMDHB refers to Counties Manukau District Health Board that administers healthcare for the South and East of the Auckland population. Ratios refer to proportion of the cohort participants divided by proportion of the ADHB & CMDHB population for each stratum.
*Refers to Europeans and others
**NZDep scale measures deprivation on an ordinal scale of 1 to 10 where 1 indicates the individual is living in a household that is in the least deprived decile of all New Zealand households

Supplement Table S2 – Socio-demographic and health factors associated with proportion of infection leading to illness among the cohort

Characteristics		Among HAI or NAI seroconverters							Among non-seroconverters						
		Total	ILI		mild illness not-ILI		Not-ILI		Total	ILI		mild illness not-ILI		Not-ILI	
		No.	No.	Row %	No.	Row %	No.	Row %	No.	No.	Row %	No.	Row %	No.	Row %
Overall		321	156	48.6 (43.0, 54.2)	91	28.3 (23.5, 33.6)	74	23.1 (18.6, 28.1)	590	131	22.2 (18.9, 25.8)	139	23.6 (20.2, 27.2)	320	54.2 (50.1, 58.3)
Age (years)	0-4	77	49	63.6 (51.9, 74.3)	17	22.1 (13.4, 33.0)	11	14.3 (7.4, 24.1)	83	40	48.2 (37.1, 59.4)	20	24.1 (15.4, 34.7)	23	27.7 (18.4, 38.6)
	5-19	117	46	39.3 (30.4, 48.8)	40	34.2 (25.7, 43.5)	31	26.5 (18.8, 35.5)	147	24	16.3 (10.7, 23.3)	51	34.7 (27.0, 43.0)	72	49.0 (40.7, 57.3)
	20-64	116	58	50.0 (40.6, 59.4)	29	25.0 (17.4, 33.9)	29	25.0 (17.4, 33.9)	328	61	18.6 (14.5, 23.2)	64	19.5 (15.4, 24.2)	203	61.9 (56.4, 67.2)
	65+	11	3	27.3 (6.0, 61.0)	5	45.5 (16.7, 76.6)	3	27.3 (6.0, 61.0)	32	6	18.8 (7.2, 36.4)	4	12.5 (3.5, 29.0)	22	68.8 (50.0, 83.9)
Sex	Female	179	92	51.4 (43.8, 58.9)	50	27.9 (21.5, 35.1)	37	20.7 (15.0, 27.3)	359	80	22.3 (18.1, 26.9)	87	24.2 (19.9, 29.0)	192	53.5 (48.2, 58.7)
	Male	142	64	45.1 (36.7, 53.6)	41	28.9 (21.6, 37.1)	37	26.1 (19.1, 34.1)	231	51	22.1 (16.9, 28.0)	52	22.5 (17.3, 28.4)	128	55.4 (48.8, 61.9)
Ethnicity	Maori	43	22	51.2 (35.5, 66.7)	14	32.6 (19.1, 48.5)	7	16.3 (6.8, 30.7)	80	17	21.3 (12.9, 31.8)	22	27.5 (18.1, 38.6)	41	51.3 (39.8, 62.6)
	Pacific	45	23	51.1 (35.8, 66.3)	8	17.8 (8.0, 32.1)	14	31.1 (18.2, 46.6)	52	15	28.8 (17.1, 43.1)	15	28.8 (17.1, 43.1)	22	42.3 (28.7, 56.8)
	Asian	78	32	41.0 (30.0, 52.7)	28	35.9 (25.3, 47.6)	18	23.1 (14.3, 34.0)	119	27	22.7 (15.5, 31.3)	30	25.2 (17.7, 34.0)	62	52.1 (42.8, 61.3)
	Other*	155	79	51.0 (42.8, 59.1)	41	26.5 (19.7, 34.1)	35	22.6 (16.3, 30.0)	339	72	21.2 (17.0, 26.0)	72	21.2 (17.0, 26.0)	195	57.5 (52.1, 62.8)
NZDep**	1 or 2	64	36	56.3 (43.3, 68.6)	16	25.0 (15.0, 37.4)	12	18.8 (10.1, 30.5)	142	26	18.3 (12.3, 25.7)	39	27.5 (20.3, 35.6)	77	54.2 (45.7, 62.6)
	3 or 4	60	28	46.7 (33.7, 60.0)	16	26.7 (16.1, 39.7)	16	26.7 (16.1, 39.7)	135	29	21.5 (14.9, 29.4)	32	23.7 (16.8, 31.8)	74	54.8 (46.0, 63.4)
	5 or 6	64	25	39.1 (27.1, 52.1)	23	35.9 (24.3, 48.9)	16	25.0 (15.0, 37.4)	106	22	20.8 (13.5, 29.7)	22	20.8 (13.5, 29.7)	62	58.5 (48.5, 68.0)
	7 or 8	63	36	57.1 (44.0, 69.5)	16	25.4 (15.3, 37.9)	11	17.5 (9.1, 29.1)	101	27	26.7 (18.4, 36.5)	20	19.8 (12.5, 28.9)	54	53.5 (43.3, 63.5)
	9 or 10	70	31	44.3 (32.4, 56.7)	20	28.6 (18.4, 40.6)	19	27.1 (17.2, 39.1)	106	27	25.5 (17.5, 34.9)	26	24.5 (16.7, 33.8)	53	50.0 (40.1, 59.9)
Underlying Condition[§]	No	275	132	48.0 (42.0, 54.1)	77	28.0 (22.8, 33.7)	66	24.0 (19.1, 29.5)	481	97	20.2 (16.7, 24.0)	115	23.9 (20.2, 28.0)	269	55.9 (51.4, 60.4)
	Yes	46	24	52.2 (36.9, 67.1)	14	30.4 (17.7, 45.8)	8	17.4 (7.8, 31.4)	109	34	31.2 (22.7, 40.8)	24	22.0 (14.6, 31.0)	51	46.8 (37.2, 56.6)

Note: ILI refers to influenza-like illness defined as *'an acute respiratory illness with a history of fever or measured temperature of ≥38°C, and cough, and onset within the past 7 days'*. Influenza-PCR-confirmed ILI is defined as PCR-confirmed influenza from those participants with ILI; Not-ILI refers those participants indicated not having ILI in the weekly text/email 'Have you had cough and fever in the previous 7 days'. Mild illness not-ILI refers to those indicated having ILI in the weekly text/email but not verified by nurses; HAI or NAI seroconverters refer to those individuals with either haemagglutination inhibition (HAI) seroconversion (4-fold or greater HAI titre rise in paired sera with the second titre at least 1:40) or neuraminidase inhibition (NAI) seroconversion (4-fold or greater NAI titre rise); Non-seroconverters refer to those individuals without any HAI or NAI seroconversion; CI refers to confidence interval;
*Refers to Europeans and others
**NZDep scale measures deprivation on an ordinal scale of 1 to 10 where 1 indicates the individual is living in a household that is in the least deprived decile of all New Zealand households
[§]Underlying condition includes one or more of the following conditions: asthma, diabetes, chronic respiratory illness, heart disease (angina, myocardial infarction/heart attack, chronic heart failure, atrial fibrillation, rheumatic heart disease, congenital heart condition) and mental illnesses.

Supplement Table S3 – Socio-demographic factors associated with ILI and influenza-PCR-confirmed ILI cases among the cohort during 27 April to 27 September 2015

Characteristics		Total	Event level				Influenza-like illness (ILI)						Influenza-PCR-confirmed ILI					
							ILI not seeing GPs			ILI seeing GPs			Influenza not seeing GPs			Influenza seeing GPs		
							Observed	Adjusted		Observed	Adjusted		Observed	Adjusted		Observed	Adjusted	
		No.	No. weekly response (%)	No. ILI (%)	No. ILI-swab tested (%)	No. Influenza-confirmed ILI (%)	No. (Row%)	No.	Incidence (%:CI)	No. (Row%)	No.	Incidence (%:CI)	No. (Row%)	No.	Incidence (%:CI)	No. (Row%)	No.	Incidence (%:CI)
Overall		911	17121 (85.4)	347 (2.0)	244 (70.3)	51 (20.9)	309 (33.9)	355	39.0 (35.8, 42.2)	38 (4.2)	43	4.7 (3.4, 6.3)	38 (4.2)	61	6.7 (5.2, 8.5)	13 (1.4)	15	1.6 (0.9, 2.7)
Age (years)	0-4	160	2923 (83.0)	114 (3.9)	90 (78.9)	17 (18.9)	104 (65.0)	125	**78.1 (70.9, 84.3)**	10 (6.3)	12	7.5 (3.9, 12.7)	15 (9.4)	20	**12.5 (7.8, 18.6)**	2 (1.3)	2	1.3 (0.2, 4.4)
	5-19	264	4912 (84.6)	79 (1.6)	36 (45.6)	15 (41.7)	68 (25.8)	79	29.9 (24.5, 35.8)	11 (4.2)	13	4.9 (2.6, 8.3)	9 (3.4)	18	6.8 (4.1, 10.6)	6 (2.3)	7	2.7 (1.1, 5.4)
	20-64	444	8484 (86.9)	144 (1.7)	110 (76.4)	18 (16.4)	128 (28.8)	144	32.4 (28.1, 37.0)	16 (3.6)	17	3.8 (2.2, 6.1)	13 (2.9)	19	4.3 (2.6, 6.6)	5 (1.1)	5	1.1 (0.4, 2.6)
	65+	43	802 (84.8)	10 (1.2)	8 (80.0)	1 (12.5)	9 (20.9)	10	23.3 (11.8, 38.6)	1 (2.3)	1	2.3 (0.1, 12.3)	1 (2.3)	1	2.3 (0.1, 12.3)	0 (0.0)	0	0.0 (0.0, 8.2)
Sex	Female	538	10101 (85.3)	215 (2.1)	150 (69.8)	35 (23.3)	191 (35.5)	219	40.7 (36.5, 45.0)	24 (4.5)	26	4.8 (3.2, 7.0)	27 (5.0)	42	7.8 (5.7, 10.4)	8 (1.5)	9	1.7 (0.8, 3.2)
	Male	373	7020 (85.5)	132 (1.9)	94 (71.2)	16 (17.0)	118 (31.6)	135	36.2 (31.3, 41.3)	14 (3.8)	16	4.3 (2.5, 6.9)	11 (2.9)	17	4.6 (2.7, 7.2)	5 (1.3)	6	1.6 (0.6, 3.5)
Ethnicity	Maori	123	2314 (85.5)	42 (1.8)	29 (69.0)	7 (24.1)	37 (30.1)	42	34.1 (25.8, 43.2)	5 (4.1)	5	4.1 (1.3, 9.2)	6 (4.9)	9	7.3 (3.4, 13.4)	1 (0.8)	1	0.8 (0.0, 4.4)
	Pacific	97	1558 (73.0)	51 (3.3)	34 (66.7)	5 (14.7)	48 (49.5)	64	**66.0 (55.7, 75.3)**	3 (3.1)	4	4.1 (1.1, 10.2)	4 (4.1)	7	7.2 (3.0, 14.3)	1 (1.0)	1	1.0 (0.0, 5.6)
	Asian	197	3579 (82.6)	67 (1.9)	43 (64.2)	9 (20.9)	61 (31.0)	71	36.0 (29.3, 43.2)	6 (3.0)	7	3.6 (1.4, 7.2)	6 (3.0)	9	4.6 (2.1, 8.5)	3 (1.5)	3	1.5 (0.3, 4.4)
	Other*	494	9670 (89.0)	187 (1.9)	138 (73.8)	30 (21.7)	163 (33.0)	181	36.6 (32.4, 41.1)	24 (4.9)	26	5.3 (3.5, 7.6)	22 (4.5)	31	6.3 (4.3, 8.8)	8 (1.6)	9	1.8 (0.8, 3.4)
NZDep**	1 or 2	206	3917 (86.4)	70 (1.8)	51 (72.9)	15 (29.4)	59 (28.6)	66	32.0 (25.7, 38.9)	11 (5.3)	12	5.8 (3.0, 10.0)	12 (5.8)	17	8.3 (4.9, 12.9)	3 (1.5)	3	1.5 (0.3, 4.2)
	3 or 4	195	3779 (88.1)	67 (1.8)	44 (65.7)	6 (13.6)	55 (28.2)	61	31.3 (24.8, 38.3)	12 (6.2)	13	6.7 (3.6, 11.1)	3 (1.5)	6	3.1 (1.1, 6.6)	3 (1.5)	3	1.5 (0.3, 4.4)
	5 or 6	170	3390 (90.6)	57 (1.7)	44 (77.2)	10 (22.7)	54 (31.8)	59	34.7 (27.6, 42.4)	3 (1.8)	3	1.8 (0.4, 5.1)	8 (4.7)	11	6.5 (3.3, 11.3)	2 (1.2)	2	1.2 (0.1, 4.2)
	7 or 8	164	3007 (83.3)	78 (2.6)	58 (74.4)	14 (24.1)	71 (43.3)	83	50.6 (42.7, 58.5)	7 (4.3)	8	4.9 (2.1, 9.4)	12 (7.3)	19	11.6 (7.1, 17.5)	2 (1.2)	2	1.2 (0.1, 4.3)
	9 or 10	176	3028 (78.2)	75 (2.5)	47 (62.7)	6 (12.8)	70 (39.8)	88	50.0 (42.4, 57.6)	5 (2.8)	6	3.4 (1.3, 7.3)	3 (1.7)	6	3.4 (1.3, 7.3)	3 (1.7)	4	2.3 (0.6, 5.7)
Underlying Condition[§]	No	756	14197 (85.4)	282 (2.0)	201 (71.3)	42 (20.9)	251 (33.2)	288	38.1 (34.6, 41.7)	31 (4.1)	35	4.6 (3.2, 6.4)	30 (4.0)	49	6.5 (4.8, 8.5)	12 (1.6)	14	1.9 (1.0, 3.1)
	Yes	155	2924 (85.7)	65 (2.2)	43 (66.2)	9 (20.9)	58 (37.4)	66	42.6 (34.7, 50.8)	7 (4.5)	8	5.2 (2.3, 9.9)	8 (5.2)	12	7.7 (4.1, 13.1)	1 (0.6)	1	0.6 (0.0, 3.5)

Note: ILI refers to influenza-like illness defined as *'an acute respiratory illness with a history of fever or measured fever of ≥38°C, and cough, and onset within the past 7 days'*. Influenza-PCR-confirmed ILI is defined as PCR-confirmed influenza from those participants with ILI; CI refers to confidence interval;

*Refers to Europeans and others

**NZDep scale measures deprivation on an ordinal scale of 1 to 10 where 1 indicates the individual is living in a household that is in the least deprived decile of all New Zealand households

[§]Underlying condition includes one or more of the following conditions: asthma, diabetes, chronic respiratory illness, heart disease (angina, myocardial infarction/heart attack, chronic heart failure, atrial fibrillation, rheumatic heart disease, congenital heart condition) and mental illnesses.

Supplement Table S4 Socio-demographic and health factors associated with HAI and/or NAI seroconversion rates among the cohort

Characteristics		Cohort	HAI		NAI		HAI & NAI		HAI or NAI	
			No.	Row% (95% CIs)	No.	Row% (95% CIs)	No.	Row% (95% CIs)	No.	Row% (95% CIs)
Total		911	221	24.3 (21.5, 27.2)	275	30.2 (27.2, 33.3)	175	19.2 (16.7, 21.9)	321	35.2 (32.1, 38.4)
Age (years)	0-4	160	48	30.0 (23.0, 37.7)	70	43.8 (35.9, 51.8)	41	25.6 (19.1, 33.1)	77	48.1 (40.2, 56.2)
	5-19	264	92	34.8 (29.1, 40.9)	102	38.6 (32.7, 44.8)	77	29.2 (23.8, 35.1)	117	44.3 (38.2, 50.5)
	20-64	444	75	16.9 (13.5, 20.7)	95	21.4 (17.7, 25.5)	54	12.2 (9.3, 15.6)	116	26.1 (22.1, 30.5)
	65+	43	6	14.0 (5.3, 27.9)	8	18.6 (8.4, 33.4)	3	7.0 (1.5, 19.1)	11	25.6 (13.5, 41.2)
Sex	Female	538	121	22.5 (19.0, 26.3)	155	28.8 (25.0, 32.8)	97	18.0 (14.9, 21.5)	179	33.3 (29.3, 37.4)
	Male	373	100	26.8 (22.4, 31.6)	120	32.2 (27.5, 37.2)	78	20.9 (16.9, 25.4)	142	38.1 (33.1, 43.2)
Ethnicity	Maori	123	32	26.0 (18.5, 34.7)	35	28.5 (20.7, 37.3)	24	19.5 (12.9, 27.6)	43	35.0 (26.6, 44.1)
	Pacific	97	33	34.0 (24.7, 44.3)	36	37.1 (27.5, 47.5)	24	24.7 (16.5, 34.5)	45	46.4 (36.2, 56.8)
	Asian	197	54	27.4 (21.3, 34.2)	68	34.5 (27.9, 41.6)	44	22.3 (16.7, 28.8)	78	39.6 (32.7, 46.8)
	Others*	494	102	20.6 (17.2, 24.5)	136	27.5 (23.6, 31.7)	83	16.8 (13.6, 20.4)	155	31.4 (27.3, 35.7)
NZDep**	1 or 2	206	45	21.8 (16.4, 28.1)	59	28.6 (22.6, 35.3)	40	19.4 (14.2, 25.5)	64	31.1 (24.8, 37.9)
	3 or 4	195	41	21.0 (15.5, 27.4)	50	25.6 (19.7, 32.4)	31	15.9 (11.1, 21.8)	60	30.8 (24.4, 37.8)
	5 or 6	170	40	23.5 (17.4, 30.6)	56	32.9 (25.9, 40.6)	32	18.8 (13.2, 25.5)	64	37.6 (30.3, 45.4)
	7 or 8	164	42	25.6 (19.1, 33.0)	53	32.3 (25.2, 40.1)	32	19.5 (13.7, 26.4)	63	38.4 (30.9, 46.3)
	9 or 10	176	53	30.1 (23.4, 37.5)	57	32.4 (25.5, 39.8)	40	22.7 (16.8, 29.6)	70	39.8 (32.5, 47.4)
Underlying condition	No	756	190	25.1 (22.1, 28.4)	237	31.3 (28.1, 34.8)	152	20.1 (17.3, 23.1)	275	36.4 (32.9, 39.9)
	Yes	155	31	20.0 (14.0, 27.2)	38	24.5 (18.0, 32.1)	23	14.8 (9.6, 21.4)	46	29.7 (22.6, 37.5)
Virus	A(H3N2)	911	151	16.6 (14.2, 19.2)	154	16.9 (14.5, 19.5)	112	12.3 (10.2, 14.6)	193	21.2 (18.6, 24.0)
	B	911	77	8.5 (6.7, 10.5)	143	15.7 (13.4, 18.2)	61	6.7 (5.2, 8.5)	159	17.5 (15.0, 20.1)

Note: HAI refers to individuals with haemagglutination inhibition seroconversion (4-fold or greater HAI titre rise in paired sera with the second titre at least 1:40); NAI refers to individuals with neuraminidase inhibition seroconversion (4-fold or greater NAI titre rise in paired sera); HAI & NAI refers to individuals with both HAI and NAI seroconversion in paired sera; HAI or NAI refers to individuals with either HAI or NAI seroconversion in paired sera (i.e. influenza infection); CI refers to confidence interval.
*Refers to Europeans and others
**NZDep scale measures deprivation on an ordinal scale of 1 to 10 where 1 indicates the individual is living in a household that is in the least deprived decile of all New Zealand households
[§]Underlying condition includes one or more of the following conditions: asthma, diabetes, chronic respiratory illness, heart disease (angina, myocardial infarction/heart attack, chronic heart failure, atrial fibrillation, rheumatic heart disease, congenital heart condition) and mental illnesses.

FULL METHODS

Ethics statement

The study protocol was approved by the Northern A Health and Disability Ethics Committee (NTX/11/11/102 AM13). Written informed consent was obtained from all participants (or guardians of children).

Study design and population

As part as ongoing influenza surveillance in Auckland, New Zealand, we included all 97,291 persons enrolled in 14 selected general practices (GPs) as our study population and sampling frame.[1] We used a stratified random sample of GP registers stratified by age (0-4, 5-19, 20-64, ≥65 years) and ethnicity (Maori, Pacific, Asian, European and others). Within

each stratum, simple random sampling was performed to select sufficient numbers of participants. Each stratum would contain more than the required number to allow for attrition. For those strata that were expected to be more difficult to recruit (i.e. young children aged 0-4 years and Maori and Pacific ethnic groups), additional higher number were selected. We estimated a minimum study size of 800 for an assumed 26% risk of infection and a two-sided 95% confidence intervals of +/- 12%, 30% risk of ILI (+/- 11%), 7% risk of influenza-PCR-confirmed ILI (+/- 29%).

Recruitment and questionnaire administration

During February to April 2015, invitation letters were sent to those randomly selected individuals including study aims, benefits, risks, processes and requirements. Individuals who agreed to participate and planned to reside the study area during the influenza season (May to September) were eligible. Individuals were excluded if they received the influenza vaccine prior to the first blood collection or they were not able to communicate in English.

We conducted telephone recruitment through computer assisted telephone interview (CATI). If a randomly selected individual declined or could not be reached after 6 attempts of call, he/she was replaced by another randomly selected person from the same stratum until successful recruitment. We administered the first questionnaire for those agreed to participate, including the participant's vaccination status, general health, contact and demographic details. During mid-October to December, the second questionnaire was administrated for respiratory illness exposure and risk factor information, including history of influenza-like illness and other acute respiratory illnesses, contact with ILI patients, general health status, living conditions and influenza vaccination during the influenza season. All participants were asked to provide two blood samples (one during March to mid-June and another during mid-October to December) by attending a nearby blood collection centre or

accessing a mobile phlebotomy service for 5 mL of venous blood or 500 µL of heel/finger-prick blood from a child less than 5 years. We reimbursed each participant with $60 after completion of two questionnaires and two blood collections.

Influenza-like illness (ILI) surveillance

We conducted ILI surveillance during the influenza season (weeks 18-39 between 27 April to 27 September 2015). ILI was defined as *'an acute respiratory illness with a history of fever or measured fever of ≥38°C, and cough, and onset within the past 7 days'.* Every week, we sent a text/email message to all participants asking '*have you had cough and fever in the previous 7 days*'. For those participants developed ILI and responded 'Yes', outreach nurses contacted them, verified their ILI status and arranged a visit to those with confirmed ILI at their daytime locations for nasopharyngeal or throat swab collections. Participants could also call outreach nurses directly for reporting ILI.

This study provided no medical advice as to what participants should do if ill, and communicated to participants to continue to use medical services as normal. Those participants who consulted their GPs with ILI were captured through the SHIVERS ILI surveillance platform with swab collection and information on influenza vaccination, morbid event and underlying conditions.[1 2]

Laboratory materials and methods

Molecular testing

Respiratory samples, collected with a COPAN flocked swab in 3 ml of viral transport medium, were packaged in a biobottle with chill-pads and couriered to the WHO National Influenza centre (NIC) at the Institute of Environmental Science and Research (ESR) daily. To detect influenza and non-influenza viruses, CDC's real-time reverse transcriptase (rRT)

PCR protocols were used as described previously.[3 4-6 7] Briefly, ribonucleic acid (RNA) was extracted from 200 µL of each sample using an automated extractor. All samples were tested for influenza A (subtypes of A(H1N1)pdm09, A(H3N2)), influenza B (lineages of B/Yamagata and B/Victoria), respiratory syncytial virus, rhinovirus, human metapneumovirus, parainfluenza types 1-3, adenovirus and enterovirus. The remaining unused samples were stored at -80°C freezer.

Serological testing – haemagglutination inhibition (HAI) assay

Blood samples, collected in a serum separation tube, were separated, stored at 2-4°C and then couriered to the NIC. Anti-haemagglutinin antibodies were detected using the haemagglutination inhibition (HAI) assay as described previously.[8] Reference antigens which were propagated in embryonated chicken eggs, A/California/7/2009 (H1N1)pdm09, A/Switzerland/9715293/2013 (H3N2), B/Phuket/3073/2013 (B/Yamagata-lineage) and B/Brisbane/60/2008 (B/Victotia-lineage) were provided by WHOCC-Atlanta and WHOCC-Melbourne. These antigens were standardized by the haemagglutination (HA) assay to 4 HA units per 25 µL using 1.0% guinea pig erythrocytes. Human serum and positive control serum were treated with receptor destroying enzyme (Denka Seiken Co. Tokyo, Japan) to inactivate non-specific inhibitors of agglutination. Samples with a 'failed' serum control in the first run were further treated with guinea-pig erythrocytes and re-tested.

Serum samples were titrated in a serial two-fold dilutions with a starting dilution of 1:10 and ending at 1:640. Paired sera were tested together in the same run. The anti-HA antibody level was measured as the reciprocal of the highest dilution titre causing complete haemagglutination inhibition of erythrocytes by the influenza virus. For those sera with HAI titre of >1:640, further serial two-fold dilution were performed in order to detect the highest dilution titre that fully prevented haemagglutination. Results were accepted if sera and

guinea-pig erythrocyte cell controls provided the correct non-agglutinated pattern and the positive controls were within two-fold of the mean titre. Samples with a HAI titre of <1:10 were assigned a titre of 1:5 for the purposes of computing seroconversion. A fourfold or greater rise in HAI antibody titres in paired sera, with the second titre at least 1:40 was considered as HAI seroconversion.

Serological testing – neuraminidase inhibition (NAI) assay

Neuraminidase inhibition (NAI) assay detects anti-NA antibodies by using peroxidase-labelled peanut agglutinin (the lectin) to bind to terminal galactose moieties that are exposed after NA enzyme cleavage.[9] Reference strains which were propagated in embryonated chicken eggs, B/Phuket/3073/2013 (B/Yamagata-lineage) and B/Brisbane/60/2008 (B/Victotia-lineage) were provided by WHOCC-Atlanta and WHOCC-Melbourne. St Jude Children's Research Hospital generated the following reassortant viruses: 8-plasmid reverse genetics system rescued reassortants bearing NA of a given vaccine strain, H6 HA of A/tk/Mass/75, and the complementary six gene segments of PR8 as previously described.[10] The viral constructs bearing NA of A/California/7/2009 (H1N1)pdm09 and A/Switzerland/9715293/2013 (H3N2) were referred to as H6N1Cal/09 and H6N2Swiss/13, respectively. All reference antigens, inactivated by beta-propiolactone (BPL), were titrated and the optimal working dilutions selected for the NAI assay.

Briefly, 96-well microtiter plates were coated with fetuin. Sera were tested in serial 2-fold dilutions from 1:10 to 1:5120. Serial dilutions of heat-inactivated sera were transferred to fetuin-coated plates, reference antigens were added, and then plates incubated for 16–18 hours at 37°C in dry heat, followed by the addition of peroxidase-labelled peanut lectin for 2 hours at room temperature. Bound lectin was detected with o-phenylenediamine dihydrochloride (OPD) (Sigma-Aldrich). Paired sera were tested

together in the same run. The anti-NA antibody level was measured as the reciprocal of the highest dilution titre that caused 50% inhibition of NA activity. Sera with a NAI titre of >1:5120 and a NAI titre of <1:10 were assigned a titre of 1:10240 and 1:5 respectively for the purposes of computing seroconversion. A 4-fold or greater increase in NAI titres in paired sera was considered NAI seroconversion.

Definition of exposure and outcome variables

'Influenza infection' was defined as either HAI or NAI seroconversion or influenza RNA detection. We also defined influenza infection based on HAI seroconversion or influenza RNA detection to compare with published rates.

'Influenza-like illness' was defined as *'an acute respiratory illness with a history of fever or measured fever of ≥38°C, and cough, and onset within the past 7 days*'. When participants responded "No" to the weekly message ''*have you had cough and fever in the previous 7 days*', they were defined as 'not-ILI'; When participants responded as "Yes" but not verified by outreach nurses as meeting ILI case definitions, these illnesses were defined as 'mild illness not-ILI'. 'not-ILI' and 'mild illness not-ILI' were combined to be called as 'no-ILI'. When outreach nurses verified participants' symptoms as meeting ILI case definitions, it was defined as 'ILI'.

'Influenza-PCR-confirmed ILI' was defined as the detection of influenza-specific RNA in a swab by rRT-PCR from those with ILI.

Statistical analysis

All analyses were performed with the statistical software Stata 14.1. Study data were collected and managed using REDCap electronic data capture tools.[11]

Participants who were vaccinated for the 2015 southern hemisphere influenza vaccines that year were excluded from the analysis as serologically defined influenza infection can't differentiate whether it is due to vaccination or natural infection.

The observed attack rates of ILI and influenza-PCR-confirmed ILI were corrected to account for: a) missed weekly reports by applying the percent of reports that were classified as ILI to those non-responders (Corrected ILI = Actual ILI x Total possible number of responses/Actual number of responses); b) missed swabs from ILI cases by applying the influenza positivity rate of those tested to those non-tested (Corrected influenza-PCR-confirmed ILI = Actual PCR-confirmed ILI x Corrected ILI/Actual number of swabs). The above methods of adjustment for the missed weekly reports and missed ILI swabs were applied on a week-by-week basis for overall and each stratum and then aggregated the relevant events for the season (22 weeks). In order to estimate the number of persons who experienced ILI or influenza-PCR-confirmed ILI events (one or more), it was then calculated by dividing the number of the relevant events by the average number of events per person for overall and each stratum: i.e. Corrected number of persons with ILI or influenza-PCR-confirmed ILI = Corrected ILI or influenza-PCR-confirmed ILI events ÷ (Actual number of ILI or influenza-PCR-confirmed ILI events/Actual number of persons with ILI or influenza-PCR-confirmed ILI events).

Two independent methods were employed to calculate the percentage of infections as measured by seroconversion that led to influenza disease: 1) dividing the corrected number of persons with influenza-PCR-confirmed ILI by the total number of unvaccinated persons who

seroconverted; 2) subtracting ILI rates among non-seroconverters from those in seroconverters. The purpose was to account for the fact that some ILI would be attributable to other non-influenza pathogens or factors unrelated to the infection or some influenza-associated ILI cases would not be confirmed by PCR due to low viral load such as late swabbing, sample type and sampling techniques.

The risk of influenza infection and of disease once infected was examined for five potential risk factors (age, sex, ethnicity, social deprivation, underlying conditions). Univariate and multivariate analysis was performed using rate ratios from a poisson regression linear model.

Finally, we weighted the attack rates in our study population by age and ethnic strata to arrive at a total infection rate for the whole Auckland region. 95% confidence intervals were calculated for the (weighted) binomial proportion.

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