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Factors associated with infection by 2009 pandemic H1N1 influenza virus during different phases of the epidemic

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SUMMARY

Objective: The focus of this study was to ascertain the factors associated with 2009 pandemic influenza H1N1 (pH1N1) infection during different phases of the epidemic.
Methods: In central Taiwan, 306 persons from households with schoolchildren were followed sequentially and serum samples were taken at three sampling time-points starting in the fall of 2008, shortly after influenza vaccination. Participants who seroconverted between two consecutive blood samplings were considered as having serological evidence of infection. A generalized estimation equation (GEE) with a logistic link to account for household correlations was applied to identify factors associated with pH1N1 infections during the pre-epidemic (April–June) and epidemic (September–October) periods.
Results: The results showed that receiving an inactivated seasonal influenza vaccine (ISIV) and having a hemagglutination inhibition assay (HI) titer of 40 or higher resulted in a significantly lower likelihood of pH1N1 infection during the pre-epidemic period only, for both children and adults (adjusted odds ratio (OR) 0.3, 95% confidence interval (CI) 0.12–0.9). Having a previous infection by pH1N1 with a baseline titer of 20 or higher resulted in a significantly lower likelihood of infection by pH1N1 during the epidemic period (adjusted OR 0.06, 95% CI 0.02–0.16).
Conclusions: Our results provide the first serological evidence to suggest a protection effect from receiving an ISIV against pH1N1 infection only when the HI titer reaches 40 or higher during the pre-epidemic period. This study gives an important insight into the control and intervention measures required for preventing infections during future influenza epidemics.

1. Introduction

On April 24, 2009, a swine-origin H1N1 influenza virus (S-OIV) emerged as a novel influenza A virus and caused widespread illness in many countries worldwide, meeting the World Health Organization (WHO) criteria for a pandemic. This is now termed pandemic influenza H1N1 (pH1N1).[1–3] Reports of antigenicity distinct from that of seasonal human influenza A and differences in the pathogenicity of the virus in animal models increase concerns for a pandemic with major public health consequences.[4,5] At the same time, the incidence of clinically severe cases appears to be similar to that for seasonal flu, with 5011 hospitalizations and 301 deaths in the USA between April 15 and July 24, according to US Centers for Disease Control and Prevention (CDC) estimates.[6] This apparent contradiction highlights the need for better insights into the risk and protection factors behind influenza virus infection.

As the pH1N1 virus spread around the world in late spring 2009, at a time when well-matched pandemic vaccines were not immediately available, the question of providing partial protection with a seasonal influenza vaccine arose. However, the lack of full

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baseline, pre-pandemic immune profiles for recipients of inactivated seasonal influenza vaccines (ISIV) and for unvaccinated individuals of various ages, resulted in inconsistent results when the effectiveness of seasonal influenza vaccination in preventing 2009 pH1N1 morbidity in the general population was evaluated, as previously published.[7–10] As experts in various fields have called for serological investigations to more accurately determine rates of infection, the stored blood bank would be very useful in unveiling the extent of ISIV protection in terms of basic research and public health, which is the focus of our current study.

The government in Taiwan initiated a pandemic H1N1 clinical surveillance system on April 29, and from that date on an increasing number of probable cases was reported, especially after May 15, which correlated with the first laboratory-confirmed imported pH1N1 case on May 19. By the end of June, there were 61 travel-related laboratory-confirmed cases.[11] The first wave of 2009 pandemic H1N1 began around July 1, peaked in the last week of August, and had subsided by the end of September. We utilized the serum banks, initially collected to evaluate the effectiveness of the ISIV, to determine the antibody level of seasonal influenza virus and pH1N1 virus before and after the different phases of the epidemic as the baseline and marker of infection, within a household study design. Our aim was to compare the risk and protection factors associated with infection during different phases of the epidemic.

2. Materials and methods

2.1. Study sites and serum collection

Taiwan has a population of over 23 million. Since 2007, all schoolchildren in grades 1–4 in Taiwan have received a free influenza vaccination (ISIV) annually from the government. In order to evaluate vaccine efficacy, students from two elementary schools located in the urban Taichung City and four schools in the rural Nantou County in central Taiwan were recruited into a 3-year study starting in the fall of 2008. Taichung City is the largest urban city in central Taiwan with a population of more than 1 million and a highly developed socio-economic structure. The two schools selected were located respectively in the north and central districts of the city, with approximately 140 000 total residents. Nearby Nantou County, with a total land size approximately 25 times larger than that of Taipei City, is the second largest county and the only landlocked county on the island of Taiwan, with a population of over 500 000, and is comparatively less developed socio-economically. The four schools in Nantou County were selected purposely from four different administrative districts in the county, namely Nantou City and Tsaotun Township each with around 100 000 residents, and the rural townships of Mingjian and Guoshing with around 40 000 and 20 000 residents, respectively.

Family members of the students were also recruited into the study to further determine the effectiveness of seasonal influenza vaccine in preventing household transmissions. The study protocol based on clinical and laboratory data was established and at least two blood samples were drawn from the study subjects, before and after an influenza season. In the fall of 2008, 454 persons from 147 households were recruited into the study. Among them, 306 persons belonging to 104 households remained in the study in 2009 and underwent all three samplings required for the analysis in this report.

To evaluate the kinetic changes in antibody responses against the influenza H1N1 virus of seasonal vaccine, wild-type, and the 2009 pH1N1 strain, only 306 study subjects who had three complete sequential blood samples taken were selected from the serum bank. The first blood samples were taken in the fall of 2008, about 2 to 3 weeks after influenza vaccination (referred to henceforth as the baseline titer); the second blood samples were taken in April–June of 2009 (referred to henceforth as the pre-epidemic period) after the 2008–09 influenza season; and the third samples were taken in September–October of 2009 (referred to henceforth as the epidemic period) before vaccination with both the 2009–10 seasonal and the 2009 pandemic influenza strains. Venous blood was taken in 5- to 10-ml plain tubes and the serum was collected after centrifugation and stored at −20 °C until use. All subjects gave informed consent to study participation and the study was approved by the Medical Ethics Committee of the China Medical University.

2.2. Data collection

Two questionnaires were conducted by trained interviewers and were used to collect basic demographic and social contact information and whether a seasonal influenza vaccination had been received in the past 2 years. Information regarding underlying diseases, including cardiovascular disease, hypertension, or diabetes mellitus, was also obtained from the adults in the family. Clinical symptoms reviews were carried out using a standardized questionnaire every 2 weeks via telephone interview. Participants were asked to report any newly experienced febrile respiratory symptoms, including fever, sore throats, and headaches during the 2008–09 influenza season. However, the clinical information during the summer season was only obtained retrospectively in December and substantial recall error was expected.

2.3. Laboratory methods

Antibody titers were measured by a hemagglutination inhibition (HI) assay following the standard protocol of the WHO.[12] The virus strain used was originally isolated from a patient infected by S-OIV H1N1, which is antigenically and genetically closely related to A/California/07/2009.

To evaluate cross-reactivity, a vaccine strain of H1N1 (A/Brisbane/59/2007) and a wild-type strain that represented more than 80% of the H1N1 circulating during the 2008–09 influenza season (A/Taiwan/606/2008) were also used. All viruses used in this study were cultured from Madin–Darby canine kidney (MDCK) cells and centrifuged at 1600 rpm, 4 °C to remove cell debris. For the HI assay, serum samples were pre-treated with receptor destroying enzyme and titrated in two-fold dilutions in phosphate-buffered saline (PBS) with an initial dilution of 1:10 and a final dilution of 1:1024. Titers were expressed as the reciprocal of the highest dilution of serum at which hemagglutination was prevented. A four-fold or greater increase in HI titer between the two consecutive serological samples was defined as evidence of H1N1 seroconversion. Samples that were negative by HI were assigned a titer of 1:5 for computational purposes in obtaining a four-fold increase of HI titers.

The HI titers against the individual virus strains used in this study were determined for pH1N1, the seasonal influenza H1N1 vaccine strain (sH1N1 v), and wild-type strain (sH1N1w). Participants who seroconverted between two consecutive blood samplings (either from the first to the second sample, or from the second to the third sample) were considered to have serological evidence of infection during the pre-epidemic or epidemic period, respectively. Geometric mean titers (GMTs) were used to avoid skewed data distribution through a log transformation and were estimated by assigning a value of 5 for titers lower than 10 and a value of 2560 for titers of 2560 or higher.

2.4. Statistical analysis

As the study design was based on the prospective family cohort and the infection status may be non-independent within a

Table 1
Univariate analysis of demographic factors associated with seroconversion during the pre-epidemic (April–June) and epidemic (September–October) periods

	Pre-epidemic period (April–June) Seroconversion				Epidemic period (September–October) Seroconversion			
	No./total	% (95% CI)	OR (95% CI)	*p*-Value	No./total	% (95% CI)	OR (95% CI)	*p*-Value
Age (years)								
5–18	26/143	18.2 (12–25)	1		62/143	43.4 (35–52)	1	
19–60	35/147	23.8 (17–31)	1.41 (0.88–2.24)	0.15	65/147	44.2 (36–52)	1.04 (0.67–1.60)	0.88
>60	5/16	31.3 (9–54)	2.05 (0.64–6.52)	0.23	9/16	56.3 (32–81)	1.68 (0.53–5.29)	0.38
Area of residence								
Urban	17/101	16.8 (10–24)	0.64 (0.26–1.62)	0.35	55/101	54.5 (45–64)	1.83 (1.03–3.24)	0.04
Rural	49/205	23.9 (18–30)	1		81/205	39.5 (33–46)	1	
Gender								
Female	35/173	20.2 (14–26)	1		82/173	47.4 (40–55)	1	
Male	31/133	23.3 (16–30)	1.20 (0.73–1.97)	0.48	54/133	40.6 (32–49)	0.76 (0.48–1.19)	0.23
Number of family members								
<4	8/33	24.2 (10–39)	1		11/33	33.3 (17–49)	1	
≥4	58/273	21.3 (16–26)	0.84 (0.23–3.08)	0.80	125/273	45.8 (40–52)	1.69 (0.65–4.38)	0.28
Size of the housing area[a]								
<30	17/66	25.8 (15–36)	1		28/66	42.4 (30–54)	1	
≥30	48/238	20.2 (15–25)	0.73 (0.29–1.84)	0.50	107/238	45.0 (39–51)	1.11 (0.57–2.15)	0.76
Personal bedroom								
No	51/218	23.4 (18–29)	1		96/218	44.0 (37–51)	1	
Yes	15/79	19.0 (10–28)	0.77 (0.38–1.54)	0.46	33/79	41.8 (31–53)	0.91 (0.52–1.59)	0.74
Number of household children aged ≤12 years								
<3	51/240	21.3 (16–26)	1		106/240	44.2 (38–50)	1	
≥3	15/66	22.7 (13–33)	1.09 (0.43–2.78)	0.86	30/66	45.5 (33–58)	1.05 (0.50–2.21)	0.89

OR, odds ratio; CI, confidence interval.
[a] Size of the housing area was measured in pin (local area unit); 1 pin is equivalent to approximately 3.3 square meters.

household, we used a generalized estimation equation (GEE) model with logistic link to detect the potential risk variables. The potential risk variables, including demographic data, household and social contact information, vaccination status, and baseline antibody titer against individual subtypes, as well as household contacts (classified according to the seroconversion status of individuals in the household: (1) any other household member seroconverted, (2) any other household member aged less than 18 years seroconverted, (3) any other household member aged older than 18 years seroconverted) were first studied using univariate analysis. To further characterize the factors associated with seroconversion of pH1N1 among the different age groups, multivariate analysis based on the significant risk factors identified in the univariate analysis and confounding variables (age, gender, area of residence, vaccination records, and baseline vaccine strain titer) was performed separately for the group of schoolchildren aged 5–18 years and the group of adults aged >19 years. The subjects aged >60 years were combined with the adult age group because of the small sample size. Stepwise logistic regression with backward selection was applied, wherein variables that did not improve the model fit at $p < 0.10$ were discarded but confounding variables were always forced into the model. In the univariate and final multivariate analysis, odds ratios (ORs) with asymptotic Wald 95% confidence intervals (CIs) and two-sided *p*-values were calculated. SAS statistical package version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for the analysis, and statistical significance was set at the 0.05 level.

3. Results

A total of 306 subjects from 104 different families were included in this study for the evaluation of risk/protection factors. The subjects included 143 schoolchildren and their siblings aged between 5 and 18 years, 147 adults aged between 19 and 60 years, and 16 people aged >60 years. Figure 1 shows the GMT against pH1N1, the seasonal influenza H1N1 vaccine strain (sH1N1 v), and wild-type strain (sH1N1w) from the sera collected during the three sampling periods. A sequential increase in GMT of pH1N1 through three different sampling periods was observed along with an increase in the titer of sH1N1w from the first to the second sampling period; however, GMT against sH1N1 v decreased from the first to the second sampling period.

Table 1 shows the results of the univariate analysis of demographic factors associated with seroconversion during the pre-epidemic period (April–June) and the epidemic period (July–October). Of the school-aged children (5–18 years), 18.2% showed seroconversion during the pre-epidemic period, and the number increased to 43.4% during the epidemic period. Similarly, 23.8% in the adult group (19–60 years) and 31.3% in the older group (>60 years) showed seroconversion during the pre-epidemic period, which increased to 44.2% and 56.3%, respectively, during the epidemic period. Those living in rural areas showed higher seroconversion (23.9%) compared to those living in urban areas (16.8%) during the pre-epidemic period, although this difference was not statistically significant. However, the opposite situation was observed during the epidemic period, with significantly higher seroconversion for subjects living in urban areas (54.5%) than for

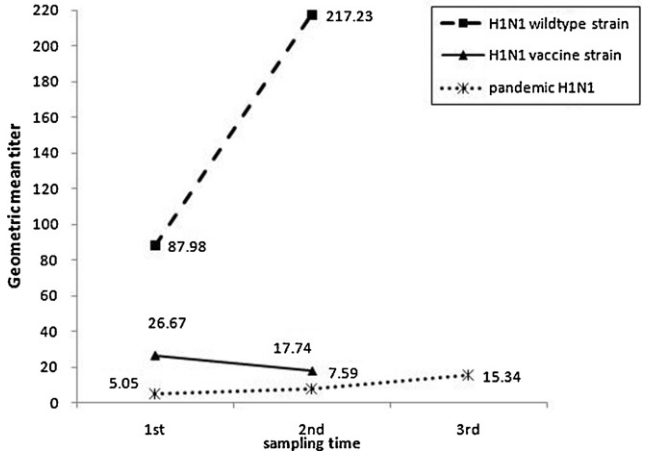

Figure 1. Geometric mean titers of seasonal H1N1 vaccine and wild-type strains and 2009 pandemic H1N1 virus measured by the hemagglutination inhibition assay from sequential sera collected at the different sampling times.

Table 2
Univariate analysis of vaccine-related factors associated with seroconversion during the pre-epidemic (April–June) and epidemic (September–October) periods

	Pre-epidemic period (April–June)				Epidemic period (September–October)			
	Seroconversion				Seroconversion			
	No./total	% (95% CI)	OR (95% CI)	*p*-Value	No./total	% (95% CI)	OR (95% CI)	*p*-Value
Vaccination 2007–08								
No	52/234	22.2 (17–28)	1		102/234	43.6 (37–50)	1	
Yes	14/71	19.7 (10–29)	0.86 (0.47–1.58)	0.63	33/71	46.5 (35–58)	1.12 (0.64–1.98)	0.69
Vaccination 2008–09								
No	47/200	23.5 (18–29)	1		89/200	44.5 (38–51)	1	
Yes	19/106	17.9 (11–25)	0.71 (0.38–1.31)	0.28	47/106	44.3 (35–54)	0.99 (0.60–1.65)	0.98
Baseline sH1N1w[b] HI titer[a]								
<40	22/90	24.4 (16–33)	1		41/88	46.6 (36–57)	1	
≥40	44/216	20.4 (15–26)	0.79 (0.44–1.41)	0.43	95/218	43.6 (37–50)	0.89 (0.56–1.40)	0.60
Baseline sH1N1v[c] HI titer								
<40	46/177	26.0 (20–32)	1		95/208	45.7 (39–52)	1	
≥40	20/129	15.5 (9–22)	0.52 (0.32–0.86)	0.01	41/98	41.8 (32–52)	0.86 (0.51–1.43)	0.55
Baseline pandemic H1N1 titer								
<20	65/303	21.5 (17–26)	1		127/224	56.7 (50–63)	1	
≥20	1/3	33.3 (0–87)	1.83 (0.17–19.51)	0.62	9/82	11.0 (4–18)	0.09 (0.04–0.20)	<0.0001
Four-fold increase of sH1N1v or sH1N1w								
No					114/236	48.3 (42–55)	1	
Yes					22/70	31.4 (21–42)	0.49 (0.28–0.87)	0.02
Other household members with seroconversion[d]								
No	14/215	6.5 (3–10)	1		36/110	32.7 (24–41)	1	
Yes	52/91	57.1 (47–67)	19.14 (7.23–50.70)	<0.0001	100/196	51.0 (44–58)	2.14 (1.04–4.39)	0.04

OR, odds ratio; CI, confidence interval.
[a] HI titer refers to the antibody titers measured by a hemagglutination inhibition (HI) assay.
[b] sH1N1w refers to the seasonal H1N1 virus wild-type strain (A/Taiwan/606/2008) used for the HI assay.
[c] sH1N1 v refers to the seasonal H1N1 virus vaccine strain (A/Brisbane/59/2007) used for the HI assay.
[d] Other household members with seroconversion: at least one other household member with seroconversion vs. no one else in the household with seroconversion.

those living in rural areas (39.5%); OR 1.83 (95% CI 1.03–3.24), this was statistically significant. In our study, males and females showed similar seroconversion rates in both the pre-epidemic and epidemic periods (23.3% vs. 20.2% pre-epidemic and 40.6% vs. 47.4% epidemic).

Moreover, the proportions of seroconversion were approximately the same, without any statistical significance, for factors associated with family contacts, including the number of household members, the size of the household area, the number of children younger than 12 years, and whether or not each household member has a personal bedroom.

Since seroconversion can be considered as an indicator of the variation in risk of infection, the effect of seasonal influenza vaccination on protection from pH1N1 infection was studied. The results are summarized in Table 2. Recipients of seasonal influenza vaccines in the 2007–08 or 2008–09 seasons were less likely to exhibit seroconversion of pH1N1, although this was without statistical significance. Interestingly, among 129 subjects with an sH1N1 v baseline titer of 40 or higher, 15.5% seroconverted to pH1N1 during the pre-epidemic period, compared to 26.0% among those with a baseline titer lower than 40; this showed significant protection (OR 0.52, 95% CI 0.32–0.86). A similar protection effect was not observed for the same baseline titer of pH1N1 or sH1N1w during the pre-epidemic period. Conversely, baseline H1N1 titers of 40 or higher against either sH1N1 v or sH1N1w were not statistically associated with the seroconversion of pH1N1 during the epidemic period (OR 0.86 and 0.89, respectively). However, 31.4% with a four-fold increase in HI titers against either sH1N1 v or sH1N1w showed seroconversion to pH1N1, compared to 48.3% without a four-fold increase who showed seroconversion (OR 0.49, 95% CI 0.28–0.87). Meanwhile, those previously infected by pH1N1 with a baseline HI titer of 20 or higher showed strong protection against infection by pH1N1 during the epidemic period (OR 0.09, 95% CI 0.04–0.2). In both the pre-epidemic and epidemic periods, there was an increased risk of pH1N1 infection if another family member showed seroconversion of pH1N1 (OR 19.14, 95% CI 7.23–50.7 for the pre-epidemic period and OR 2.14, 95% CI 1.04–4.39 for the epidemic period).

The results of multivariate analysis are shown in Table 3. We observed a higher likelihood of infection during the pre-epidemic period if other household members had seroconverted (adjusted OR 25.76, 95% CI 9.64–68.81), but no such association characterized the epidemic period. Since vaccination with ISIV is highly associated with HI titers of 40 or higher against sH1N1 v, the interaction between these two factors was taken into consideration in the multivariate analysis. The results showed that receiving a vaccination along with having an HI titer of 40 or higher resulted in a significantly lower likelihood of pH1N1 infection during the pre-epidemic period (adjusted OR 0.33, 95% CI 0.12–0.90). This protection effect was not observed during the epidemic period; instead, having been previously infected by pH1N1 and having a baseline titer of 20 or higher showed a significantly lower likelihood of pH1N1 infection (adjusted OR 0.06, 95% CI 0.02–0.16).

In order to understand whether children mediate a higher risk of household transmission of influenza virus, we further characterized the factors associated with seroconversion of pH1N1 in the different age groups; multivariate analysis was performed separately for the schoolchildren aged 5–18 years and the adults aged >19 years. Subjects aged >60 years were combined with the adult age group because of the small sample size. The results from the analysis in the adult age group are shown in Table 4; the results for the schoolchildren age group are not represented in a table as they were found to be similar to the results from the overall cohort. Having a baseline HI titer of 40 or higher for sH1N1 v was still strongly associated with a lower likelihood of pH1N1 infection during the pre-epidemic period in the adult age group (adjusted OR 0.32, 95% CI 0.11–0.91). This effect again was not observed during the epidemic period, and the conditions instead were having a previous infection and a baseline titer of 20 or higher to pH1N1 (adjusted OR 0.02, 95% CI 0.00–0.18). Interestingly, living with another adult household member with seroconversion (adjusted

Table 3
Multivariate analysis for factors associated with seroconversion (four-fold increase) during the pre-epidemic and epidemic periods for all subjects ($N = 305$[a])

Variable		Pre-epidemic period (April–June)				Epidemic period (September–October)			
		beta	SE	*p*-Value	OR (95% CI)	beta	SE	*p*-Value	OR (95% CI)
Age, years	>18 vs. 5–18	0.68	0.44	0.12	1.96 (0.83–4.62)	0.077	0.33	0.81	1.08 (0.57–2.04)
Gender	Male vs. female	0.35	0.40	0.38	1.42 (0.65–3.08)	−0.313	0.27	0.25	0.73 (0.43–1.24)
Area of residence	Urban vs. rural	−0.47	0.36	0.19	0.62 (0.31–1.27)	0.544	0.28	0.05	1.72 (1.00–2.97)
Vaccination 2007–08	Yes vs. no	0.55	0.48	0.25	1.73 (0.68–4.39)	0.303	0.38	0.43	1.35 (0.64–2.87)
Vaccination 2008–09 + baseline sH1N1v[c] HI titer	Non-vaccinated, HI <40[b]	Referent group			1.00	Referent group			1.00
	Non-vaccinated, HI ≥40	−0.86	0.69	0.21	0.42 (0.11–1.63)	−0.456	0.49	0.35	0.63 (0.24–1.66)
	Vaccinated, HI <40	0.005	0.47	0.99	1.01 (0.40–2.53)	−0.292	0.42	0.48	0.75 (0.33–1.69)
	Vaccinated, HI ≥40	−1.12	0.52	0.03	0.33 (0.12–0.90)	−0.356	0.52	0.49	0.70 (0.25–1.94)
Baseline pandemic H1N1 HI titer (2nd sampling)	≥20 vs. <20	–[e]	–[e]	–[e]	–[e]	−2.829	0.50	<0.0001	0.06 (0.02–0.16)
Other household members with seroconversion[d]	Yes vs. no	3.25	0.50	<0.0001	25.76 (9.64–68.81)	0.407	0.38	0.28	1.50 (0.71–3.17)

SE, standard error of beta estimate; OR, odds ratio; CI, confidence interval.
[a] One observation missing for vaccination in 2007–08.
[b] HI titer refers to the antibody titers measured by a hemagglutination inhibition (HI) assay.
[c] sH1N1 v refers to the seasonal H1N1 virus vaccine strain (A/Brisbane/59/2007) used for HI assay.
[d] Other household members with seroconversion: at least one other household member with seroconversion vs. no one else in the household with seroconversion.
[e] Not included in the multivariate model because of insufficient data.

Table 4
Multivariate analysis for factors associated with seroconversion (four-fold increase) during the pre-epidemic and epidemic periods for adult subjects ($N = 156$[a])

Variable		Pre-epidemic period (April–June)				Epidemic period (September–October)			
		beta	SE	*p*-Value	OR (95% CI)	beta	SE	*p*-Value	OR (95% CI)
Gender	Male vs. female	0.76	0.45	0.10	2.13 (0.88–5.20)	0.09	0.40	0.81	1.10 (0.51–2.38)
Area of residence	Urban vs. rural	−0.34	0.44	0.44	0.71 (0.30–1.69)	0.40	0.38	0.29	1.50 (0.71–3.17)
Vaccination 2007–08	Yes vs. no	−0.88	1.16	0.45	0.42 (0.04–4.05)	−1.66	1.08	0.12	0.19 (0.02–1.58)
Vaccination 2008–09	Yes vs. no	0.13	0.74	0.86	1.14 (0.27–4.82)	0.28	0.88	0.75	1.33 (0.24–7.48)
Baseline sH1N1v HI titer[b]	≥40 vs. <40	−1.15	0.54	0.03	0.32 (0.11–0.91)	−0.04	0.65	0.95	0.96 (0.27–3.46)
Seroconversion of sH1N1v or sH1N1w[c]	Yes vs. no	–[f]	–[f]	–[f]	–[f]	−0.69	0.48	0.15	0.50 (0.20–1.29)
Baseline pandemic H1N1 HI titer (2nd sampling)	≥20 vs. <20	–[f]	–[f]	–[f]	–[f]	−3.85	1.09	<0.001	0.02 (0.00–0.18)
Other household adults (age >18 years) with seroconversion[d]	Yes vs. no	2.20	0.66	0.001	9.04 (2.46–33.22)	0.57	0.49	0.24	1.77 (0.68–4.60)
Having underlying diseases[e]	Yes vs. no	1.38	0.56	0.01	3.97 (1.34–11.80)	1.38	0.66	0.04	3.97 (1.08–14.55)

SE, standard error of beta estimate; OR, odds ratio; CI, confidence interval.
[a] Nine observations missing for underlying diseases.
[b] HI titer refers to the antibody titers measured by a hemagglutination inhibition (HI) assay.
[c] Seroconversion of sH1N1 v or sH1N1w: having four-fold increase in HI titer against either the seasonal H1N1 virus vaccine strain (A/Brisbane/59/2007) or the seasonal H1N1 wild-type stain (A/Taiwan/606/2008) from the first and second follow-up samples.
[d] Other household members with seroconversion: at least one other household member aged older than 18 years with seroconversion vs. no one else in the household with seroconversion.
[e] Underlying diseases refers to having one of the following three diseases: cardiovascular disease, hypertension, or diabetes mellitus.
[f] Not included in the multivariate model because of insufficient data.

OR 9.04, 95% CI 2.46–33.22), or having underlying diseases (adjusted OR 3.97, 95% CI 1.34–11.80), resulted in a higher risk of infection by pH1N1 in the pre-epidemic period. Meanwhile, having underlying diseases also resulted in a higher likelihood of infection in the adult age group during the epidemic period (adjusted OR 3.91, 95% CI 1.08–14.55).

Different methods were also compared in this study. In order to understand whether children mediate a higher risk of household transmission of influenza virus, we further classified other household members with seroconversion into groups of school-children (<18 years old) and adults (>18 years old). Results showed that having children with seroconversion in the household

Table 5
Multivariate analysis for factors of household children associated with seroconversion (four-fold increase) during the pre-epidemic period for all subjects ($N = 305$[a])

Variable		beta	SE	*p*-Value	OR (95% CI)
Age, years	>18 vs. 5–18	0.14	0.43	0.74	1.15 (0.50–2.67)
Gender	Male vs. female	0.23	0.32	0.47	1.26 (0.67–2.36)
Area of residence	Urban vs. rural	−0.46	0.34	0.18	0.63 (0.32–1.24)
Vaccination 2007–08	Yes vs. no	0.03	0.59	0.96	1.03 (0.32–3.30)
Vaccination 2008–09	Yes vs. no	0.27	0.72	0.71	1.31 (0.32–5.37)
Baseline A/H1N1 vaccine strain HI titer[b]	≥40 vs. <40	−1.02	0.50	0.04	0.36 (0.14–0.95)
Other household children (age ≤18 years) with seroconversion	Yes vs. no	3.24	0.49	<0.0001	25.58 (9.77–66.96)

SE, standard error of beta estimate; OR, odds ratio; CI, confidence interval.
[a] 1 observation missing for vaccination 2007–2008.
[b] HI titer refers to the antibody titers measured by a hemagglutination inhibition (HI) assay.

Table 6
Multivariate analysis for factors of household adults associated with seroconversion (four-fold increase) during the pre-epidemic period for all subjects (N = 305[a])

Variable		beta	SE	p-Value	OR (95% CI)
Age, years	>18 vs. 5–18	0.81	0.38	0.03	2.24 (1.07–4.68)
Gender	Male vs. female	0.26	0.38	0.50	1.30 (0.61–2.75)
Area of residence	Urban vs. rural	−0.53	0.40	0.18	0.59 (0.27–1.28)
Vaccination 2007–08	Yes vs. no	0.88	0.38	0.02	2.40 (1.15–5.02)
Vaccination 2008–09	Yes vs. no	−0.33	0.37	0.37	0.72 (0.35–1.49)
Baseline A/H1N1 vaccine strain HI titer[b]	≥40 vs. <40	−0.85	0.31	0.006	0.43 (0.24–0.78)
Other household adults (age >18 years) with seroconversion	Yes vs. no	2.67	0.49	<0.0001	14.41 (5.52–37.66)

SE, standard error of beta estimate; OR, odds ratio; CI, confidence interval.
[a] 1 observation missing for vaccination 2007–2008.
[b] HI titer refers to the antibody titers measured by a hemagglutination inhibition (HI) assay.

Table 7
Multivariate analysis for factors associated with seroconversion (four-fold increase) during the epidemic period

Variable		Total (N = 305)[a]				Age >18 years (N = 156)[b]			
		beta	SE	p-Value	OR (95% CI)	beta	SE	p-Value	OR (95% CI)
Age, years	>18 vs. 5–18	0.03	0.31	0.92	1.03 (0.56–1.90)	–	–	–	–
Gender	Male vs. female	−0.40	0.26	0.11	0.67 (0.41–1.10)	−0.26	0.38	0.49	0.77 (0.37–1.62)
Area of residence	Urban vs. rural	0.65	0.27	0.02	1.91 (1.12–3.27)	0.45	0.34	0.19	1.56 (0.81–3.03)
Vaccination 2007–08	Yes vs. no	0.40	0.38	0.30	1.49 (0.71–3.12)	−0.59	0.87	0.50	0.55 (0.10–3.02)
Vaccination 2008–09	Yes vs. no	−0.15	0.35	0.67	0.86 (0.44–1.70)	0.29	0.82	0.72	1.34 (0.27–6.63)
Baseline A/H1N1 vaccine strain HI titer[c]	≥40 vs. <40	−0.14	0.36	0.69	0.87 (0.43–1.74)	0.14	0.62	0.83	1.15 (0.34–3.88)
Four-fold increase of seasonal H1N1 (vaccine or wild-type strain)	Yes vs. no	−0.80	0.30	0.008	0.45 (0.25–0.81)	−1.04	0.43	0.02	0.35 (0.15–0.81)
Other household members with seroconversion	Yes vs. no	0.74	0.37	0.05	2.10 (1.01–4.33)	–	–	–	–
Other household adults (age >18 years) with seroconversion	Yes vs. no	–	–	–	–	1.17	0.45	0.01	3.23 (1.33–7.85)

SE, standard error of beta estimate; OR, odds ratio; CI, confidence interval.
[a] 1 observation missing for vaccination 2007–2008.
[b] 9 observations missing for underlying diseases.
[c] HI titer refers to the antibody titers measured by a hemagglutination inhibition (HI) assay.

presented a 25.6-fold higher risk of infection by pH1N1 (95% CI 9.77–66.96), compared to adults with seroconversion in the household (adjusted OR 14.41, 95% CI 5.52–37.66), with statistical significance in both models (Tables 5 and 6). Moreover, having a four-fold increase in the HI titer against sH1N1 v or sH1N1w during the pre-epidemic period resulted in a lower likelihood of infection by pH1N1 during the epidemic period, with OR of 0.45 (95% CI 0.25–0.81) (Table 7).

4. Discussion

To the best of our knowledge, this is the first cohort study designed to distinguish the effect of receiving an ISIV on protection against pH1N1 infection using serological assays. Our study shows that the risk of infection by pH1N1 was 0.33-fold lower (95% CI 0.12–0.90) among vaccine recipients if the baseline HI titer was 40 or higher, compared to those without vaccinations and a baseline titer below 40, during the pre-epidemic period in April–June. However, the protection effect of the baseline HI titer by vaccination was not significant during the epidemic period due to the waning of antibody induced by vaccination after 6 months. Instead, the risk of infection by pH1N1 among those who had previously been infected by pH1N1 (pH1N1 baseline titer of 20 or higher during the second sampling period) was 16.7-fold lower than in those who had not been previously infected (95% CI 0.02–0.16). Having another household member with seroconversion or having underlying diseases were shown to be important risk factors for infection by pH1N1, similar to the results of previous studies.[13,14]

The assessment of the potential risk and protection factors for influenza virus infection could be affected by the ascertainment bias of case status. Early data based on a frequency-matched case–control study from Mexico revealed some protection against pH1N1 infection, particularly severe forms of the disease, among ISIV recipients, although it was unclear whether these observations were affected by biases in case ascertainment.[7,9] Classifying the case status by the appearance of respiratory symptoms could lead to misclassification because of the substantial proportion of subclinical infections.[15–17] The detection of virus in nasopharyngeal and oropharyngeal specimens from those with symptoms resembling infection could be affected by the sensitivity and specificity of the polymerase chain reaction (PCR) assay.[18,19] Using serological cohorts is one of the best ways to differentiate infection status associated with risk and protection factors. The study conducted by Chen et al. failed to demonstrate a protection effect of receiving ISIV against pH1N1 infection, without measuring the HI titer against sH1N1 v and sH1N1w.[13] Also, rather surprisingly, the Canadian sentinel study showed that receiving an ISIV in the previous season appeared to increase the risk of pH1N1 illness, even after adjustment for co-morbidities, age, and geography.[10] Again, their study failed to measure the HI titer against different strains of H1N1. The causal-inference relationship was established in our study through the prospective cohort design, by measuring the kinetic changes of antibody titer against different H1N1 strains, which emphasizes the valuable information obtained from the serum bank.

Previous studies have suggested that household transmission with children presents a higher risk of pH1N1 infection.[20,21] Interestingly, by using other household members older than 18 years of age with seroconversion as a co-variable (Tables 5 and 6), significant factors included having an sH1N1 v baseline titer of 40 or higher and being older than 18 years and the recipient of the 2007–08 seasonal vaccine during the pre-epidemic period. Having a four-fold increase in the HI titer against sH1N1 v or sH1N1w during the pre-epidemic period also resulted in a lower likelihood of pH1N1 infection during the epidemic period, as previously published[22] (Table 7). It is tempting to speculate on the

significance of our findings on the partial cross-protection effect of ISIV or the infection by sH1N1w in terms of the disease severity and death rate of S-OIV as compared with seasonal human H1N1 influenza virus. It was initially feared that the current S-OIV would be much more lethal than seasonal H1N1 influenza. In fact, although S-OIV isolates were consistently found to replicate better in the lung tissue of animal models,[23–25] the potential pathogenic nature of S-OIV is not supported by the currently available data. The data suggest that there are large numbers of suspected infections, but a disproportionately low number of deaths associated with S-OIV in the USA. We propose that the divergence in disease severity observed in most animal studies and found in the human population could be due to the contribution of a pre-existing T-cell-mediated immunity that lessens disease severity, as suggested in previous studies.[26–29]

In conclusion, our study demonstrated that the risk of pH1N1 infection was associated with seroconversion in another family member, especially children, and the presence of underlying diseases. The target population for the pandemic influenza vaccine should be either schoolchildren, which would stop transmission, or those in the adult age group with underlying diseases. Focusing on these groups would reduce the morbidity and mortality in the general population. At the same time, our study confirmed that partial protection from receiving an ISIV occurred only if there was an HI titer of 40 or higher after vaccination. Although the household study design might limit the applicability of our study to the general population, the results obtained from this current study may assist in the implementation of intervention and control strategies in future influenza virus pandemics.

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