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MAJOR ARTICLE

Protective Efficacy of Seasonal Influenza Vaccination against Seasonal and Pandemic Influenza Virus Infection during 2009 in Hong Kong

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(See the editorial commentary by Glezen, on pages 1380–1382.)

Background. The relationship between seasonal influenza vaccine and susceptibility to 2009 pandemic A/H1N1 virus infection is not fully understood.

Methods. One child 6–15 years of age from each of 119 households was randomized to receive 1 dose of inactivated trivalent seasonal influenza vaccine (TIV) or saline placebo in November 2008. Serum samples were collected from study subjects and their household contacts before and 1 month after vaccination (December 2008), after winter (April 2009) and summer influenza (September–October 2009) seasons. Seasonal and pandemic influenza were confirmed by serum hemagglutinination inhibition, viral neutralization titers, and reverse-transcription polymerase chain reaction performed on nasal and throat swab samples collected during illness episodes.

Results. TIV recipients had lower rates of serologically confirmed seasonal A/H1N1 infection (TIV group, 8%; placebo group, 21%; $P = .10$) and A/H3N2 infection (7% vs 12%; $P = .49$), but higher rates of pandemic A/H1N1 infection (32% vs 17%; $P = .09$). In multivariable analysis, those infected with seasonal influenza A during the study had a lower risk of laboratory-confirmed pandemic A/H1N1 infection (adjusted odds ratio [OR], 0.35; 95% confidence interval [CI], 0.14–0.87), and receipt of seasonal TIV was unassociated with risk of pandemic A/H1N1 infection (adjusted OR, 1.11; 95% CI, 0.54–2.26).

Conclusions. TIV protected against strain-matched infection in children. Seasonal influenza infection appeared to confer cross-protection against pandemic influenza. Whether prior seasonal influenza vaccination affects the risk of infection with the pandemic strain requires additional study.

Clinical trials registration. ClinicalTrials.gov number NCT00792051.

Trivalent inactivated influenza vaccine (TIV) is effective in preventing infection and illness associated with influenza A and B viruses in children during seasons when the vaccine components closely match circulating strains [1]. On the basis of evidence from ecological studies [2, 3], intervention trials [4–10], and simulation models [11–14], some health authorities have recommended vaccination of school-age children against seasonal and pandemic influenza, not only to directly protect those children, but also to confer indirect protection on the general community by reducing transmission [15, 16]. Approximately one-third of transmission is thought to occur within households [17, 18], and children are more likely than adults to transmit infection to household contacts [19, 20]. However, there have been few detailed individual-based studies of the indirect benefits to household contacts of vaccinating children [9, 10]. We therefore designed a randomized controlled trial to assess whether vaccinating children against seasonal influenza protects their household contacts. We began a pilot study of 120 households in 2008–2009, to be followed by a main study of 800 households in 2009–2010.

The pandemic (H1N1) 2009 virus emerged in North America and rapidly spread worldwide [21]. There has

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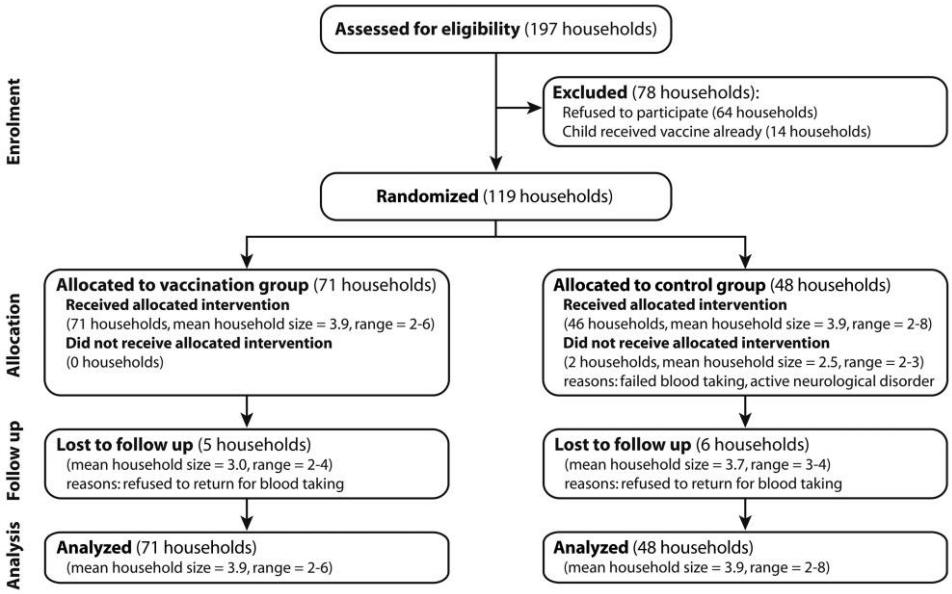

Figure 1. Flow of participants through the study.

been intense interest in the effect of seasonal influenza vaccination on the risk of pandemic influenza infection, following results from a Canadian study that suggested that seasonal vaccine was associated with increased risk of pandemic influenza [22]. Other studies have suggested that seasonal vaccine may confer no protection [23–25] or partial protection against the pandemic virus [26–28]. Taking advantage of the pilot study that was implemented during 2008–2009, and which thus cov-

Table 1. Baseline Characteristics of Children who Received Trivalent Influenza Vaccine (TIV) or Placebo and their Household Contacts

Group, characteristic	TIV group	Placebo group
Study subjects		
No. of study subjects	71	48
Male sex	41 (58)	23 (48)
Age group		
6–8 years	20 (28)	16 (33)
9–11 years	42 (59)	28 (58)
12–15 years	9 (13)	4 (8)
Received influenza vaccination for 2007–2008 season	8 (11)	7 (15)
Household contacts		
No. of household contacts	189	123
Male sex	86 (46)	52 (42)
Age group		
<15 years	47 (25)	26 (21)
16–45 years	94 (50)	69 (56)
>45 years	48 (25)	28 (23)
Received influenza vaccination for 2007–2008 season	24 (13)	12 (10)
Received influenza vaccination for 2008–2009 season	9 (5)	5 (4)
Households		
No. of households	71	48
No. of individuals per household, mean ± SD	3.7 ± 0.9	3.6 ± 1.0
Size of residence, mean ± SD, m^2	48 ± 22.8	49 ± 23.6

NOTE. Data are no. (%) of study subjects, household contacts, or households, unless otherwise indicated. SD, standard deviation.

The figure is available in its entirety in the online edition of *Clinical Infectious Diseases*.

Figure 2. Time line of vaccination and serum sample collection versus local surveillance data on influenza virus activity during the study period. Weekly proportions of positive influenza isolates are reported by Queen Mary Hospital, Hong Kong.

ered the first wave of the pandemic, we investigated the effect of vaccination against seasonal influenza on the risk of pandemic influenza infection. The results for the original research question concerning direct and indirect benefits of vaccinating children in the household setting will be separately reported on completion of the main phase.

PATIENTS AND METHODS

Recruitment and follow-up of participants. Invitation letters were distributed via schools located within 3 km of our study clinic in Kowloon and to the families of members of a local birth cohort [29]. Households who expressed an interest in the study were assessed for their eligibility to participate and were invited to our study clinic. Eligible households included at least 1 child aged 6–15 years who did not have any contraindications against injection of inactivated influenza vaccine, including allergy or hypersensitivity to eggs or other substances contained in the vaccine. Children who were prescribed immunosuppressive treatment or were otherwise immunocompromised were excluded.

One eligible child from each household was randomized to receive either a single dose of TIV (0.5 mL Vaxigrip; Sanofi Pasteur) or 0.5 mL of saline solution intramuscularly. The 2008–2009 TIV used in our study included the strains A/Brisbane/59/2007(H1N1)-like, A/Brisbane/10/2007(H3N2)-like, and B/Florida/4/2006. We hypothesized that vaccine-naive children aged 6–8 years in Hong Kong may be more influenza-experienced than those in Western temperate climates [30] and thus might only require 1 vaccine dose, because a prior influenza infection could have already primed their immune system.

Serum specimens were collected from study subjects at baseline immediately before vaccination (November–December 2008), 1 month after vaccination, after the winter influenza season ("mid-season"; April 2009) and at the end of the follow-up period (August–October 2009). Serum specimens were also collected from all household contacts at baseline, at mid-season, and post-season.

All subjects and household contacts were instructed to record the presence of any systemic and respiratory symptoms in a symptom diary daily throughout the study. Telephone calls were made monthly outside influenza seasons and fortnightly within season to monitor for any acute respiratory illnesses. Households were also reminded to report any acute respiratory illnesses to the study hotline as soon as possible after illness onset. Home visits were triggered by the presence of any 2 symptoms

Table 2. Antibody Titers against Seasonal A/H1N1, Seasonal A/H3N2, and Pandemic A/H1N1 Virus before and 1 Month after Receipt of Trivalent Inactivated Vaccine (TIV) or Placebo

Virus, antibody	TIV group (n = 71)	Placebo group (n = 48)	P[a]
Seasonal A/H1N1			
Antibody titer ≥1:40 before vaccination, % of subjects	49	59	.44
Antibody titer ≥1:40 one month after vaccination, % of subjects	93	68	<.01
Geometric mean titer increase from before to 1 month after vaccination	45	1.8	<.01
Seasonal A/H3N2			
Antibody titer ≥1:40 before vaccination, % of subjects	45	55	.39
Antibody titer ≥1:40 1 month after vaccination, % of subjects	97	61	<.01
Geometric mean titer increase from before to 1 month after vaccination	46.2	1.4	<.01
Seasonal B			
Antibody titer ≥1:40 before vaccination % of subjects	86	93	.32
Antibody titer ≥1:40 one month after vaccination % of subjects	99	91	.15
Geometric mean titer increase from before to 1 month after vaccination	7.8	1.1	<.01
Pandemic A/H1N1			
Antibody titer ≥1:40 before vaccination % of subjects	0	0	>.99
Antibody titer ≥1:40 1 month after vaccination % of subjects	0	0	>.99
Geometric mean titer increase from before to 1 month after vaccination	1.3	1.1	.02

NOTE. Antibody titers to the vaccine strains A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006 were measured by hemagglutination inhibition, and antibody responses to A/California/04/2009 (H1N1) were measured by viral neutralization.

[a] P values were calculated by χ^2 tests and Wilcoxon signed-rank tests.

The figure is available in its entirety in the online edition of *Clinical Infectious Diseases*.

Figure 3. Antibody responses against seasonal and pandemic influenza viruses before (*gray circles*) and 1 month after (*black circles*) receipt of inactivated trivalent seasonal influenza vaccine (TIV) or placebo. Medians values and interquartile ranges are indicated by tick marks and vertical lines, and *P* values for comparisons between groups are displayed.

or signs of fever ⩾37.8°C, chills, headache, sore throat, cough, presence of phlegm, coryza, or myalgia in any household member. During home visits, nasal and throat swab samples were collected from all household members regardless of illness. Home visits were repeated at 3-day intervals until acute illnesses resolved. Households were compensated with supermarket vouchers (or book tokens for children) worth US$65 for enrolment in the study, plus vouchers of US$13 for each serum specimen provided and US$6.5 for each home visit.

Ethics. All subjects aged ⩾18 years gave written informed consent. Proxy written consent from parents or legal guardians was obtained for subjects aged ⩽17 years of age, with additional written assent from those 8–17 years of age. The study protocol was approved by the Institutional Review Board of the University of Hong Kong.

Outcome measures. The primary outcome measure was influenza virus infection in study subjects and their household contacts indicated by a 4-fold or greater increase in antibody titer. Secondary outcome measures included (1) reverse-transcription polymerase chain reaction (RT-PCR)–confirmed influenza, (2) acute respiratory illness (ARI) as determined by self-reported symptoms (at least any 2 of temperature ⩾37.8°C, headache, sore throat, cough, presence of phlegm, coryza, and myalgia), and (3) influenza-like illness (ILI), defined as temperature ⩾37.8°C plus cough or sore throat [19]. Acute reactions were recorded by parents for 4 days after vaccination.

Sample size justification. This study was designed as a pilot study for a larger trial and thus was not powered to detect indirect benefits. The sample size of 120 families was chosen to allow us to estimate attack rates in study subjects of ~20% to a precision of ±7%, and to confirm the logistical arrangements and the feasibility and acceptability of the study protocol.

Randomization. Randomization lists were prepared by a biostatistician (B.J.C.). Eligible study participants were randomly allocated to the TIV group or placebo group in the ratio 3:2 using a random number generator (R software). A block-randomization sequence was generated with randomly permuted block sizes of 5, 10, and 15. More households were allocated to the TIV group to enhance the acceptability of the study to participants.

Blinding. Blinding of households and study nurses was achieved by identical repackaging of the TIV and placebo into numbered syringes by a trained nurse not involved in vaccine administration. A research assistant who had no access to the randomization list allocated unique numbers to participating households based on their order of attendance, and these were subsequently matched to vaccine packages. Allocation of TIV or placebo was concealed from participating households, study nurses, and laboratory staff and was only revealed to investigators after completion of follow-up.

Laboratory methods. All serum specimens were tested for antibody responses to the vaccine strains A/Brisbane/59/2007(H1N1), A/Brisbane/10/2007(H3N2), and B/Florida/4/2006-like (Yamagata-lineage) by hemagglutination inhibition (HAI) and for antibody responses to A/California/04/2009(H1N1) by viral microneutralization (VN) using standard methods. We chose to use VN tests rather than HAI tests for pandemic A/H1N1 after preliminary studies that showed that VN was more sensitive than HAI for the detection of antibody responses in pandemic A/H1N1 infections [31]. The serum samples were tested in serial doubling dilutions from an initial dilution of 1/10. Nose and throat swab samples collected during home visits were tested by RT-PCR for influenza A and B viruses [19]. Additional technical details of the laboratory procedures are given in the Appendix, which appears only in the online version of the journal.

Statistical analysis. Rates of adverse reaction events experienced by subjects within 4 days after administration of TIV or placebo were compared with use of Fisher's exact tests. To assess vaccine immunogenicity, pre- and post-vaccination titers and ratios of pre- to post-vaccination titers were compared using Wilcoxon signed-rank tests. The proportions of study subjects with antibody titer ⩾1:40 post-vaccination were compared between the intervention group and the control group using χ^2 tests.

Post-season antibody titers were compared with mid-season titers, which were in turn compared with post-vaccination antibody titers (or baseline titers in household contacts), to determine serologic evidence of infection during the summer and winter influenza seasons, respectively. Rates of influenza infection determined by serological testing, RT-PCR, and clinical illness were compared by χ^2 tests and Fisher's exact tests, and 95% confidence intervals (CIs) were obtained using the exact

The figure is available in its entirety in the online edition of *Clinical Infectious Diseases*.

Figure 4. Reported frequencies of mild and moderate adverse reactions for 4 days after receipt of seasonal trivalent influenza vaccine (TIV) or placebo. Mild reactions were those that were easily tolerated and did not interfere with usual activities, moderate reactions were those that interfered with usual activities, and severe reactions were those that disabled usual activities.

Table 3. Attack Rates of Laboratory-Confirmed Influenza Infections and Acute Respiratory Illnesses in Children who Received Trivalent Inactivated Vaccine (TIV) or Placebo and their Household Contacts

Variable	TIV group	Placebo group	*P*
Study subjects			
No. of study subjects	71	48	
Serologically confirmed infection[a]			
Seasonal A/H1N1	0.08 (0.02–0.15)	0.21 (0.09–0.32)	.10
Seasonal A/H3N2	0.07 (0.01–0.13)	0.12 (0.03–0.22)	.49
Pandemic A/H1N1	0.32 (0.22–0.43)	0.17 (0.06–0.27)	.09
Seasonal B	0.03 (0.00–0.07)	0.08 (0.01–0.16)	.36
RT-PCR–confirmed infection			
Seasonal A/H1N1	0.03 (0.00–0.07)	0.04 (0.00–0.10)	.91
Seasonal A/H3N2	0.01 (0.00–0.04)	0.02 (0.00–0.06)	.66
Pandemic A/H1N1	0.03 (0.00–0.07)	0.00 (0.00–0.07)	.66
Seasonal B	0.00 (0.00–0.05)	0.02 (0.00–0.06)	.84
ILI[b]	0.35 (0.24–0.46)	0.38 (0.24–0.51)	.95
ARI[c]	0.66 (0.55–0.77)	0.67 (0.53–0.80)	.89
Household contacts			
No. of household contacts	189	123	
Serologically confirmed infection[a]			
Seasonal A/H1N1	0.13 (0.08–0.17)	0.14 (0.08–0.20)	.91
Seasonal A/H3N2	0.21 (0.15–0.26)	0.16 (0.10–0.23)	.41
Pandemic A/H1N1	0.17 (0.12–0.23)	0.14 (0.08–0.20)	.48
Seasonal B	0.06 (0.02–0.09)	0.10 (0.05–0.15)	.28
RT-PCR–confirmed infection			
Seasonal A/H1N1	0.01 (0.00–0.03)	0.01 (0.00–0.02)	.71
Seasonal A/H3N2	0.02 (0.00–0.04)	0.01 (0.00–0.02)	.66
Pandemic A/H1N1	0.03 (0.00–0.05)	0.01 (0.00–0.02)	.47
Seasonal B	0.00 (0.00–0.02)	0.00 (0.00–0.03)	>.99
ILI[b]	0.16 (0.11–0.22)	0.11 (0.06–0.17)	.29
ARI[c]	0.42 (0.35–0.49)	0.39 (0.30–0.48)	.64

NOTE. Data are percentage of study subjects or household contacts (95% confidence interval), unless otherwise indicated. ARI, acute respiratory infection; ILI, influenza-like illness; RT-PCR, reverse-transcription polymerase chain reaction.

[a] Winter infection was confirmed by a 4-fold increase in antibody titers from after vaccination to mid-season; summer infection was confirmed by a 4-fold increase in antibody titers from mid-season to post-season. Results displayed reflect either winter or summer infection in the aggregate (see Table 5 for winter and summer results separately).

[b] ILI was defined as temperature ≥37.8°C plus cough or sore throat.

[c] ARI was defined at least any 2 of fever ≥37.8°C, chills, headache, sore throat, cough, presence of phlegm, nasal congestion, runny nose, and muscle or joint pain.

binomial method or the Wald approximation, where appropriate. In an analysis that was not specified in our study protocol (because the study was designed before the pandemic), multivariable logistic regression models were used to study risk of laboratory-confirmed pandemic influenza infection adjusting for age, sex, receipt of TIV, laboratory-confirmed seasonal influenza infection, and date of study completion. Infection with a specific influenza strain can lead to increases in antibody titers to other heterologous strains (ie, cross-reactions) [32], and we identified 15 individuals with 4-fold or greater increases in antibody titers to >1 influenza strain during either the winter or summer seasons. We adjusted for cross-reactions by classifying the most likely virus infection during a season based on RT-PCR confirmation, where available, or otherwise by assuming that the infecting virus was that for which the geometric antibody titer increase was greatest.

All analyses of study outcomes were performed under the principle of intention-to-treat [33]. We used multiple imputation with 10 imputations to account for a small amount of missing data [34]. Statistical analyses were conducted in R, version 2.8.1 (R Development Core Team).

Table 4. Rates of Laboratory-Confirmed Influenza Infections and Acute Respiratory Illnesses in Study Subjects and Household Contacts, Stratified into Winter 2008–2009 and Summer 2009 Influenza Seasons

This table is available in its entirety in the online version of the journal.

Table 5. Rate of Influenza-Like Illness (ILI) in Study Subjects who received Trivalent Inactivated Vaccine (TIV) or Placebo and had Serologically Confirmed Influenza

This table is available in its entirety in the online version of the journal.

RESULTS

Invitation letters were sent to a convenience sample of 20 primary and 5 secondary schools. In 3 schools that agreed to participate, letters were distributed to the parents of 2190 children, and 54 households were enrolled. Fifteen hundred invitations were sent to households of children who are members of a local birth cohort, and 51 households were enrolled. A further 14 households were enrolled through personal referral.

Figure 1 shows the flow of participating households throughout the study. A total of 119 households were enrolled and randomized. Subjects and household contacts in the TIV and control groups had similar baseline characteristics (Table 1). Children from 2 households did not receive the intervention and withdrew from the study: 1 child with a history of epileptic seizures was assessed by the study nurse to be contraindicated against giving blood specimens and receiving vaccination at the time of presentation, and blood specimens could not be obtained from another child. The households of 11 of 117 children who received the intervention did not complete the study. Following the principle of intention-to-treat, we included all 119 households in the primary analyses. Figure 2 shows the timeline of vaccination and serum sample collection versus local surveillance data on influenza activity during the study period [35].

A single dose of TIV led to substantial and statistically significant increases in antibody titers to the seasonal strains among study subjects (Table 2; Figure 3). Children who received TIV had statistically significant but limited geometric mean increases in antibody titers to pandemic A/H1N1 virus following receipt of TIV, and all post-vaccination titers were below 1:40. No serious adverse reactions were observed, and pain at the injection site was the only adverse event for which there was a statistically significant difference between arms (Figure 4).

Children who received TIV had lower rates of serologically confirmed seasonal influenza infection, compared with the placebo group, although reductions were not statistically significant (Table 3). By the end of the study period, 8% and 7% of the children in the TIV group had serologically confirmed infection with seasonal influenza A/H1N1 and A/H3N2, compared with 21% and 12%, respectively, in the control group. The attack rates stratified into winter and summer seasons are reported in Table 4. Children who received TIV had a non–statistically significant higher rate of pandemic A/H1N1 infection during the summer season, compared with children who received placebo ($P = .09$) (Table 3). After adjusting for potential cross-reactions, we estimated that 31% of children who received TIV were infected with pandemic A/H1N1, compared with 12% of children who received placebo ($P = .04$). No differences were observed in the rates of RT-PCR–confirmed influenza, ILI, or acute respiratory illness during the study (Table 3). Among those who had serologically confirmed pandemic A/H1N1 virus infection, no difference in ILI rates was observed between study subjects who received TIV and those who received placebo (Table 5).

We did not identify any statistically significant differences in attack rates of seasonal and pandemic influenza infection or clinical influenza among the household contacts of children who received TIV or placebo, although our study had limited power to detect such differences (Table 3). Results were similar when stratified by winter and summer seasons (Table 4).

Of 91 children and their household contacts who had laboratory-confirmed (by serological testing or RT-PCR) seasonal influenza infection during the follow up period, 7 (8%; 95%

Table 6. Factors Associated with the Risk of Laboratory-Confirmed Pandemic Influenza

Risk factor	No. of study subjects	Adjusted odds ratio[a] (95% confidence interval)
Age <16 years	192	6.60 (2.17–20.13)
Age 16–45 years	163	2.53 (0.80–7.99)
Age >45 years	76	1.00
Female sex	229	1.00
Male sex	202	0.97 (0.55–1.70)
No laboratory-confirmed seasonal influenza A infection	277	1.00
Laboratory-confirmed seasonal influenza A infection	93	0.35 (0.14–0.87)
Did not receive seasonal influenza vaccine	271	1.00
Received seasonal influenza vaccine prior to 2008–2009 season	106	1.11 (0.54–2.26)
Completed study before 1 October 2009	221	1.00
Completed study between 1 October and 20 October 2009	156	2.77 (1.53–4.99)

[a] Adjusted for age, sex, laboratory-confirmed seasonal influenza A infection, receipt of TIV, and date of study completion.

Table 7. Factors Associated with the Risk of Laboratory-Confirmed (by Reverse-Transcription Polymerase Chain Reaction or Serological Testing) Pandemic Influenza, with Analysis Stratified by Vaccines and Household Contact Status

This table is available in its entirety in the online version of the journal.

CI, 3%-15%) had confirmed pandemic influenza infection by RT-PCR or serological testing. This was a significantly lower pandemic strain attack rate than that among the 276 subjects who did not have laboratory-confirmed seasonal influenza infection, of whom 56 (20%; 95% CI, 16%-26%) had laboratory-confirmed pandemic influenza infection ($P = .01$). In an adjusted model, individuals who had laboratory-confirmed seasonal influenza infection had a substantially and statistically significantly lower risk of pandemic influenza infection (Table 6). Receipt of TIV did not statistically significantly affect the risk of pandemic influenza infection in the adjusted model. Adjusted estimates were similar in a sub-analysis restricted to the children who received TIV or placebo although the protective effect of seasonal influenza infection was not statistically significant, although it did remain significant in analysis of their household contacts (Table 7). In a sensitivity analysis, results were similar when an 8-fold or greater increase in antibody titer was used to define infection (data not shown). Our sample size was insufficient to allow us to distinguish whether seasonal A/H1N1 or A/H3N2 infections were associated with greater cross-protection against pandemic influenza, and both were associated with a similar nonsignificant protective effect against pandemic A/H1N1 infection (Table 8). Among individuals infected with seasonal influenza between baseline and mid-season, few had antibody titers $\geqslant$1:40 against pandemic A/H1N1 virus at mid-season, although there was some evidence of cross-reactive antibody to pandemic A/H1N1 virus following seasonal A/H1N1 infection (Figure 5).

DISCUSSION

From January through September 2009, the predominant circulating strains of influenza in Hong Kong matched the vaccine strains in our study, except for antigenically drifted A/Perth/16/2009-like (H3N2) viruses, which circulated in the summer. Our results are consistent with the prior expectation that administration of TIV would be effective in preventing serologically confirmed seasonal influenza infection in school-age children (Table 3) [1]. Pandemic A/H1N1 was the predominant influenza strain in Hong Kong from mid-August 2009 onwards (Figure 2) [36]. During the study period, we estimated that 31% of the children who received TIV and 12% of the children who received placebo were infected with the pandemic virus ($P = .04$). Furthermore, subjects who had seasonal influenza infection during our study were found to have a significantly lower risk of subsequent infection with pandemic A/H1N1 virus (Table 4), although we could not distinguish whether greater protection was associated with seasonal A/H1N1 or A/H3N2 (Table 8).

Our results mirror those from an earlier trial conducted in the United Kingdom, which found that children who received influenza A vaccine had a lower risk of A/England infection in 1972 but later appeared to lack cross-protection against A/Port Chalmers in 1974, compared with other children who had received influenza B vaccine [37]. Similarly, in the 1957 Cleveland Family Study cohort, adults with laboratory-confirmed influenza during the period 1950–1957 had significantly lower rates of pandemic influenza A/H2N2 infection in the 1957–1958 pandemic [38]. Cross-protection against heterologous strains following infection has also been demonstrated in animal models for A/California/04/2009(H1N1) in guinea pigs [39] and other influenza viruses in pigs, chickens, mice, and cotton rats [40–45]. There are few data on the duration of cross-protective immunity following infection, although 1 study conducted in UK boarding schools found that prior influenza A/USSR(H1N1) infection in 1978 protected against infection and clinical illness from an antigenic drift variant A/England/83(H1N1) 5 years later [46, 47].

Although seasonal influenza infection does appear to confer cross-protection against pandemic H1N1, our results are consistent with those of other studies that have found little cross-reactive antibody response to pandemic A/H1N1 virus following seasonal influenza vaccination (Figure 3) [23, 48]. We found statistically significant cross-reactive antibody responses to pandemic influenza A/H1N1 following seasonal A/H1N1 infection, although few individuals had antibody titers $\geqslant$1:40 (Figure 5). The cross-protection observed in our study may be associated with mechanisms such as cell-mediated immunity [45, 49–53] or nonneutralizing antibodies via antibody dependent cell cytotoxicity [54, 55]. It is recognized that inactivated influenza vaccines are poor at inducing efficient $CD8^+$ T cell responses in humans [56]. However, natural seasonal influenza infection can elicit cross-reactive T cell responses against new virus subtypes [53], and the presence of cross-reactive cytotoxic T cells inversely relates to the amount of virus shedding in infected individuals [57]. Experimental studies in mice have suggested that, although natural infection with seasonal influenza virus

Table 8. Factors Associated with the Risk of Laboratory-Confirmed Pandemic Influenza, with Prior Seasonal Influenza Infection Stratified into Laboratory-Confirmed Influenza A/H1N1 and A/H3N2 Infection

This table is available in its entirety in the online version of the journal.

The figure is available in its entirety in the online edition of *Clinical Infectious Diseases*.

Figure 5. Antibody titers to pandemic A/H1N1 virus by viral neutralization after laboratory-confirmed seasonal influenza A infection during the winter 2008–2009 influenza season. Medians values and interquartile ranges are indicated by tick marks and vertical lines, and *P* values for comparisons between groups are displayed.

provides partial protection against the development of severe disease after challenge with a pathogenic H5N1 virus, prior vaccination with inactivated seasonal vaccine not only fails to elicit such cross-subtype protection but impairs the development of such T cell–mediated protection that arises from subsequent natural infection with seasonal influenza [58]. These findings are analogous to the observations in our study. We investigated the possibility of nonsterilizing cross-immunity by TIV against pandemic influenza, but we did not find any evidence for different ILI attack rates between study subjects who received TIV or placebo and had confirmed pandemic A/H1N1 infection (Table 6).

Our study has several limitations. First, our pilot study has a small sample size and, in particular, was underpowered to detect indirect benefits of vaccination. Second, although 40% of the participants had serologically confirmed influenza infections during our study, we only obtained RT-PCR confirmation of influenza infections in 15% because of a lack of timely identification of illnesses and possible under reporting. In other prospective cohort studies, 10%–16% of serologically confirmed infections were confirmed by RT-PCR [7,27] We had insufficient sample size to explore which seasonal strains conferred greater cross protection. In our main phase, during 2009–2010, we have increased the frequency and intensity of telephone follow-up to detect ARIs sooner, facilitate more home visits, and allow virologic confirmation of a greater proportion of infections. Finally, we may have failed to detect some seasonal influenza infections in vaccinees, because increases in antibodies associated with influenza infection might be obscured by higher post-vaccination titers, which may have decreased over time.

In conclusion, administration of TIV in children aged 6–15 years can protect against seasonal influenza infection, whereas our results show that seasonal influenza infection can confer cross-protection against pandemic A/H1N1 infection. Therefore, by protecting against strain-matched seasonal infection, administration of seasonal TIV might lead to increased vulnerability to antigenically different influenza strains [49]. Alternative vaccines, such as live attenuated vaccines [59, 60] or adjuvanted vaccines [61, 62], may confer greater cross-protection against heterologous strains and avoid this potential disadvantage of TIV.

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