MAJOR ARTICLE

Protective efficacy against pandemic influenza of seasonal influenza vaccination in children in Hong Kong: a randomized controlled trial

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Seasonal influenza vaccination administered towards the end of the first wave of the pandemic appeared to provide school-age children with moderate protection against infection with the 2009 pandemic influenza A(H1N1) virus.

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ABSTRACT

Background: The efficacy of seasonal influenza vaccination against 2009 pandemic influenza A(H1N1) remains unclear.

Methods: One child aged 6-17 years in each of 796 households was randomized to receive 2009-10 seasonal trivalent inactivated influenza vaccine (TIV) or saline placebo between August 2009 and February 2010. Households were followed up with serology, symptom diaries and collection of respiratory specimens during illnesses. The primary outcomes were influenza infection confirmed by reverse transcription polymerase chain reaction (RT-PCR) or a 4-fold or greater rise in serum antibody titer measured by hemagglutination inhibition assay.

Results: Receipt of TIV led to 8-13 fold mean geometric rises in antibody titers against seasonal A and B viruses, but only 1.5-fold mean geometric rises against the pandemic A(H1N1) virus that was not included in the vaccine. Children who received TIV had reduced risk of seasonal influenza B confirmed by RT-PCR with vaccine efficacy estimate of 66% (95% confidence interval, CI: 31%, 83%). Children who received TIV also had reduced risk of pandemic influenza A(H1N1) indicated by serology, with vaccine efficacy estimate of 47% (95% CI: 15%, 67%).

Conclusions: Seasonal TIV prevented pandemic influenza A(H1N1) and influenza B infections in children. Pandemic A(H1N1) circulated at the time of vaccination and for a short time afterwards with no substantial seasonal influenza activity during that period. The potential mechanism for seasonal TIV to provide protection, possibly short-lived, for children against pandemic A(H1N1) infection despite poor cross-reactive serological response deserves further investigation.

INTRODUCTION

While seasonal influenza vaccination is effective in reducing influenza-related morbidity in children aged 6 years or older [1,2] it is not currently recommended to this group in Hong Kong. In an influenza pandemic, a vaccine against the novel virus may not be available for approximately 6 months. Thus, if previously available seasonal influenza vaccines are even moderately effective against the pandemic strain, they may have a role to play in mitigating the first wave of a pandemic.

The 2009 pandemic influenza A(H1N1) (pH1N1) virus was antigenically distinct from seasonal influenza A(H1N1) viruses that circulated before the pandemic and that were included in seasonal trivalent inactivated influenza vaccines (TIV) in 2008-09 and 2009-10 in the Northern hemisphere and 2009 in the Southern hemisphere. Seasonal influenza vaccination only marginally increased cross-reactive antibody to the pH1N1 virus [3]. It was therefore thought that seasonal influenza vaccination would have low effectiveness against pH1N1. Estimates of seasonal vaccine effectiveness against pH1N1 have varied substantially [4-16]. A monovalent pH1N1 vaccine was distributed in Hong Kong only in January 2010 at the end of the first wave of infections, and uptake of the vaccine in the general community was below 1% [17].

Following a pilot study in 2008-09 [18], we conducted a large community-based, randomized controlled trial in Hong Kong during the 2009-10 influenza season. The aim of our study was to evaluate the efficacy of TIV for prevention of laboratory-confirmed influenza in children. The study was also designed to

evaluate indirect benefits to household members, which will be reported separately.

METHODS

Recruitment and follow-up of participants

Invitation letters were distributed to participants of our previous studies, via schools, and to the families of members of a local birth cohort [19]. Eligible households included at least one child aged 6-17 who did not have any contraindications against injection of TIV and was not immunocompromised. One eligible child from each household was randomized to receive either a single dose of TIV (0.5ml VAXIGRIP, Sanofi Pasteur) or placebo (0.5ml saline solution) intramuscularly. The 2009-10 TIV used in our study included the strains A/Brisbane/59/2007(H1N1)-like, A/Brisbane/10/2007(H3N2)-like, and B/Brisbane/60/2008-like.

Serum specimens were collected from study subjects at baseline prior to vaccination (August 2009–February 2010), one month after vaccination, after the winter/spring influenza season for 25% of subjects ("mid-study", April–May 2010), and at the end of the follow-up period (August–December 2010), which would also capture influenza infections that occur during the summer in Hong Kong. Enrolment of subjects, vaccinations and serum collections were performed by trained research staff at a study clinic. All subjects and their 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 biweekly to monitor for any acute upper respiratory tract infections (URTIs).

Households were also reminded to report any acute URTIs to the study hotline as soon as possible after illness onset. Home visits were triggered by the presence of any two symptoms or signs of fever ≥37.8°C, chills, headache, sore throat, cough, presence of phlegm, coryza or myalgia in any household member and repeated at three-day intervals until acute URTIs resolved. During home visits, pooled nose and throat swabs were collected from all household members regardless of illness. Households were compensated with supermarket vouchers (and book tokens for children) worth US$65 for participation in the study, US$13 for each serum specimen provided and US$6.5 for each home visit.

Ethics

Proxy written consent from parents or legal guardians was obtained for the subjects who were all aged 17 years and younger, with additional written assent from those aged 8 to 17 years. The study protocol was approved by the Institutional Review Board of Hong Kong University.

Outcome measures

The primary outcome measures were influenza virus infection confirmed by reverse-transcription polymerase-chain-reaction (RT-PCR), or indicated by a four-fold or greater increase in antibody titer from post-vaccination to the end of the study. Secondary outcomes included (1) acute respiratory illness (ARI) defined as at least any two of fever ≥37.8°C, headache, sore throat, cough, myalgia [18], and (2) febrile acute respiratory illness (FARI) defined as fever ≥37.8°C plus cough or sore throat [18,20,21]. We defined episodes of ARI and FARI as periods of 1 or more day when participants met the criteria for ARI or FARI, respectively, and

episodes occurring within 7 days were merged together. RT-PCR-confirmed infections and illness episodes were analyzed for each subject from 14 days after vaccination until collection of the final serum specimen. Acute reactions were recorded by parents on a scale of none/mild/moderate/severe for four days following vaccination.

Sample size justification

Assuming conservatively that 10% of subjects in the control arm would be infected with a prevalent influenza strain [22], inclusion of 800 subjects would have 75% power to detect a vaccine efficacy of 50% or greater, with a 5% type I error rate. An unbalanced randomization scheme was proposed, where more subjects were included in the intervention arm to enhance acceptability.

Randomization, allocation concealment and blinding

Randomization lists were prepared by a biostatistician (VJF). Eligible study participants were randomly allocated to the TIV 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.

Blinding of households and study nurses was achieved by identical re-packaging of TIV/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/placebo was concealed to 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) and B/Brisbane/60/2008-like (Victoria-lineage), as well as the prevalent seasonal strain A/Perth/16/2009-like(H3N2) plus the pandemic strain A/California/7/2009(H1N1) by hemagglutination inhibition assays using standard methods as previously described [18,21]. The sera were tested in serial doubling dilutions from an initial dilution of 1:10. Pooled nose and throat swabs were tested by RT-PCR for influenza A and B using standard methods as previously described [18,21].

Statistical analysis

Risks of adverse reactions were compared between children who received TIV/placebo with Fisher's exact tests. To assess vaccine immunogenicity, the proportions of subjects with antibody titer ≥1:40 pre- and post-vaccination were compared between children who received TIV/placebo using chi-squared tests, and ratios of pre- to post-vaccination titers were compared using Wilcoxon signed-rank tests.

Post-study antibody titers were compared with post-vaccination antibody titers to determine serologic evidence of infection during the study period. For the subset of subjects with mid-study sera available, overall estimates of cumulative incidence of infection were compared with estimates based on post-study vs mid-

study and mid-study vs post-vaccination. We used Poisson regression offset by the duration of follow-up to estimate the incidence rate ratio of confirmed influenza, ARI and FARI in TIV/placebo recipients. Vaccine efficacy was estimated as one minus the incidence rate ratio. All analyses of study outcomes were performed under the principle of intention-to-treat [23]. We used multiple imputation with 10 imputations to account for a small amount of missing data [24]. Statistical analyses were conducted in R version 2.11.0 (R Development Core Team, Vienna, Austria).

RESULTS

Figure 1 shows the flow of subjects throughout the study. Subjects in the TIV and placebo group had similar characteristics (Table 1). One subject withdrew from the study after randomization but before the intervention was administered, and 13 of the 795 subjects who received the intervention did not complete the study. Following the principle of intention-to-treat we included all 796 subjects in the primary analyses.

Immune response to vaccine

A single dose of TIV led to substantial and statistically significant increases in antibody titers to seasonal influenza strains among study subjects (Table 2). There was no statistically significant difference in geometric mean rises in antibody titers to pH1N1 following receipt of TIV or placebo, while around half of the subjects had post-vaccination titers at 1:40 or above against pH1N1.

Reported reactogenicity

Myalgia and local reactions including swelling, redness, and pain/soreness at the injection site were more frequently reported following receipt of TIV (Table S1). Only a small number of participants reported severe systemic or local adverse reactions. No serious adverse events were reported following vaccination.

Incidence of influenza

Vaccinations were administered towards the end of the first wave of pH1N1. During the winter/spring influenza season in early 2010 there was circulation of pH1N1 and seasonal influenza B, while the summer influenza season occurred slightly later than in previous years [18,25,26] and was dominated by seasonal influenza A(H3N2) (Figure 2).

A total of 757 ARI episodes were reported by subjects during the follow-up period, and we were able to confirm 56 influenza A and B infections by RT-PCR on 477 swabs collected during 229 episodes. We were also able to confirm 2 pH1N1 infections from 738 swabs collected from subjects when a household contact reported illness but the subject was not ill. Among the 58 confirmed influenza A and B infections, the most common symptom was cough (81%), while 72% of the confirmed infections were associated with a febrile illness (Table S2). Fever, ARI and FARI were reported more commonly by individuals with confirmed seasonal influenza A(H3N2) and B than pH1N1.

Vaccine efficacy

Subjects who received TIV had significantly lower risk of seasonal B infection confirmed by RT-PCR or serology, with vaccine efficacy estimates of 66% (95% confidence interval, CI: 31%, 83%) and 83% (95% CI: 46%, 95%) respectively (Table 3). Subjects who received TIV also had significantly lower incidence rates of pH1N1 infection by serology, with vaccine efficacy of 47% (95% CI: 15%, 67%). Stratifying subjects by month of entry to the study, we did not observe statistically significant differences in VE against pH1N1 (data not shown). There were no statistically significant differences in incidence rates of seasonal influenza A(H3N2) by RT-PCR or serology, or in incidence rates of ARI or FARI.

In the subset of subjects who provided mid-study sera, estimation of cumulative incidence of infection in either winter or summer influenza seasons were 1.4 to 2 times higher than overall estimates based only on the baseline and post-study serology (Table S3). In this subset the estimate of vaccine effectiveness against pH1N1 was 59% (20%, 79%) over the winter 2009-10 influenza season when pH1N1 circulated.

No substantial antibody cross-reactivity between seasonal and pandemic influenza was observed. None of 8 subjects with seasonal A(H3N2) infections and only 2 of 35 subjects with seasonal B infections confirmed by RT-PCR were found to have a 4-fold or greater rise in antibody titer against pH1N1. Only a small proportion of subjects with 4-fold or greater rises in antibody titer against pH1N1 also had a 4-fold or greater rise in antibody against influenza A(H3N2) (12%) and B (13%).

We investigated the correlation between post-vaccination antibody titer against pH1N1 and subsequent serologic evidence of pH1N1 infection. Comparing TIV and placebo recipients with post-vaccination antibody titer ≤1:20, cumulative incidence of pH1N1 infection was significantly lower among the TIV recipients (Table S4). Incidence of pH1N1 was low among TIV and placebo recipients with higher post-vaccination antibody titers against pH1N1.

DISCUSSION

We report the results of a large randomized controlled trial of seasonal TIV during a period when pH1N1 as well as seasonal influenza viruses circulated in the community. We observed statistically significant protection in TIV recipients against influenza B, but non-significant protection against influenza A(H3N2) (Table 3), similar to our pilot study [18]. Suboptimal vaccine efficacy against A(H3N2) could have been associated with an imperfect match between the vaccine component and the circulating A/Perth/16/2009-like strains [18,21], as well as the longer interval between receipt of TIV and circulation of A(H3N2) (Figure 2). Despite evidence of protection against confirmed influenza, there was no evidence that TIV protected children against ARI or FARI (Table 3). A similar observation was reported in our pilot study [27].

Because antibody titers tend to rise substantially shortly after vaccination and then decline, serology can underestimate the cumulative incidence of influenza infection in vaccinees and bias estimates of vaccine efficacy [2,28]. However receipt of seasonal TIV did not raise antibody titers against pH1N1 substantially (Table 2) and therefore the estimates of cumulative incidence of pH1N1 infections

based on serology may be more accurate than for seasonal influenza. According to the serologic data, children who received TIV appeared to have greater protection against pH1N1 with vaccine efficacy 47% (95% CI: 15%, 67%) (Table 3). Protection occurred despite poor serological response induced by TIV against pH1N1 (Table 2) [3,29,30], and was conferred even to TIV recipients who did not achieve post-vaccination antibody titers of 1:40 or above (Table S4).

There is ongoing controversy over the change in risk of pH1N1 associated with receipt of seasonal TIV. Some observational studies of seasonal TIV have reported evidence of protection against illness associated with pH1N1 [4-6], while other studies have found no change in risk of illness associated with pH1N1 [7-14]. However, observational studies in Canada and Japan reported that receipt of seasonal TIV was associated with an increased risk of illness associated with pH1N1 infection [15,16]. In our own pilot study in Hong Kong in 2008-09, TIV in winter 2008-09 was associated with reduced seasonal influenza infections in spring 2009 but increased pH1N1 infection in Autumn 2009 [18], and we hypothesized that the effect was due to immunity against pH1N1 conferred by seasonal influenza infections [31-37]. In the present study pH1N1 circulated during and immediately after the vaccination period (Figure 2) and therefore we would not expect immunity associated with seasonal influenza infections to play any role in changing the risk of pH1N1.

We can speculate on two possible explanations for our findings, both of which deserve further investigation. First, the lack of apparent cross-reactive serological responses against the novel pH1N1 virus [3,29,30] may underestimate a true

protective effect of TIV. The criteria used for seasonal vaccine immunogenicity, i.e. that a titer of 1:40 or higher is associated with 50% protection against seasonal influenza infection, is based on limited evidence primarily from challenge studies in young adults [38], and may not apply to a novel virus. Moreover, it appeared during the pandemic that adults had some degree of protection against pH1N1 as indicated by low secondary infection risks in households of index cases with confirmed infection [21,39], and low cumulative incidence of infection at a population level in those aged 30-50 years [40] despite very low seroprevalence of pH1N1 antibody against pH1N1 before the pandemic [18,21,40]. Second, TIV could have conferred temporary non-specific protection against influenza, which waned over a shorter time-frame than the protection against strains included in the vaccine, and protected TIV recipients against pH1N1 which circulated at the beginning of our study period (Figure 2). This would mirror the hypothesis of temporary immunity following infection [18,41], and potential mechanisms worthy of further exploration would include cell-mediated or innate immune responses to TIV [42]. Recent studies have demonstrated antibodies binding to the stalk of the haemagglutinin that confer broad heterosubtypic immunity [43,44]. If such antibodies are elicited following vaccination but are short lived, this may also explain the observations from our study.

Since vaccination was carried out in the period following the peak of the first wave of pH1N1 in Hong Kong during which approximately 45% of school-age children were infected [40], it may be argued that the protective effect of TIV on pandemic infection was mediated by boosting low or waning levels of immunity in those already infected by the pandemic H1N1 virus. The observation of protection even

in those who had no detectable antibody 1 month after vaccination makes this explanation less plausible (Table S4).

There are some limitations of our study. First, given the circulation of pH1N1 and seasonal A(H3N2) viruses that were not included or antigenically well-matched in the seasonal TIV used in our study, our study had reduced power to confirm lower vaccine efficacy against these strains. Second, while 18% of participants had evidence of influenza infection indicated by serology during the study, we were only able to confirm 17% of those infections by RT-PCR (Table 3), which is similar to the proportion of influenza infections that could be confirmed by virologic testing in our pilot study and other community-based cohort studies [11,18,45]. Identification of acute URTIs and timely collection of respiratory specimens is difficult even with the intense follow-up in our cohort study, and confirming a greater proportion of infections remains an important consideration when designing community-based studies of influenza infection and transmission. Rise in antibody may be detectable even in those in whom the vaccine protects from disease though failing to protect from infection. On the other hand, serologic indication of infection via a 4-fold rise in antibody titer across a period of influenza activity may not have ideal sensitivity and specificity to identify influenza infections due to variability in antibody titers over time for other reasons unrelated to influenza infection status, changes in cross-reactive antibody associated with other infections, and imperfect reliability or repeatability of serologic tests. Third, as discussed above we may have failed to detect some seasonal influenza infections in children who received TIV [28].

In conclusion, receipt of seasonal TIV prevented seasonal influenza B and pH1N1 infections in school-age children. Apparent protection against pH1N1 was only detected by serology but not by RT-PCR (Table 3), and occurred despite the poor cross-reactive serological responses induced by the seasonal TIV against pH1N1 (Table 2). These results may have implications for the use of seasonal influenza vaccines in future pandemics even if initial studies were to suggest that seasonal vaccine was not immunogenic against the novel virus. The importance of the timing of the seasonal vaccination in relation to pandemic activity was also highlighted in this study. However the mechanism for protection remains unclear and seasonal TIV might be less effective against influenza A subtypes that human populations have not previously been exposed to, such as H5N1 and H9N2.

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COMPETING INTERESTS

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FIGURE LEGENDS

Figure 1. Flow chart of participants through the study.

Footnote to Figure 1: Breakdown of background of 796 enrolled households. Invitation letters were sent to a convenience sample of 311 primary and 379 secondary schools. In 79 schools that agreed to participate, letters were distributed to the parents of 59,630 children, and 656 households were enrolled. 1,150 invitations were sent to households of children who are members of a local birth cohort, and 55 households were enrolled. A further 85 households continued from the preceding pilot study [18] and were re-enrolled and re-randomized.

Figure 2. Timeline of subject recruitment and follow-up in 2009-10, compared to influenza virus detections in the reference laboratory for Hong Kong Island at Queen Mary Hospital. Seasonal influenza A(H1N1) viruses were not detected in Hong Kong after October 2009, apart from one detection in the public health reference laboratory (in 6802 specimens tested) in April 2010. Victoria-lineage strains predominated among the influenza B viruses detected, and Yamagata-lineage strains also circulated.

Table 1. Baseline characteristics of children who received TIV or placebo and their household contacts

Characteristics	***Missing data***	**TIV**		**Placebo**	
Study subjects		(n=479)		(n=317)	
Female	*0*	231	(48%)	141	(44%)
Age group:	*0*				
6-8 years*		170	(35%)	104	(33%)
9-11 years		152	(32%)	107	(34%)
12-17 years		157	(33%)	106	(33%)
Received influenza vaccination for 2008-09 season	*24*	119	(26%)	90	(29%)
Households					
Mean number of members (sd)	*0*	3.8	(1.0)	3.8	(1.0)
Mean flat size, square feet (sd)	*7*	519	(229)	516	(263)

*including one child aged 5 years and 356 days.

Table 2. Immunogenicity of 2009-10 seasonal TIV against seasonal and pandemic influenza strains.

Virus	TIV (n=479)	Placebo (n=317)	p-value*
Seasonal influenza A(H1N1)			
Pre-vaccination antibody titer ≥1:40	56%	52%	0.34
Post-vaccination antibody titer ≥1:40	94%	54%	**<0.01**
GMT increase after vaccination	10.0	1.2	**<0.01**
Seasonal influenza A(H3N2)			
Pre-vaccination antibody titer ≥1:40	58%	64%	0.08
Post-vaccination antibody titer ≥1:40	96%	64%	**<0.01**
GMT increase after vaccination	13.2	1.1	**<0.01**
Seasonal influenza B/Brisbane			
Pre-vaccination antibody titer ≥1:40	15%	13%	0.59
Post-vaccination antibody titer ≥1:40	70%	15%	**<0.01**
GMT increase after vaccination	7.9	1.0	**<0.01**
Pandemic influenza A(H1N1)			
Pre-vaccination antibody titer ≥1:40	39%	37%	0.59
Post-vaccination antibody titer ≥1:40	51%	47%	0.38
GMT increase after vaccination	1.5	1.3	0.18

Abbreviation: GMT = geometric mean titer.

*p-values were calculated by chi-squared tests and Wilcoxon signed-rank tests.

Antibody titers <1:10 were imputed as 1:5.

Table 3: Incidence rates per person-year of infection of laboratory-confirmed influenza by RT-PCR and serology, acute respiratory illness and febrile acute respiratory illness among study subjects who received TIV or placebo.

Outcome	TIV (n=479)		Placebo (n=317)		p-value*	Vaccine effectiveness*	
	Incidence rates (per person-yr)	(95% CI)	Incidence rates (per person-yr)	(95% CI)		VE	(95% CI)
By RT-PCR							
pandemic A(H1N1)	0.02	(0.01, 0.04)	0.02	(0.01, 0.04)	0.61	-0.32	(-2.86, 0.55)
seasonal A(H3N2)	0.01	(0.00, 0.03)	0.01	(0.00, 0.03)	0.90	-0.10	(-3.60, 0.74)
seasonal B	0.03	(0.02, 0.05)	0.08	(0.05, 0.12)	**<0.01**	0.66	(0.31, 0.83)
By serology							
pandemic A(H1N1)	0.09	(0.06, 0.14)	0.17	(0.12, 0.24)	**0.01**	0.47	(0.15, 0.67)
seasonal A(H3N2)	0.05	(0.02, 0.09)	0.07	(0.04, 0.12)	0.21	0.35	(-0.28, 0.67)
seasonal B/Brisbane	0.02	(0.01, 0.06)	0.11	(0.08, 0.16)	**<0.01**	0.83	(0.46, 0.95)
ARI†	1.02	(0.93, 1.12)	1.02	(0.91, 1.14)	0.96	0.00	(-0.16, 0.13)
FARI†	0.43	(0.37, 0.49)	0.50	(0.43, 0.59)	0.59	0.15	(-0.06, 0.31)

* p-values estimated from Poisson regression models, and vaccine effectiveness estimated as 1 – incidence rate ratio.

† Acute respiratory illness (ARI) is defined as at least two of body temperature ≥37.8°C, cough, sore throat, headache, and myalgia; Febrile acute respiratory illness (FARI) is defined as body temperature ≥37.8°C plus cough or sore throat.

Table S1. Adverse reactions reported by children who received TIV or placebo.

Reactions*	**TIV (n = 457/479 returned card)**						**Placebo (n = 306/317 returned card)**						***p*-value**[†]
Systemic reactions	**Mild**		**Moderate**		**Severe**		**Mild**		**Moderate**		**Severe**		
Fever (over 37.8°C)	13	(3%)	5	(1%)	2	(0%)	9	(3%)	2	(1%)	1	(0%)	0.96
Shivering/chills	24	(5%)	4	(1%)	0	(0%)	9	(3%)	4	(1%)	0	(0%)	0.26
Feeling Tired	81	(18%)	18	(4%)	1	(0%)	53	(17%)	7	(2%)	0	(0%)	0.54
Headache	39	(9%)	9	(2%)	1	(0%)	20	(7%)	5	(2%)	1	(0%)	0.71
Cough	57	(12%)	5	(1%)	0	(0%)	29	(9%)	6	(2%)	1	(0%)	0.22
Muscle pain	78	(17%)	19	(4%)	2	(0%)	33	(11%)	3	(1%)	0	(0%)	<0.01
Local reactions at injection site													
Swelling	70	(15%)	3	(1%)	1	(0%)	13	(4%)	2	(1%)	0	(0%)	<0.01
Redness	54	(12%)	0	(0%)	0	(0%)	17	(6%)	0	(0%)	1	(0%)	<0.01
Bruising (>5mm)	26	(6%)	2	(0%)	0	(0%)	15	(5%)	2	(1%)	0	(0%)	0.82
Pain/Soreness	188	(41%)	38	(8%)	3	(1%)	57	(19%)	7	(2%)	0	(0%)	<0.01

* Mild: symptoms are easily tolerated and do not interfere with any usual activities; Moderate: symptoms interfere with usual activities;

Severe: symptoms are severe and cannot carry out usual activities

† By Fisher's exact test.

Table S2. Signs, symptoms and syndromes associated with influenza A and B infections confirmed by RT-PCR in study subjects.

	Influenza infection confirmed by RT-PCR*						p-value
	pH1N1 (n=15)		sH3N2 (n=8)		B (n=35)		
Cough	10	(67%)	7	(88%)	30	(86%)	0.25
Runny nose	9	(60%)	7	(88%)	26	(74%)	0.40
Body temp ≥37.8°C	7	(47%)	6	(75%)	29	(83%)	0.03
Sore throat	7	(47%)	5	(62%)	25	(71%)	0.23
Headache	6	(40%)	3	(38%)	18	(51%)	0.70
Chills	5	(33%)	3	(38%)	13	(37%)	1.00
Muscle pain	5	(33%)	0	(0%)	11	(31%)	0.19
Asymptomatic†	2	(13%)	0	(0%)	0	(0%)	0.08
ARI†	9	(60%)	7	(88%)	32	(91%)	0.03
FARI†	5	(33%)	6	(75%)	27	(77%)	0.01

* pH1N1 = 2009 pandemic influenza A(H1N1); sH3N2 = seasonal influenza A(H3N2)

† Asymptomatic infections defined as subjects with RT-PCR confirmed influenza infection but not reporting any signs or symptoms including body temperature ≥37.8°C, chills, headache, sore throat, cough, coryza or myalgia within 5 days of the collection date of the specimen that was positive by RT-PCR. Acute respiratory illness (ARI) defined as at least two of body temperature ≥37.8°C, cough, sore throat, headache, and myalgia; Febrile acute respiratory illness (FARI) defined as body temperature ≥37.8°C plus cough or sore throat.

Table S3. Comparisons of estimates of the cumulative incidence of infection among participants during the winter, summer and overall study period for the subset of 284 participants who provided mid-study sera in April-May 2010. Cumulative incidence of infection was estimated by the proportion of subjects with a 4-fold or greater rise in antibody titer (1) from baseline to mid-study; (2) from mid-study to post-study; (3) from baseline to mid-study or from mid-study to post-study; (4) from baseline to post-study.

Outcome	**TIV (n=171)**		**Placebo (n=113)**		**p-value**	**Vaccine effectiveness***	
	Cum. inc.	**(95% CI)**	**Cum. inc.**	**(95% CI)**		**VE**	**(95% CI)**
(1) Winter 2009-10							
pandemic A(H1N1)	0.07	(0.03, 0.11)	0.18	(0.11, 0.25)	0.01	0.59	(0.20, 0.79)
seasonal A(H3N2)	0.04	(0.01, 0.07)	0.03	(0.00, 0.06)	0.74	-0.56	(-4.92, 0.59)
seasonal B/Brisbane	0.07	(0.03, 0.11)	0.12	(0.06, 0.17)	0.28	0.39	(-0.30, 0.71)
(2) Summer 2010							
pandemic A(H1N1)	0.05	(0.02, 0.09)	0.06	(0.02, 0.11)	0.92	0.15	(-1.22, 0.67)
seasonal A(H3N2)	0.09	(0.05, 0.13)	0.05	(0.01, 0.10)	0.41	-0.63	(-3.10, 0.35)
seasonal B/Brisbane	0.02	(0.00, 0.05)	0.02	(0.00, 0.04)	0.92	-0.32	(-6.10, 0.75)
(3) Winter 2009-10 or summer 2010							

pandemic A(H1N1)	0.12	(0.07, 0.17)	0.24	(0.16, 0.32)	**0.01**	0.50	(0.15, 0.71)
seasonal A(H3N2)	0.12	(0.07, 0.17)	0.08	(0.03, 0.13)	0.35	-0.53	(-2.23, 0.27)
seasonal B/Brisbane	0.09	(0.05, 0.14)	0.13	(0.07, 0.20)	0.41	0.29	(-0.38, 0.63)
(4) Overall 2009-10							
pandemic A(H1N1)	0.06	(0.02, 0.10)	0.18	(0.11, 0.25)	**<0.01**	0.66	(0.29, 0.83)
seasonal A(H3N2)	0.03	(0.00, 0.06)	0.03	(0.00, 0.06)	0.86	-0.10	(-3.46, 0.73)
seasonal B/Brisbane	0.01	(0.00, 0.02)	0.09	(0.04, 0.14)	**<0.01**	0.93	(0.50, 0.99)

* Vaccine effectiveness estimated as 1 – cumulative incidence ratio.

Table S4. Cumulative incidence of infection of 2009 pandemic influenza A(H1N1) (pH1N1) among children who received TIV or placebo, stratified by post-vaccination antibody titer against pH1N1.

Post-vaccination antibody	**TIV (n=426)**			**Placebo (n=289)**			**p-value***
titer against pH1N1	**n**	**Cum. Inc.**	**(95% CI)**	**n**	**Cum. Inc.**	**(95% CI)**	
≤1:20	215	0.15	(0.10, 0.20)	153	0.27	(0.21, 0.35)	<0.01
1:40	38	0.00	(0.00, 0.09)	26	0.04	(0.00, 0.20)	0.41
1:80	70	0.00	(0.00, 0.05)	50	0.02	(0.00, 0.11)	0.42
≥1:160	111	0.00	(0.00, 0.03)	66	0.00	(0.00, 0.05)	1.00

Cum. inc. = cumulative incidence of pH1N1 infection during follow-up assessed by a 4-fold or greater rise in antibody titer against pH1N1.

* p-values calculated by chi-squared tests and Fisher's exact tests.

Figure 1

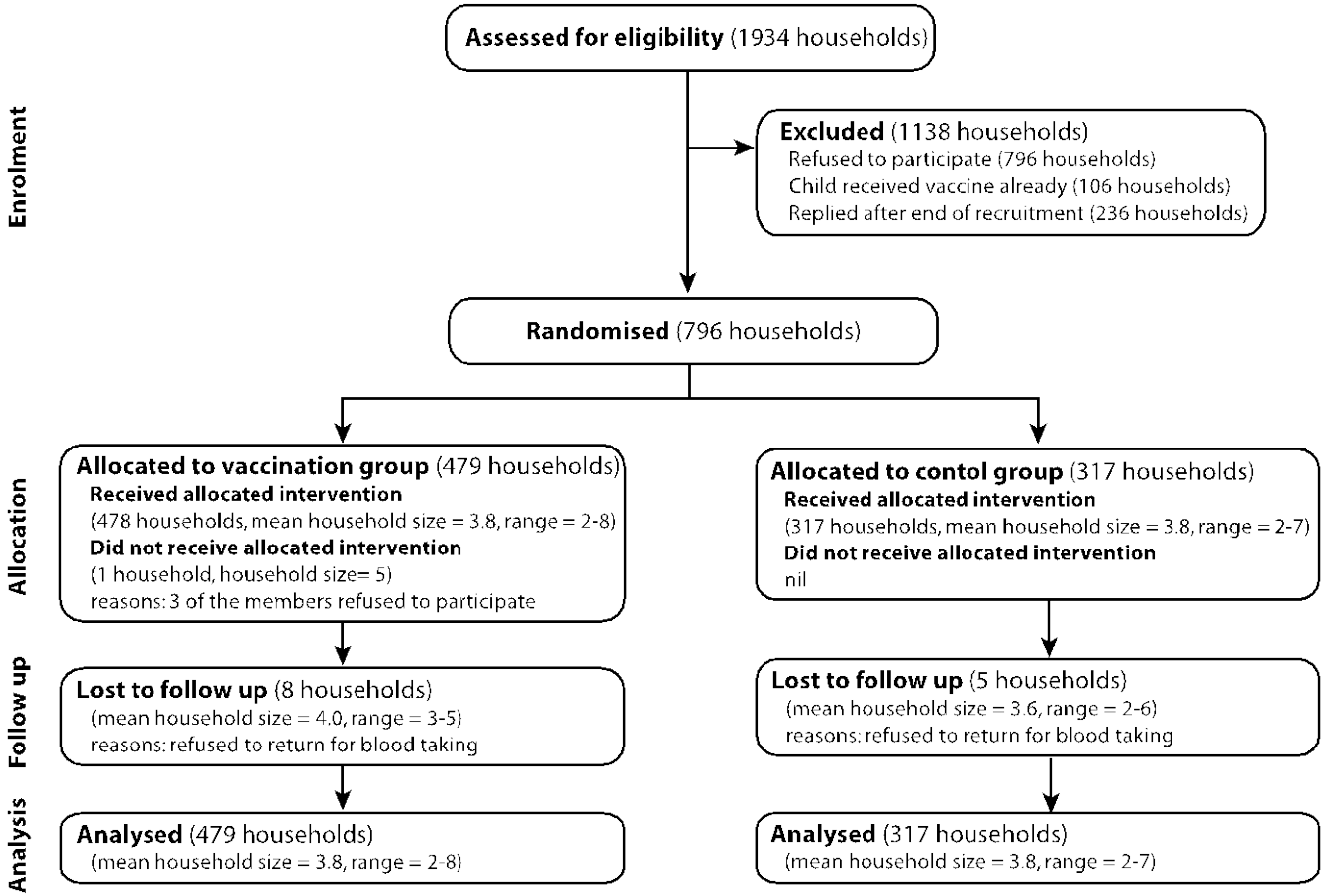
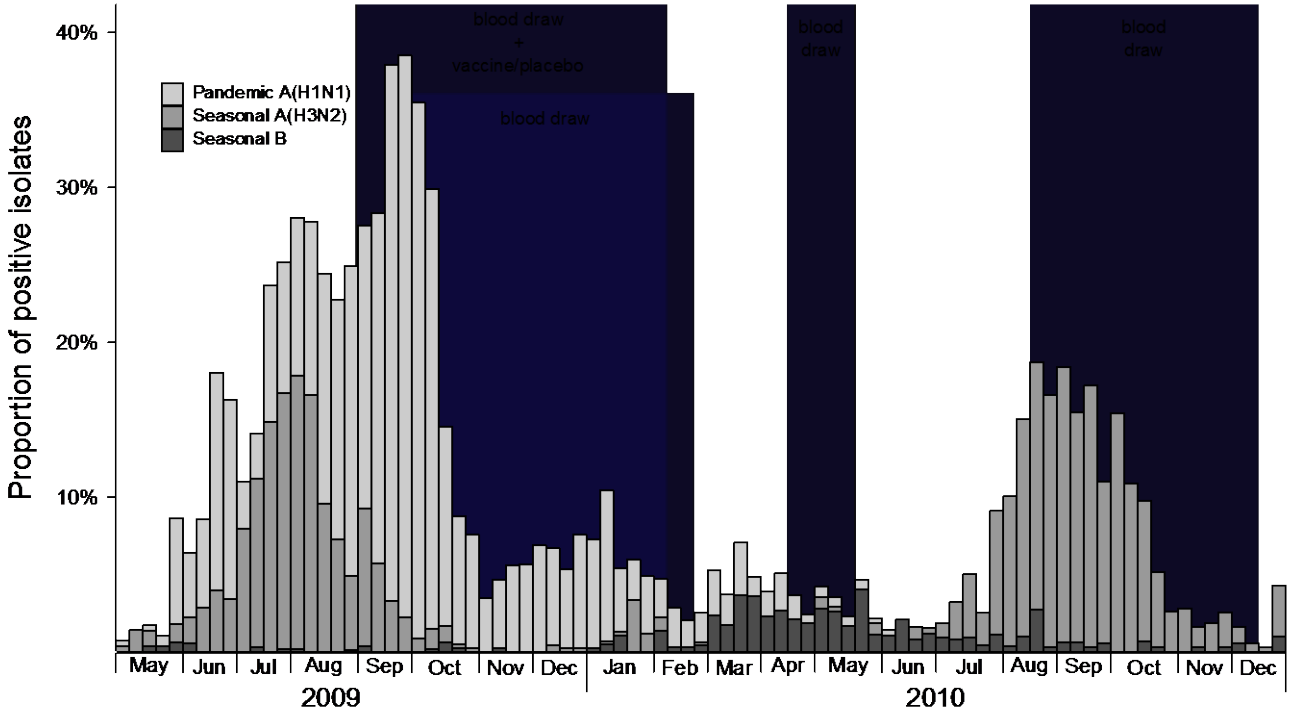

Enrolment
Assessed for eligibility (1934 households)
Excluded (1138 households)
Refused to participate (796 households)
Child received vaccine already (106 households)
Replied after end of recruitment (236 households)
Randomised (796 households)
Allocation
Allocated to vaccination group (479 households)
Received allocated intervention
(478 households, mean household size = 3.8, range = 2-8)
Did not receive allocated intervention
(1 household, household size= 5)
reasons: 3 of the members refused to participate
Allocated to contol group (317 households)
Received allocated intervention
(317 households, mean household size = 3.8, range = 2-7)
Did not receive allocated intervention
nil
Follow up
Lost to follow up (8 households)
(mean household size = 4.0, range = 3-5)
reasons: refused to return for blood taking
Lost to follow up (5 households)
(mean household size = 3.6, range = 2-6)
reasons: refused to return for blood taking
Analysis
Analysed (479 households)
(mean household size = 3.8, range = 2-8)
Analysed (317 households)
(mean household size = 3.8, range = 2-7)

Figure 2


blood draw
+
vaccine/placebo
blood draw
blood
draw
blood
draw
Pandemic A(H1N1)
Seasonal A(H3N2)
Seasonal B
Proportion of positive isolates
40%
30%
20%
10%
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
2009
2010