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# 2007-2008年台灣地區國小一、二年級學童接種流感疫苗的效益研究

# Evaluation of Human Influenza Mass-vaccination among Grade 1-2 Elementary Schoolchildren during 2007-2008 in Taiwan

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# Chinese Abstract 中文摘要

流行性感冒是每年冬季好發的呼吸道傳染病，孩童是流感病毒的易感受者之一，易在得流感之後傳播病毒給同處的其他孩童或家中同住的年長者與幼童，所以我國的防疫政策每年為滿 6 到 24 個月大的幼童免費接種季節性流感疫苗，以增幼童的免疫保護。惜台灣在 2006~07 年的 B 型流感流行導致許多孩童的重症與死亡，因此衛生單位考量流感在孩童中的傳播及預防的重要性，首度在 2007~08 年針對全國的國小一、二年級學童開始接種免費流感疫苗，為此本研究目的是在學童接種流感疫苗前後，以血清流行病學探究學童接種流感疫苗的免疫效果和不良反應，並評估接種疫苗對學童及其家人的疫苗效益(vaccine effectiveness)。

做法上，以台灣的都會區台北市一所、鄉村區宜蘭三所和離島區金門兩所國小的一、二年級學童為研究對象，經過家長簽署同意後，敬邀學童參與研究，在各校的流感疫苗接種日前的二至三週內、接種後的一和四個月，各抽血 3~5 cc，以進行流感病毒三疫苗株 [A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), B/Malaysia/2506/2004]的血球凝集抑制抗體[hemagglutination inhibition (HI) antibody]測試。此外，經由家長填寫問卷蒐集學童的基本人口學、流感的危險及保護因子、接種疫苗後的不良反應與學童及其家戶成員在此流感流行季中發生感冒的症狀等資料，以學生t檢定(student t test)、卡方檢定($\chi^2$ test)和費歇爾精確檢定(Fisher exact test)進行統計分析，以確切明瞭流感疫苗對預防其感染、發病與傳播家人罹病的群體免疫之三方面成效及其相關聯的危險與保護因子。

在總計 677 位持續參加研究的學童(590 名有三次的血清及 87 名有前兩次的血清)中，572 位(84.5%)學童有施打 2007~08 年的流感疫苗，比較學童有無接種疫苗，發現此二群在各校中的分布、每日睡眠時數、參與課餘活動、家中照顧者的教育程度、研究期間的缺席及 2006~07 年接種流感疫苗均達統計差異($p<0.05$)。

疫苗施打後一到三個月內，比較疫苗接種者與非接種者兩組在三種的疫苗效益，發現：(1) 疫苗『防止感染』(血清流感抗體四倍上升)的效益分別是：A/H1N1 流感為-29.32% [1.41% (7/498) vs. 1.09% (1/92)]、A/H3N2 流感為 86.14% [0.60% (3/498) vs. 4.35% (4/92)] ($p<0.05$)和 B 型流感為 38.42% [2.01% (10/498) vs. 3.26% (3/92)]；(2) 以防止學童發生類流感(influenza-like illness, ILI)的臨床症狀(出現一項全身性合併一項呼吸道症狀)評估所得的疫苗效益是 31.60% [19.74% (108/547) vs. 28.87% (28/97)] ($p<0.05$)；及(3) 在流感流行季間因呼吸道症狀而缺席或住院的疫苗效益各是 43.89% [16.61% (90/542) vs. 29.59% (29/98)] ($p<0.05$)和 45.76% [1.11% (6/542) vs. 2.04% (2/98)]。仔細探究，發現疫苗施打後一至三個月內，27 名流感血清抗體四倍上升的自然感染學童之三種效益，在：(1) 防止學童得類流感的疫苗效益是 53.95% [26.32% (5/19) vs. 57.14% (4/7)]；(2) 降低學童在流感流行季間缺席或住院的疫苗效益各是 41.67% [50.00% (9/18) vs. 85.71% (6/7)] 和 63.16% [5.26% (1/19) vs. 14.29% (1/7)]；及(3) 減少學童家人得呼吸道病徵的疫苗效益是 42.28% [15.28% (11/72) vs. 26.47% (9/34)]。

比較有、無接種疫苗學童在打疫苗後一個月的免疫效果，針對 A/Solomon Islands/3/2006 (H1N1)、A/Wisconsin/67/2005 (H3N2) 和 B/Malaysia/2506/2004 三株流感疫苗的組成病毒株可發現，有打疫苗學童的血清保護率、血清抗體四倍上升率和幾何平均效價均比沒打疫苗學童顯著地高 ($p<0.05$)，即學童在此三流感疫苗病毒株的：(1)血清保護率(sero-protection rate: 抗體≧1:40)各是 94.06% (538/572) vs. 75.24% (79/105)、97.73% (559/572) vs. 85.71% (90/105)、69.06% (395/572) vs. 31.43% (33/105)；(2) 血清抗體四倍上升比率(percentage of 4-fold serotiter rise)各是 53.67% (307/572) vs. 3.81% (4/105)、70.80% (405/572) vs. 3.81% (4/105)及 34.44% (197/572) vs. 0% (0/105)；(3) 血清抗體的幾何平均效價(geometric mean titer, GMT)各是 161.11 vs. 45.63、329.00 vs. 63.64 及 48.57 vs. 20.42。整體而言，學童對 A/H3N2

流感病毒的抗體反應最好，但對 B 型流感病毒為最差。有 15.2% (71/648)學童紀錄在接種部位的疼痛反應，但大部分的學童在接種流感疫苗後均無不良反應。

在打疫苗後一至四個月流感血清抗體呈四倍上升而得流感感染的 27 名學童中（包含 1 名感染兩型病毒者），觀察其抗體上升前之疫苗後一個月的抗體分布，可發現感染學童的血清抗體均較低，從≦1:10 至 1:80 不等，以抗體值 1:40 (35.7%, 10/28)和 1:20 (28.6%, 8/28)為最多，顯示在疫苗接種後，抗體較低之學童在流行季中會有相對較高的機會感染到流感病毒。

針對前兩年未接種過流感疫苗，於 2007~08 年首次接種學童在都會區、鄉村和離島之血清抗體幾何平均效價，在接種前學童對A/H3N2 和B型流感即有地區間的差異($p<0.05$)，以都會區之幾何平均效價最高，離島最低；在接種疫苗後一個月和流行季結束之抗體衰退時，都會區的幾何平均效價仍較其他兩區高，且對A/H1N1 和A/H3N2 流感病毒均呈現地區間的差異($p<0.05$)。此外，研究結果也顯示研究學校的疫苗接種比率越高，學童中血清抗體四倍上升證實之流感侵襲率呈現較低的趨勢($R^2=0.26$)。

本研究的結論是流感疫苗接種者在接種後一個月的血清抗體在上述三種血清評估值上均比未接種者高($p<0.05$)，且至流行季末的疫苗接種後四個月時，仍維持比未接種學童為高($p<0.05$)，顯示疫苗接種所產生的血清抗體能夠維持並保護學童到流感季結束。另方面，往後應強化疫苗中 B 型流感病毒的免疫效果，或者進行補接種。未來我們仍需要藉由血清流行病學的長期追蹤研究，比較不同年度流行的野生病毒株特性，觀察疫苗介入的遠程效果，深信此台灣公共衛生經驗能為流感病毒在孩童間的傳播提供最佳的防疫實證。

關鍵字：流感疫苗接種、兒童、免疫效果、疫苗效益、血清流行病學、台灣

# English Abstract

Elementary schoolchildren, an influenza susceptible group, would spread influenza virus easily to other children or household members leading to cluster cases or an epidemic if they were infected. An outbreak of influenza B virus caused many severe cases and deaths among young children during 2006-2007 in Taiwan. Therefore, the first influenza mass-vaccination program to grade one and two elementary schoolchildren free of charge was initiated before the 2007-2008 influenza season. Therefore, the aims of this seroepidemiologic study were to evaluate the immunogenicity, reactogenicity, and vaccine effectiveness among those schoolchildren received the seasonal influenza vaccine and their family members as well.

Grade 1 and 2 schoolchildren at six schools, including one in metropolitan Taipei, three in rural Yilan, and two in Kinmen islet were enrolled, after receiving the informed consents from their parents. Serum samples of the participants collected at the three time-points (2-3-week pre-vaccination, 1-month and 4-month post-vaccination) were tested for their presence of hemagglutination inhibition (HI) antibody and serotiters against the 2007 WHO recommended three vaccine strains [A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004]. In addition, their demographic information, risk and protective factors related to influenza infection,

reactogenicity following the vaccination, and clinical manifestations of influenza-like illness (ILI) from themselves and their household members were also obtained through questionnaire. Student t, $\chi^2$, and Fisher exact tests were used to investigate the vaccine efficacy and effectiveness in protecting from the infection, ILI and further transmission to the household members and their associated risk/protective factors.

Of 677 participants in the cohort study (590 children with triple serum samples and 87 children with their first two consecutive serum samples), 572 (84.5%) children received the 2007-08 influenza vaccine. Comparing vaccinated and unvaccinated schoolchildren, the school's 2007-08 influenza vaccination coverage rates, children's history of receiving 2006-07 influenza vaccine, daily sleeping hours, post-school activities, school absenteeism during the study period, and education levels of their guardian/parents were significantly different between these two groups ($p<0.05$).

Three measures were used to evaluate unfluenza vaccine by comparing vaccinated and unvaccinated schoolchildren from 1-month to 4-month post-vaccination. The results showed that: (1) vaccine efficacy in preventing influenza infection [eg. 4-fold HI antibody (Ab) serotiter rise] were -29.32% [1.41% (7/498) vs. 1.09% (1/92)], 86.14% [0.60% (3/498) vs. 4.35% (4/92)] ($p<0.05$), and 38.42% [2.01% (10/498) vs. 3.26% (3/92)] for A/H1N1, A/H3N2, and B viruses, respectively；(2) vaccine effectiveness (VE) in declining ILI

was 31.60% [19.74% (108/547) vs. 28.87% (28/97)] ($p<0.05$); and (3) VE in reducing respiratory-illness-related absenteeism and hospitalization were 43.89% (16.61% vs. 29.59%)($p<0.05$) and 45.76% (1.11% vs. 2.04%), respectively. Further vaccine evaluation among the 27 influenza infected children with 4-fold HI Ab serotiter rises from 1-month to 4-month post-vaccination demonstrated that VE in decreasing ILI was 53.95% [26.32% (5/19) vs. 57.14% (4/7)]; in reducing respiratory-illness-related absenteeism, and hospitalization were 41.67% (50.00% vs. 85.71%) and 63.16% (5.26% vs. 14.29%), respectively; and in decreasing ILI among household members was 42.28% (15.28% vs. 26.47%).

Overall, vaccinated children had higher values of the following three serological measures of HI Ab against all the three human influenza vaccine strains of 2007-08 (A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2005) at 1-month post-vaccination than those in unvaccinated children ($p<0.05$): (1) percentages of sero-protection (serotiter $\geqq$1:40) [94.06% (538/572) vs. 75.24% (79/105), 97.73% (559/572) vs. 85.71% (90/105), and 69.06% (395/572) vs. 31.43% (33/105)], (2) percentages of HI Ab 4-fold serotiter rises from pre-vaccination to 1-month post-vaccination [53.67% (307/572) vs. 3.81% (4/105), 70.80% (405/572) vs. 3.81% (4/105), and 34.44% (197/572) vs. 0% (0/105)], and (3) geometric mean titers (GMT) [161.11 vs. 45.63,

329.00 vs. 63.64, and 48.57 vs. 20.42], respectively. The immunogenicity was highest against A/H3N2 but lowest against B viruses. In addition, 15.2% (71/468) schoolchildren reported pain at injection site as the most reactogenicity, but most children had no severe adverse reactions after receiving the influenza vaccine.

Among the 27 influenza-infected schoolchildren with 4-fold rises of HI Ab serotiters from 1-month to 4-month post-vaccination, including one child involved infections of the two subtypes (A/H3N2 and B virus), their 1-month post-vaccination serotiters prior to the infection were lower, ranged from ≦1:10 to 1:80. The two most frequent serotiters were children with serotiters of 1:40 [35.7% (10/28)] and 1:20 [28.6% (8/28)]. These results imply that schoolchildren with lower HI Ab serotiters were most likely to be infected with influenza virus during influenza epidemic season.

To understand geographical variations in anti-influenza HI Ab, we analyzed the GMTs of vaccinated children without taking influenza vaccine in 2005-06 and 2006-07 living in metropolitan city, rural area, and isolated islet. At pre-vaccination, children in metropolitan Taipei City showed the significantly highest GMT whereas children in Kinmen islet revealed significantly the lowest GMT for A/H3N2 and B viruses ($p<0.05$). At 1- and 4-month post-vaccination (ending of epidemic period), children in Taipei City still remained the highest GMT against A/H1N1 and A/H3N2 influenza viruses ($p<0.05$).

Furthermore, the schools with the higher vaccine coverage rates had the trends in lower influenza virus infection rate during 2007-08 influenza season ($R^2$=0.26).

In conclusion, the above mentioned three measures of anti-influenza HI Ab in vaccinated children were significantly higher than non-vaccinated at 1-month ($p<0.05$) and 4-month post-vaccination ($p<0.05$), implying that the immunogenicity of vaccine could sustain till the end of 2007-08 influenza season. Future efforts can enhance the immunogenicity of vaccine B virus or provide another booster. In addition, longitudinal seroepidemiological studies to evaluate the long-term effectiveness of vaccine to protect against the various circulating wild-type influenza viruses in different years are needed. We believe Taiwan's experience in mass-vaccination of influenza in schoolchildren and subsequent studies can provide evidence-based public health policy to minimize influenza transmission among children.

Key words: influenza vaccination, children, immunogenicity, vaccine effectiveness, seroepidemiology, Taiwan

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# Chapter 1 Introduction

Influenza is a viral disease transmitting through air-droplets. Close and longer contact to the patient with influenza virus generally can spread the virus very easily. The disease can cause not only high morbidity but also high mortality among high risk groups. To prevent influenza, vaccination is an approach to induce protective immunity. However, not everyone can get vaccine shots during each influenza season. Influenza vaccine coverage rates varied in different countries.

In Taiwan, 6-23-month children and over 65-year elders are two major target groups to receive the inactivated human influenza vaccine annually since they contribute to high morbidity and mortality of influenza (Simonsen L, et al. 1998; Monto AS, et al. 1993). In addition, influenza patients of these two age groups, who are more likely to share common environment at home, may be more likely to transmit the virus to each other and/or other household members. In spite of 6-23-month children, young children attending day-care centers or going to school are also crucial to contract the disease (Hurwitz ES, et al. 2000), because they play or study with others who could directly spread or carry the virus back to their homes.

During the 2006-2007 influenza season in Taiwan, there were an outbreak of influenza type B, which resulted in pediatric fatal cases and several school classes were even closed. The Immunization Advisory Committee discussed this issue and recommended the initiation of influenza vaccination for schoolchildren. In 2007-08 influenza season, health authorities in Taiwan planned to launch a free influenza mass-vaccination program for nationwide children at 1-2 grades at elementary schools. Since this is the first time to initiate the influenza vaccination among schoolchildren, the

specific aims of this study are: (1) to understand the effectiveness, (2) to measure the immunogenicity, and (3) to record the reactogenicity of the influenza vaccine provided to those schoolchildren from this new public health policy.

The risk or protective factors, for example, the previous vaccination history, exercise hours, sleeping hours, and ethnicity, should be determined between vaccinated and unvaccinated groups. The vaccine effectiveness in preventing natural infection, in lowering rate of absenteeism or hospitalization, and in reducing ILI among schoolchildren or household contacts would be also measured. We hope this study can provide scientific base for public health administrators to decide whether such a program should be continued or even extended to other grades of schoolchildren in future years.

# Chapter 2 Literature Review

## 2.1 Public Health Importance of Influenza in Children

Influenza is a respiratory infectious disease which often attacks young children and elderly people in winter season. When children acquire influenza virus infection, they might develop symptoms like a common cold. If they were not well treated and their parents neglect the situation, severe complications would threaten their life. Other socio-economic impact, like the children absenteeism of schools or day-care centers and the work loss of parents for taking care of the sick children, would be additional expenses spent for children with influenza (Heikkimen T, et al. 2004; Principi N, et al. 2004) Thus, influenza in children should be addressed as one important disease among health care workers, parents, and public health administrators.

## 2.2 General Epidemiology and Clinical Manifestations of Influenza in Children

### 2.2.1 Clinical Manifestations of Influenza in Children

The clinical symptoms after influenza virus infection are similar to a common cold, involving, fever, cough, running nose, throat pain, malaise, and myalgia**.** Children infected with influenza virus infection can develop similar clinical symptoms like adults, but they might have higher possibility to develop gastrointestinal tract syndromes such as diarrhea (Wang YH, et al. 2003). In addition, severe complications, for example, acute otitis media, pneumonia, sinusitis, had been documented to attack healthy children (Heikkinen T, et al. 2004).

### 2.2.2 Epidemiology of Influenza in Children

#### A. Incidence Rate and Attack Rate

Infants and young children, with lower immunity, are susceptible to influenza virus infection. Among different age groups, the attack rate of preschool and school children is the highest for over 30% (Monto AS, et al. 1993). The influenza surveillance conducted during the 2005-2006 influenza season in Taiwan also showed that schoolchildren had about 25.36% naturally infection rate (Lin CY. 2006). With such high attack rates, influenza accounted for more than 7% of all pediatric respiratory infections (Heikkimen T, et al. 2003)

#### B. Seasonality

Since influenza virus transmits through aerosol droplets and causes respiratory infection, it has higher activity during winter season. Influenza season in Taiwan involves two peaks: 1) winter flu: the large peak starts from every November to February of the following year, and 2) summer flu: the other small peak starts from March to June of each year (Hsieh YC, et al. 2005).

#### C. Influenza Cluster Cases

When children have influenza, they will shed with higher amount of the virus and longer duration than adults. They could disseminate the virus for 10-14 days after the onset of symptoms (Nicholson KG. 1998). Under these circumstances, it is much easier to spread influenza virus from infected children to other healthy children, their siblings, family members, elderly people, or child-care workers. Once influenza viruses transmit through the daily-contact web, it might initiate outbreaks at schools and households to affect the daily life of healthy children and their care-takers (Neuzil KM, et al. 2002).

## D. Risk and Protective Factors

### 1. Risk Factors

Age of the sick person or the contacts is an important risk factor among household influenza transmission (Viboud C, et al. 2004). Children aged 6-15 years had hazard ratio 1.68 (95% CI=1.07-2.65, p=0.02) times higher than those aged older than 15 years to transmit the disease. On the contrary, healthy household aged 6-15 years had also slightly hazard ratio 1.12 (95% CI=0.73-1.71, p=0.60) times higher in contracting influenza than those aged older than 15 years. The younger the patient or the household contact is, the higher the chance to transmit or to contract the virus will be.

The socioeconomic status of children is another predictor to influence the morbidity of respiratory disorders. One study from 225 children with 9.5 mean age surveyed in Poland showed that material condition, the mother's education, and socioeconomic status of the children were significant factors to affect the respiratory disease morbidity of the children (Pawlinska-Chmara R, et al. 2007). Better material condition (p=0.028), higher education levels of the mother (p=0.011), and the higher socioeconomic status of the children (p=0.045) caused lower respiratory illness incidence, including influenza. Although the growth conditions of the children such as body mass index and height, of the children had no statistical significance to their respiratory morbidity, children who were shorter and fatter suffered more often from respiratory illness.

### 2. Protective Factors

Children with history of prior previous influenza vaccination is an important protective factor to prevent from the influenza because their induced baseline antibody

serotiters were much higher after vaccination and thus were more capable to defend against the influenza virus than those without immunization history (Neuzil KM, et al. 2001).

## 2.3 Influenza Vaccines

### 2.3.1 Pediatric Influenza Vaccines

**A. Types of Pediatric Influenza Vaccines and Reactogenicity**

Vaccination is one way to prevent influenza. Influenza vaccination is annually provided for children in October-November, the time before the influenza season. For those children who could not receive influenza vaccine before the influenza season, they are generally recommended to have vaccine shots before the end of each year to induce protective immunity as early as possible.

Two types of commercialized influenza vaccines have been used in many countries (Wright PF. 2006). One is a traditional trivalent inactivated influenza vaccine (TIV), and the other one is recently developed trivalent live-attenuated influenza vaccine (LAIV). TIV is injected intra-muscularly and some people might present swelling and pain for a few days after the vaccine injection (Belshe RB, et al. 2000). It has been distributed to many countries in the world, including Taiwan. LAIV is delivered through intra-nasal sprayer to aim at simulating the natural infection of influenza (Ambrose CS, et al. 2006). It also reduces the pain and psychological fear to have a vaccine shot like TIV. Those people who are allergic to egg proteins are not recommended to receive the influenza vaccine because the vaccines are produced by eggs. The recipients of both types of influenza vaccines might develop influenza-like illness (ILI) within days after the

vaccination while the human immune system produces antibody against the vaccine antigen. The ILI and other symptoms/signs that are probably related to the influenza vaccination are called reactogenicity. The severity of reactogenicity differs from person to person. Fever, malaise, myalgia are often recorded as reactogenicity after trivalent influenza vaccines (Neuzil KM, et al. 2001).

**B. Selection of Virus Strains for Influenza Vaccine**

Since influenza virus constructs with segmented RNA genomes which easily cause nucleotide changes and sometimes amino acid changes called “antigenic drift”, the selection of virus strains as the three components of influenza vaccine- subtype A/H1N1, A/H3N2, and B viruses have to be reevaluated every year depending on the dominant circulating virus strains of these three viruses. World Health Organization (WHO) collects influenza virus strains from the widely distributed “Influenza Collaborating Laboratories” to predict the probable circulating virus strains for the coming influenza season. For the northern hemisphere, WHO announces the recommended influenza vaccine components in each February and the vaccine companies subsequently follow the recommendation to manufacture the human seasonal influenza vaccines.

Although WHO coordinated the global influenza virus data, several factors, including the type/subtype of the virus and geographical variations, may affect the matching between the predicted influenza vaccine strain and the up-coming circulating epidemic strains. Among three influenza components in the vaccine, A/H3N2 viruses, the most virulent subtype, vary most frequently and cause more often epidemics than A/H1N1 subtype and B type viruses (Frank AL, et al. 1985). In Canada, analysis of seasonal changes of wild-type influenza viruses from 1980 to 1992 found that the similarity

between the wild-type circulating viruses and the predicted vaccine strains was highest for A/H1N1 subtype (99%), and 65% for both A/H3N2 subtype and B type (Ellis E, et al. 1998).

Despite of the subtype variability, geographical difference is another factor to influence the effectiveness of influenza vaccine. For example, East Asia is the epicenter of influenza, the newly arisen influenza viruses often show up earlier than those in eastern countries (Cox NJ, et al. 1994). From the epidemiological point of view, it is essential to fully understand the dynamic changes of circulating influenza virus strains and then according to these findings to predict probable circulating strains on national and regional basis.

**C. The Schedule and Doses of Pediatric Influenza Vaccine Immunized at Different Ages**

Infants and young children are recommended to receive annual influenza vaccination. In the USA, the Advisory Committee on Immunization Practices (ACIP) of the Centers for Diseases Control and Prevention (US-CDC) has initiated an influenza vaccine policy for 24-59-month old children in 2002. Later in 2004, the vaccination program extends to include 6-23-month old children (US-CDC, 2003).

In general, one dose of influenza vaccine is recommended for children, similar to the vaccination program for adults and elderly in the USA. However, two doses of influenza vaccine with the time interval of four weeks are recommended to be delivered before December of the year for younger than 9-year old children who have never been vaccinated before to induce protective humoral immunity (US-CDC, 2007). In global perspective, six additional countries (Australia, Canada, Chile, Korea, Mexico, and

Taiwan) have provided free influenza vaccine for this age group (Macroepidemioogy of Influenza Vaccination (MIV) study group, 2005). For the inactivated influenza vaccines intramuscularly given to children aged older than 3 in USA and Taiwan, it contains 15 $\mu$g antigen of hemagglutinin (HA) protein per 0.5 ml (US-CDC, 2007).

In Taiwan, nation-wide mass-immunization of inactivated influenza vaccine as public health policy with free charge has been implemented to initially aim at 6-23-month old children since 2004. Because more pediatric severe hospitalized and fatal influenza cases due to influenza B viruses occurred and several school classes were stopped in Taiwan in early 2007, the nation-wide mass-vaccination of influenza started to cover grade 1-2 elementary pupils since 2007, and extended to involve grade 1-4 elementary schoolchildren in 2008.

### 2.3.2 Influenza Vaccine Policy and Coverage Rates for Children in Taiwan

Very few papers published about the influenza vaccine coverage in children in Taiwan. In one survey conducted in young children in 2004 in Taiwan, the influenza vaccine coverage rates for 240 participated children younger than 3 years were 62.50% (150/240). Of 150 vaccinated children, higher percentage of children [64% (96/150)] received two doses of human influenza vaccine (Wu SC, et al. 2005). The influenza vaccine coverage rates for 6-23-month-old children who had received before and received one dose, first-time received but only received one dose, or complete two doses in 2005 were 72.90%, 64.0% and 46.50%, respectively (Chang CW, et al. 2007). Such influenza vaccine coverage rates among those children of the same age group who had received before and received one dose, first-time received but only received one dose, or complete two doses were quite the same in 2006 (about 70%, 60% and 40%,

respectively) but even dropped to about 60%, 40% and 30%, respectively in 2007. These decreases may be due to the misunderstanding after mass media broadcasting on minute mercury content in influenza vaccine (Asia Media).

The influenza vaccine coverage rates among grade 3-5 elementary schoolchildren delivered in 2005 was carefully evaluated by subsequent influenza serologic surveillance and found that such rates were much higher in schoolchildren in urban area than those in rural area and the vaccine coverage rate was the lowest in isolated Kinmen islet before the nationwide free vaccination program for schoolchildren started in 2007 (Lin CY. 2007). In other words, the influenza vaccine coverage rate among schoolchildren in the era of self-payment was quite low (mean: 6.10%) and varied from 0-19.44%.

In 2007, Taiwan government initiated an influenza mass-vaccination program for grade 1-2 elementary schoolchildren. This nation-wide vaccination policy in schoolchildren is the first large-scale influenza vaccination in Asia after Japan's public health program to vaccinate for 6-15-year-old children during the years of 1962-1987 (Reichert TA, et al. 2001). Among those vaccine recipients, the coverage rate of influenza vaccine was almost 70% (TW-CDC, 2008).

## 2.4 Methods to Evaluate Vaccine

### 2.4.1 Vaccine Efficacy and Vaccine Effectiveness

In vaccine evaluation studies, there are direct or indirect protection related to the vaccine used. The direct protection is to evaluate the reduction of illness or disease at an individual level of the vaccine recipients. The indirect protection is to assess the

decreasing of incidence rate of the disease to be protected by the studied vaccine at a population level. To measure the public health impact of mass-vaccination, two terms, “vaccine efficacy” and “vaccine effectiveness”, are usually used.

“Vaccine efficacy” is often used in vaccine clinical trials to evaluate the immunity effect of the vaccine given to study participants directly derive at an individual level. However, “vaccine effectiveness” has been applied to measure the direct and indirect protection of the tested vaccine at a population level through observational epidemiological studies. Thus, herd immunity can be obtained from calculating vaccine effectiveness (VE). Although the two terms have different meanings, the distinction between them has been misused under certain circumstances in several studies (Chen RT, et al. 1996). In this thesis study, we used the term of “vaccine efficacy “shown by those serologic data that can be obtained from each participant of the study versus the term of “vaccine effectiveness” revealed by clinical data that are related to transmission of the virus in population without individual information.

In evaluating vaccine, researchers can use the concept of “reducing relative risk” to calculate “vaccine efficacy” or “vaccine effectiveness”. By calculating the incidence (I) in both vaccinated group ($I_{vaccinated}$) and unvaccinated (control) group ($I_{unvaccinated}$), the vaccine efficacy or vaccine effectiveness can be calculated as follows:

$$\frac{\text{Attack rate of unvaccinated children} - \text{Attack rate of vaccinated children}}{\text{Attack rate of unvaccinated children}} \times 100\%$$

.

### 2.4.2 Vaccine Efficacy and Vaccine Effectiveness Studies to Evaluate Trivalent Human Influenza Vaccine

#### A. The Choice of End-point Definition

Using vaccine efficacy and vaccine effectiveness to evaluate vaccine, we should consider the different outcome measures of the end-points that may affect higher or lower sensitivity or specificity. In influenza, the end points can be either influenza virus infection or clinical illness of influenza. In general, laboratory-based serological tests or virus isolation methods or molecular diagnosis by reverse-transcriptase polymerase chain reaction (RT-PCR) can help to determine the presence of influenza virus infection whereas a set of syndrome called “influenza-like illness (ILI)” involving clinical symptoms/signs such as fever, cough, sore throat and others can measure clinical illness of influenza. Both virus isolation and molecular diagnosis are more suitable to be applied to clinical settings where ILI patients are identified and clinical specimens are obtained. However, successful isolation of influenza virus through virological surveillance depends on good specimen collection and laboratory skills, awareness of physician, and best timing of virus shedding while patients having hospital visits. On the other hand, seroepidemiologic investigation offers more precise measures of the sero-prevalence and sero-incidence rates of influenza virus infection against specific type/subtype/strains of influenza viruses at a population level. Healthy individuals with influenza virus infection produce humoral immunity and thus will increase their antibody serotiters after the infection. Therefore, serotiters with 4-fold increase in serological tests from pre- to post-influenza season can be used to assess individuals having influenza virus infection.

Virological end-point definition of having influenza infection is believed to have the highest sensitivity among the three, because certain proportion of the infected persons may not develop clinical symptoms or detectable humoral immunity after influenza virus infection. Therefore, researchers should comprehensively consider the advantage

and disadvantage of each end-point to be used, choose the best available method to measure having influenza infection or illness, and then interpret the results carefully to meet the objective of the study.

**B. Evaluation Studies of Trivalent Human Influenza Vaccine**

**1. Benefit for Vaccinated Schoolchildren and Community**

During the 1968-1969 influenza season when Hong Kong pandemic flu attacked, one community study was conducted to measure the effectiveness of vaccination among schoolchildren receiving TIV in Michigan, USA (Monto AS, et al. 1970). Schoolchildren from kindergarten to high school in the intervention area were vaccinated subcutaneously and those schoolchildren in the control area were unvaccinated for comparison. The results found that the percentage of schoolchildren's absenteeism at schools and the incidence rate of respiratory illness at community were higher in the control area than those in the intervention area. The ratio of excess attack rates was 3.0 times higher in the control area than that in the intervention area. The data proved that the influenza vaccination in schoolchildren had modification effect of influenza outbreak not only in the schoolchildren themselves but also in the community as well. The community gained protection from the vaccination of the schoolchildren.

The second successful example to prove the effectiveness of seasonal influenza vaccine is mass-vaccination program of schoolchildren in Japan from 1960s to 1980s for more than 25 years. Retrospective analysis revealed that all-cause mortality rates declined for total population in Japan in those years implemented schoolchildren's influenza vaccination compared to those in US without such a public health policy (Reichert TA, et al. 2001). Interestingly, the all-cause mortality rates and the mortality

rates of pneumonia and influenza in 1-4-year-old children increased after the stop of mass-vaccination health policy in 1994 (Sugaya N, et al. 2005). Comparing the trends in those rates through consecutive years showed that the mass-vaccination of schoolchildren was associated with the reducing influenza-associated mortality rates.

**2. Influenza Vaccine Efficacy**

Several clinical trial studies had measured the influenza vaccine efficacy ranged from 56% to 100%. A randomized, double blinded, placebo-controlled study calculated that the vaccine efficacy of one-dose inactivated trivalent influenza vaccine (TIV) among 6-9-year-old US schoolchildren in preventing laboratory-confirmed influenza illness (serological or culture evidence) was 56% in 1985 (Cruber WC, et al. 1990). The vaccine efficacy of TIV influenza vaccine in another study among 3-9-year-old 63 children against the drifted influenza A virus was also 56% (Clover RD, et al. 1991), based on preventing serological- or culture-proved influenza virus infection. However, the vaccine efficacy of TIV influenza vaccine among 64 children aged 6-10 years reached 100% against the influenza vaccine strains (Piedra PA, et al. 1991) through a randomized double blinded, placebo-controlled study using the same outcome end-pint measures. However, the vaccine efficacy should be lower than 100% if it would be measured by serologic tests against the circulating wild-type influenza viruses.

Influenza vaccine efficacy varied by the types/subtypes of influenza viruses have also been demonstrated in different pediatric studies. In a 5-year prospective study enrolled 259 healthy American children aged 6-10 years who received trivalent inactivated influenza vaccine from 1985 to 1990, the vaccine efficacy against wild-type circulating strains was quite similar for H1N1 and H3N2 viruses [eg. 76.1% (95% CI= 53.0, 87.9)

for A/H1N1 epidemic years and 73.8% (95% CI= 37.4, 89.1) for A/H3N2 epidemic years] in preventing the infection shown by serological evidence with 4-fold serotiter increase (Neuzil KM, et al. 2001). Another prospective non-randomized TIV vaccine controlled trial study recruited 137 Japanese children with moderate to severe asthma during the 1992-1993 influenza season and showed that, the vaccine efficacy against the drifted A/H3N2 virus, matched B virus, and both of them were 67.5%, 43.7%, and 42.1%, respectively ($p<0.1$) (Sugaya N, et al. 1994). In other words, influenza inactivated vaccine may not be 100% protective for asthma children. Since age is a strong confounder, data analysis stratified by age of children with asthma found that the vaccine efficacy for younger than 7 year-old children who received two doses of TIV subunit-antigen influenza vaccine was lower for both drifted A/H3N2 and matched B viruses than that for children aged ≧7 years (A/H3N2: 53.5% vs. 78.1%; B: 22.3% vs. 60.0%) (Sugaya N, et al. 1994). Interestingly, 2-14-year-old asthma Children still had influenza virus infection with their serotiter 1:128 against the drifted A/H3N2 virus and serotitier 1:256 against B virus at 3-4 weeks post-vaccination (pre-epidemic season). These results further demonstrated that the vaccine efficacy of the influenza split antigen vaccine was not high enough to provide protection for children, especially those younger than aged 7. The vaccine efficacy of two-dose TIV among 145 US children, 24-60 months old attended day care centers during the 1996-1997 influenza season was quite low for H3N2 [0.31 (95% CI= -0.95, 0.73)] and B [0.45 (95% CI= 0.05, 0.66)] viruses, based on the influenza virus infection shown by 4-fold serotiter rises , evaluated the efficacy of two dose of TIV (Hurwitz ES, et al. 2000). Most importantly, children with pre-vaccination serotiter ≦1:5, who were less likely to induce 4-fold serotiter increases to the vaccine strains or high serotiters at post-vaccination, were more likely to acquire further influenza virus infection shown by serological evidence against

A/H1N1, A/H3N2, and B vaccine virus strains. Therefore, the factor of the lower serotiters at pre-vaccination might reduce the serotiters at post-vaccination, protection against influenza virus infection, and the overall vaccine efficacy as well.

In recent years, a review article published in 2004 pooled five studies to analyze the vaccine efficacy among <9 years-old children who received two doses of TIV. The results found that the overall vaccine efficacy in the first year of vaccination was 63% (95% CI= 45, 70), assessed by culture positivity or serotiter increases (Zangwill KM, et al. 2004).

In summary, several factors including the end-points measured, the vaccine strains matched or mismatched with the wild-type circulating influenza viruses, the numbers of doses, the type of vaccine used, the distributions of age and gender in the study population, all together should be considered in comparing the vaccine efficacy among different studies.

**3. Influenza Vaccine Effectiveness**

The vaccine effectiveness of different influenza vaccines for younger children has been mostly evaluated by clinical respiratory illness after the influenza season. During the 1995-1996 influenza season in Sardinia, Italy, the vaccine effectiveness for 344 1-6-year-old children who received two doses of inactivated-split influenza vaccine compared with unvaccinated was 67% [12.4% (22/177) vs. 37.7% (63/167), 95% CI= 59%~74%], measured by ILI including fever and cough or sore throat lasting longer than 72 hours (Colombo C, et al. 2001).

In the following 1996-1997 influenza season, the overall vaccine effectiveness of

trivalent influenza vaccine among 127 24-60-month-old day-care children and their 328 household contacts ranged 50%~80%, based on the same respiratory clinical criteria as the above-mentioned study (Hurwitz ES, et al. 2000). The school-aged contacts without receiving influenza vaccine of the vaccinated children had significantly lower attack rates of respiratory illness than those of the unvaccinated children (p=0.007~0.010). Besides, the vaccine effectiveness was 45%~72% in reducing the respiratory-related morbidity among the unvaccinated household members aged 5-17 years. The study provided the solid evidence that young children with influenza vaccination protected from the household contacts from influenza-related morbidity and respiratory illness effectively.

## 2.5 Immunogenicity and Reactogenicity of Trivalent Influenza Vaccines in Children

### 2.5.1 Immunogenicity of Trivalent Influenza Vaccines (TIV) in Children

Immunogenicity of TIV is frequently evaluated by seroconversion rate (from seronegative to seropositive), sero-protection rate (serotiter $\geqq$1:40), geometric mean titer (GMT) and sero-incidence rate of influenza virus infection demonstrated by 4-fold serotiter rise. Sero-protection rate is commonly used by vaccine industry whereas GMT can provide more quantitative information, particularly above what levels of the serotiter that would be protective from influenza virus infection. Furthermore, the combination of GMT and sero-incidence can offer how the baseline serotiter might be associated with the new infection.

Sero-protection rates are closely related to the baseline serotiter before vaccination. A 5-year follow-up study from 1985 to 1990 involved 259 6-10-year-old children whose serotiters at pre-vaccination baseline were higher than 1:10 (eg. seropositive), showed much higher boosting effect of the levels of antibody at post-vaccination after receiving one dose of TIV than sero-negative children, regardless of A/H1N1, A/H3N2, or B viruses (Neuzil KM, 2001). The higher the serotiters before vaccination, the better seroprotection rates will be.

In most studies, sero-protection rates of TIV against the influenza vaccine strain right after the first dose of TIC were quite high as 83%~100% for 42 French children at 8-10 years of age in 1991, 1993, and 1995 (Lina B, et al. 2000). However, two doses of TIV for 83 German children of 6-month to 12-year of age did increase the sero-protection rates for all three components of influenza vaccine viruses (Schmitt-Grehé S, et al. 2001). The sero-protection rates at post-vaccination from the first dose to the second dose were from 75% to 96% for A/H1N1 virus, from 83% to 100% for A/H3N2 virus, and from 33% to 96% for B virus (Schmitt-Grohé S, et al. 2001). In other words, the sero-protection rate was mostly increased for B virus. Similar findings on significant higher sero-protection rates were revealed among Seattle children after receiving two doses of TIV than those vaccinated with one dose TIV for A/H1N1 ($p<0.001$), A/H3N2 ($p=0.01$), and B ($p<0.001$) viruses (Neuzil KM, et al. 2006).

The major public health question is that under what conditions that 2-dose trivalent influenza vaccine (TIV) is recommended based on what type of data. One Seattle study on 222 children during the 2004-05 influenza season demonstrated that 5-8-year-old children, whose serotiters were $\geqq$ 1:10 before the vaccination, induced sufficient antibody serotiters even after a single dose TIV with higher sero-protection rates and

higher GMTs against each vaccine strains at post-vaccination (Neuzil KM, et al. 2006). On the other hand, children with serotiter <1:10 (eg. sero-negative) before the vaccination would need 2 doses of TIV to induce sufficient serotiers. These results supported the recommendation that children younger than 9 years of age should receive two doses TIV for the first time to have adequate immune response. To determine the serotiter threshold for sero-protection, immunogenicity of TIV among 97 German children at 6-9-years of age was analyzed during 2005-06 influenza season. The sero-protection rates were 64.9% (95% CI= 54.6, 74.4) for A/H1N1, 93.8% (95% CI= 87.0, 97.7) for A/H3N2, and 71.1% (95% CI= 61.1, 79.9) for B viruses. The GMT of B viruses was the lowest [97.7 (95% CI=68.6, 139.2)], compared to A/H1N1 [290.4 (95% CI= 165.5, 509.3)], and A/H3N2 [381.2 (95% CI= 281.3, 516.6)] viruses. Detail analyses found that one dose vaccine given to children aged ≧8 years were sufficient for them to have ≧70% sero-protection, which met the criteria of the European Committee for Human Medicinal Products (CHMP) for assessing influenza vaccine in adults aged 18-60 years. Again, younger children need to have two doses of TIV and the baseline serotiter prior to vaccination influenced the GMT and sero-protection rates at post-vaccination. These results explain the age difference in vaccine efficacy that older age may encounter more frequent of prior infection and thus booster more immunologic memory responses after vaccination. In fact, one US study recruited 21 young children aged 3-9 years during the 2003-2004 influenza season showed that older children, whose baseline GMT were higher at pre-vaccination, had more percentage of serotiters above the levels of sero-protection but lower rates of their serotiters with ≧4-fold increase (Zeman AM, et al. 2007). However, immunogenicity of TIV was generally higher in children than in adults or elderly (Zhu FC, et al. 2008).

In summary, several factors determine the immunogenicity of influenza vaccine, including the baseline levels of serotiters before vaccination (Neuzil KM, et al. 2001), number of doses of vaccine (Schmitt-Grohé S, et al. 2001), history of vaccination and influenza virus infection (Zeman AM, et al. 2007), age (Neuzil KM, et al. 2006; Zeman AM, et al. 2007; Zhu FC, et al, 2008), and type of vaccine (Zhu FC, et al. 2008; Ashkenazi S, et al. 2006; Fleming DM, et al. 2006).

### 2.5.2 The Usual Reactogenicity after Vaccination

Safety is the most important public health issue in vaccine evaluation. Both direct and indirect measures of safety have been used. Safety data of TIV after injection are usually recorded as clinical symptoms signs developed after the vaccination to directly measure the reactogenicity and adverse events related to the vaccine occurring from the study participants. Both local and systematic reactions should be assessed very carefully by experienced physicians. Besides direct measures, the indirect measure such as absenteeism at one-day post-vaccination had been employed in one study conducted during the 1968-1969 influenza season (Monto AS, et al. 1970).

Local reactions are generally mild and last only for a few days whereas systematic reactions can be more severe. In influenza vaccine, pain at injection site was mostly reported local reaction, regardless one or two doses delivered (Neuzil KM, et al. 2006; Schmidt-Ott R, et al. 2007; Zhu FC, et al. 2008). In addition, redness, swelling, indurations, itching were often recorded local reactions as well.

Fever and headache were mostly presented two major systematic reactions after vaccination. Other symptoms developed after vaccination such as cough, coryza, sore

throat, and malaise were often recognized (Lina B, et al. 2000; Neuzil KM, et al. 2001; Schmidt-Ott R, et al. 2007; Zhu FC, et al. 2008). Young children received vaccine were more often to develop systematic reactions (Neuzil KM, et al. 2001). Although adverse events related to vaccine have been reported in several studies (Schmidt-Ott R, et al. 2007; Mitchell DK, et al. 2005), most of them were excluded as non-relevant to vaccine shots.

In summary, because of annual change of virus components in TIV and different characteristics of study participants, reactogenicity and safety of each TIV should be monitored. The safety data are needed to verify the quality of each TIV and the immunologic fitness of vaccine antigen to vaccine recipients.

# Chapter 3 Objectives, Specific Aims and Hypotheses

## 3.1 Objective

The objective of this study aimed to evaluate the efficacy and effectiveness of human influenza vaccine against the different types and subtypes of influenza viruses and the reactogenicity after the vaccination among grade 1-2 elementary schoolchildren who were first enrolled to participate influenza mass-vaccination program during the 2007-2008 influenza season.

## 3.2 Specific Aims

Five major specific aims in the study were:

1. To measure the effectiveness of influenza vaccine in reducing serological-confirmed influenza virus infection, influenza-like illness (ILI) cases, and absenteeism and hospitalization rates between vaccine recipients and unvaccinated.
2. To evaluate the possible effectiveness of influenza vaccine in providing cross protection for family members from influenza-like illness (ILI).
3. To monitor the serotiter changes from pre-vaccination to post-vaccination against different types and subtypes of human influenza viruses (A/H1N1, A/H3N2, and B) among schoolchildren at schools in different geographical areas.
4. To search for factors involved in anti-influenza antibody persistence and waning.
5. To assess the post-vaccination reactogenicity among schoolchildren.

## 3.3 Hypotheses

Our proposed hypotheses were as followed:

1. The 2007-2008 influenza vaccine would provide sufficient levels of antibody against the influenza vaccine strains.
2. The immunogenicity against the three vaccine viruses was different among schoolchildren attended at different schools located in various geographical areas.
3. Influenza vaccination for schoolchildren might decrease the occurrence of ILI at both individual and household levels.
4. The higher the serotiter at 1-month post-vaccination, the less likely of that individual to acquire the influenza virus infection during the time periods between 1- and 4-month post-vaccination.
5. The reactogenicity after vaccination would differ from person to person.

# Chapter 4 Materials and Methods

## 4.1 Study Design and Study Populations

A cohort follow-up study design was used to follow schoolchildren from September, 2007 (pre-influenza season) to April, 2008 (post-influenza season). We enrolled grade 1-2 elementary schoolchildren from one school in Taipei City, three schools in Yilan County, and two schools in Kinmen County. The study areas were chosen because of their regional importance; Taipei is a metropolitan city with high population density that influenza virus might spread easily and people live in Taipei City have shared more medical and public health resources would be healthier than people live in other areas. Yilan, a rural area of eastern Taiwan with many rice paddies and ponds for the habitats of migrating birds and duck farms, has had very frequent travelers coming back and forth between Taipei City and Yilan County since the opening of Syue-Shan tunnel in June, 2006. Several low pathogenic avian influenza (LPAI) virus strains of A/H5N2 had been isolated in 2004, 2005 and 2006. Kinmen, an isolated islet located in western Taiwan and closer to Xiamen of mainland China, many people have travelled between the two places to make it serve as the sentinel sites for possible novel influenza virus that would come from China into Kinmen. In fact, highly pathogenic avian influenza virus H5N1 was identified in Kinmen from the red-face ducks（紅面鴨）smuggled from China in December of 2003 and the virus was proved to be closely related to H5N1 in China (Lee MS, et al. 2007).

Six elementary schools participated in the study after our visit and the agreement of the school principals. Serum samples at three time-points, 2-3-week before vaccination, 4-7-week post-vaccination, and about 4-month (15-20 weeks) post-vaccination, were collected from schoolchildren recruited in the study after obtaining the informed

consent of their parents or guardians (Table 1) prior to blood taking in each time-point. The first serum samples were collected prior to the influenza vaccination during October-November of 2007. Local department of health administered the influenza vaccination at schools and provided one dose of inactivated influenza vaccines to those children whose parents agreed to receive vaccination. For the schoolchildren in the study, most of them received the *Fluvirin* influenza vaccine with 10 doses per pack produced by Novartis Vaccines and Diagnostics Limited, UK, and only few students received influenza vaccine produced by other companies because of receiving vaccine outside of schools. Four weeks later, the second serum samples were collected at 4-7-week post-vaccination during December, 2007-January, 2008. In Taiwan, the human influenza season generally started in November-December and peaked around January-February (Hsieh YC, et al. 2005). After the influenza season, the third serum samples were then obtained with informed consents during March-April of 2008. Only schoolchildren who participated at the previous time-points were followed in the cohort study and the serum samples were taken only after their parents' signed informed consents.

## 4.2 Data Collection

Data about demographic information, protective and risk factors related to influenza infection of the study subjects, including history of past influenza vaccination, daily sleeping hours, nutrition-taking were collected using questionnaire (Appendix E.1~3) filled out by schoolchildren's parents or guardians. In addition, data of schoolchildren's 2007-2008 influenza vaccine history, height, and weight were provided by school nurses. After their influenza vaccination, reactogenicity of the influenza vaccine from

schoolchildren were recorded by the specific designed table (Appendix E.2). The symptoms and signs related to influenza-like illness (ILI) were also recorded by using questionnaire filled out by parents or school teachers. To measure the effect of household protection, family size and basic information related to each family member were recorded by schoolchildren's parents.

The study protocol was approved by the Ethics Committee of the Taipei City Hospital (Official no: TCHIRB-970209-E) and the College of Public Health at National Taiwan University (Official approved on October 1, 2007).

## 4.3 Laboratory Methods

### 4.3.1 Serum Samples Treatment

Whole blood Serum samples with 3~5 cc were collected in serum tubes and stored at low temperature after blood-taken. The samples were centrifuged at 4℃, 1,200 rpm/ 10 min within 24 hr to separate serum from blood cells. They were collected in 1.5 ml microtubes and then stored at -20℃ until testing.

### 4.3.2 Receptor Destroying Enzyme (RDE) Treatment

To remove non-specific receptors which might interfere with serum antibody and antigen reaction, serum samples were treated with receptor destroying enzyme (RDE, SEIKEN cat# 340122) using the protocol in the Appendix D.1 prior to the testing by hemagglutination inhibition (HI) assay.

### 4.3.3 Identification of Non-specific Agglutinin in Treated Serum Samples

To check non-specific receptors were completely destroyed after RDE treatment, the serum samples should be tested for identification of non-specific agglutinin as the protocols in Appendix D.2. PBS solution was used as a negative sample.

### 4.3.4 Human Influenza Virus Strains

The influenza viruses used in the serological testing were the 2007-08 influenza vaccine strains, A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 viruses recommended by the WHO for northern hemisphere. The viruses were obtained from Taiwan-CDC and then prepared for two passages in MDCK cell-culture.

### 4.3.5 Hemagglutination (HA) Assay

The HA assay was initially used to measure antigen concentration of influenza viruses. An HA unit is defined as the amount of virus needed to agglutinate an equal volume of a standardized RBC suspension. Duplicated antigen samples were tested in HA assay (Appendix D.3). After determining the HA titer of human influenza viruses, the final concentration of HA used in the HA assay was 8 HA units/50ul.

### 4.3.6 Hemagglutination Inhibition (HI) Assay

The rationale of this test is that the anti-influenza HI specific antibody can agglutinate the hemagglutinin (HA) protein on the surface of influenza virus and thus causes hemagglutination inhibition so that RBCs are no longer able to interact with the virus HA antigen. Duplicated serum samples were tested in HI assay (Appendix D.4) simultaneously with virus control, positive and negative serum controls. Positive serum controls were serum with higher serotiter and negative serum controls were PBS

solution.

## 4.4 Outcome Analyses

### 4.4.1 Outcome Measures

Four serologic measurements were used to evaluate the 2007-2008 influenza vaccine for participants only. The clinical measurements involved both systematic and local symptoms/signs were applied for ILI. Eight symptoms and signs including : (1) four systematic symptoms/signs (fever, chills, myalgia or joint pain, and tiredness) and (2) four respiratory symptoms/signs (sore throat, rhinorrhea or nasal congestion, cough, and raucous). Vaccine efficacy was evaluated by comparing anti-influenza HI antibody at an individual level of each participant with paired or triple serum taken between the vaccinated and unvaccinated groups, using serological measures. Vaccine effectiveness was calculated as the comparison of clinical attack rates between the vaccinated and unvaccinated group with the following formula.

$$\frac{\text{Attack rate of unvaccinated children} - \text{Attack rate of vaccinated children}}{\text{Attack rate of unvaccinated children}} \times 100\%$$

The four serologic measures were defined as follows,

1. Natural infection of influenza was defined as anti-influenza HI antibody with at least 4-fold serotiter rise against the testing influenza viruses from pre-vaccination to 1-month post-vaccination for unvaccinated schoolchildren or from 1-month to 4-month post-vaccination for all schoolchildren.
2. Geometric mean titer (GMT) was calculated and the serotiter of schoolchildren $<$ 1:10 was regarded as 1:5 for calculation.

3. Sero-protection rate was defined as the percentage of the number of schoolchildren with their serotiters ≧1:40 divided by the total number of participants in each analyzed group at each time-point.
4. Serotiter fold-changes was defined as the participant's serotiter at 1-month post-vaccination divided by the serotiter at pre-vaccination, and the participant's serotiter at 4-month post-vaccination divided by the serotiter at 1-month post-vaccination. After calculating the serotiter fold-changes, the percentage of serotiter 4-fold rise was obtained from the percentage of schoolchildren with serotiter equal to and higher than 4-fold rise.

**4.4.2 Data Analyses**

To realize the variations of receiving influenza vaccination, demographic, risk and protective factors related to influenza infection were compared between vaccinated and unvaccinated children. Schoolchildren presented anti-influenza HI Ab serotiter 4-fold rise or higher, ILI, absenteeism and hospitalization were further calculated to obtain the vaccine efficacy and vaccine effectiveness of 2007-08 human influenza vaccine between vaccinated and unvaccinated children. Moreover, to understand the household protection provided from vaccinated schoolchildren, influenza infected children with their anti-influenza HI Ab serotiter 4-fold rise were used to compare ILI presentation among their household members of vaccinated and unvaccinated schoolchildren. The HI serotiters were further used to analyze sero-protection and GMTs between vaccinated and unvaccinated schoolchildren and among three studied areas or six studied schools at pre-vaccination, 1-month and 4-month post-vaccination. At last, frequency of reactions after receiving the influenza vaccine were recorded to estimate safety concerns about the vaccine.

## 4.5 Statistical Methods

To evaluate influenza vaccine, the definition of vaccine effectiveness (VE) mentioned above was used to calculate the VE in reducing influenza morbidity between vaccine and non-vaccine recipients, and the 95% confidence interval of vaccine effectiveness was also obtained.

For descriptive and univariate analysis, student t test and analysis of variance (ANOVA) were used for continuous dependent variables and categorical independent variables, respectively. $\chi^2$ test was used for categorical dependent and independent variables. After univariate analysis, significant parameters were selected for multivariate analysis. Logistic regression was used to analyze impact of influenza vaccine injection on protective or risk factors. The significant $\alpha$ level was 0.05 and SAS 9.1 software was used for statistical analysis.

# Chapter 5 Results

## 5.1 Characteristics of the Study Cohort in Taiwan

### 5.1.1 Participation Rates and Follow-up Rates

The target population involved 2003 schoolchildren in total from the six selected elementary schools in Taipei, Yilan, and Kinmen, including one larger school in each of the three study areas, two other smaller schools in Yilan, and one other smaller school in Kinmen (Table 1, Figure 1). After the first blood-taken at pre-vaccination, 931 students were enrolled into the study with their informed consent from their parents/guardians from Oct 30, 2007 to November 12, 2007, and the overall participation rate was 46.50%. At 1-month post-vaccination, there were 688 schoolchildren still followed with total follow-up rate of 73.90% from December 9, 2007 to January 4, 2008, and overall participation rate of 34.35%. Later, at 4-month post-vaccination, 598 schoolchildren left at the end of the influenza season, and the total follow-up rate decreased to 64.23% from March 19, 2008 to April 3, 2008, and the overall participation rate dropped to 29.86% (Table 2).

In general, schools in Yilan (35.63%~47.57%)had higher participation rates than schools in Taipei (20.40%) and Kinmen (22.40%~23.33%), despite of the time-points of blood-collection. In addition, children in Yilan (69.51%~73.13%) had higher follow-up rates than children in Taipei (57.63%) and Kinmen (55.34%~57.53%) as well. Because the collected serum were used for serological assay to measure the immunogenicity of the trivalent influenza vaccine given during the 2007-2008 influenza season, only the available paired or triple serum samples of schoolchildren collected at three time-points of pre-vaccination, 1-month and 4-month post-vaccination or the first two time-points were chosen for the study samples for further analysis. Besides, among all 688 children,

11 children were excluded from analysis because their delayed time schedule of influenza vaccination or unknown date of influenza vaccination. Finally, 677 schoolchildren, involved 590 schoolchildren with all-three serum samples and 87 schoolchildren with the first two paired serum sample were compiled for data analyses.

### 5.1.2 Influenza Vaccine Coverage Rates and Relationship with Natural Infection Rates

The overall vaccination coverage rate among the six elementary schools was 63.92% (1279/2003), ranged from 46.67% (84/180) at KM-JH school to 83.50% (172/206) at YL-LZ school (Table 2). The vaccine coverage rates varied at different schools with statistical significance ($p<0.0001$). YL-LZ School located in rural area and YL-LT school located in suburban area had relatively high influenza vaccination rates with 83.50% (172/206) and 72.10% (323/448), respectively, and the two schools in Kinmen had relatively low influenza vaccination rates with 50.10% (255/509) for KM-JJ school and 46.67% (84/180) for KM-JH school.

Of the total 677 schoolchildren with available serum samples and information for data analysis in the study, the vaccine coverage rates were 85.06% for 87 paired serum samples collected at only the first two time-points and 84.41 % for 590 triple serum samples obtained at all the three time-points.

To search for possible association between vaccine coverage rates and influenza infection rates among children with evidence of 4-fold anti-influenza HI antibody serotiter rise, the two rates among the six elementary schools were plotted (Figure 2, Table 3). The results showed that vaccine coverage rates of total target population involved 2003 students were weakly correlated with the influenza infection rates

identified from the positive 27 influenza virus infected children at the six schools ($R^2$=0.15, p=0.44). However, such a correlation became higher ($R^2$=0.26, p=0.30) between vaccine coverage rates of the studied 677 participants and the influenza infection rates (n=27), indicating lower vaccine coverage rates might lead to subsequent higher influenza infection rates.

### 5.1.3 Demographic Analysis of the Participants

Of 677 participants, there were 572 (84.5%) students received the influenza vaccine and 105 (15.5%) unvaccinated children during the 2007-2008 influenza season (Table 4). The demographic analyses showed that there were no differences in the mean age, mean BMI, grade, gender, blood-type, and ethnicity between the vaccinated and unvaccinated schoolchildren (p>0.01). However, the only variable significantly exhibited the difference between these two groups was the percentage of vaccinated children in the six schools (p<0.0001). The three schools of YL-SS, KM-JJ, and KM-JH had higher unvaccinated schoolchildren than the other three schools enrolled into our study population.

### 5.1.4 Risk Factor Analysis between Vaccinated and Unvaccinated Children

Based on the demographic information, children's daily life and medical history provided by schoolchildren's parents and guardians, both risk or protective factors between the vaccinated and unvaccinated schoolchildren were analyzed (Table 5). In risk factors, unvaccinated groups had significantly higher percentage of school absenteeism during the influenza season, lower education levels of schoolchildren's parents/guardians, less post-school activities and slightly longer mean of daily sleeping hours than vaccinated group (p<0.02). In protective factors, vaccinated group had only

significantly higher percentage in history of the 2006~2007 influenza vaccine than unvaccinated group (p=0.0455). In addition, unvaccinated group also had higher disease history than vaccinated (58.5% vs. 48.1%, p=0.0798)

### 5.1.5 Distribution of Anti-influenza HI Antibody Serotiters and GMTs between the Vaccinated and Unvaccinated Children

The distribution of HI serotiters of anti-influenza antibody among schoolchildren stratified by with and without receiving the 2007-2008 human influenza vaccine at each of the three time-points was shown at Table 6. At pre-vaccination, most anti-influenza HI antibody serotiters were less than 1:80 regardless of vaccination for A/H1N1 (p=0.95), A/H3N2 (p=0.98) and B viruses (p=0.03). At 1-month post-vaccination, higher percentage of HI serotiters $\geq$1:160 for A/H1N1, $\geq$1:320 for A/H3N2, and $\geq$ 1:80 for B viruses increased strikingly for vaccinated than unvaccinated schoolchildren. At 4-month post-vaccination, such patterns still remained but with declining trends of serotiters, particularly for B virus. In other words, vaccination indeed increased the distribution trends in higher serotiters at 1-month post-vaccination ($p<0.05$ for all three viruses). Although antibody waning did occur at the ending period of influenza epidemic season, quite high percentage of higher serotiters for most frequent A/H3N2 subtype can still be maintained ($p<0.05$ for all three viruses). The most percentage of serotiter against A/H1N1 virus in vaccinated children changed from 1:40 (n=275, 48.1%) at pre-vaccination, to 1:160 (n=108, 18.9%) at 1-month post-vaccination, and finally to 1:80 (n=139, 27.9%) at 4-month post-vaccination. The most percentage of serotiter against A/H3N2 virus in vaccinated children changed from 1:40 (n=204, 35.7%) at pre-vaccination, to 1:320 (n=145, 25.4%) at 1-month post-vaccination, and then stayed as 1:160 (n=121, 24.3%) at 4-month post-vaccination. However, the most percentage of

serotiter against B virus in vaccinated children had little change with lower serotiters, comparing those with A/H1N1 or A/H3N2 virus, from 1:20 (n=183, 32.0%) at pre-vaccination, to 1:40 (n=149, 26.1%) at 1-month post-vaccination, and then to the same level as 1:40 (n=143, 28.7%) at 4-month post-vaccination.

Using cumulative percentage of serotiter distribution against the three human vaccine viruses between vaccinated and unvaccinated children**,** the differences in patterns looked more clear (Figure 3). In vaccinated children, the most percentages of serotiter at two post-vaccination time-points were much higher than those at pre-vaccination against the three influenza vaccine viruses. By contrast, the serotiter distribution against the same three influenza vaccine strains in unvaccinated children remained almost little change at each of these three same time-points that the curve almost overlapped each other.

The GMTs of schoolchildren at pre-vaccination for vaccinated and unvaccinated children were similar, but vaccinated schoolchildren had higher GMTs against the three human influenza vaccine viruses than unvaccinated schoolchildren at 1-month post-vaccination (A/H1N1 virus: 161.11 vs. 45.63, A/H3N2 virus: 329.00 vs. 63.64, and B virus: 48.57 vs. 20.42, all $p<0.05$) (Table 6).

## 5.2 Vaccine Efficacy and Vaccine Effectiveness

### 5.2.1 Influenza Virus Infection Prevented

First of all, since not all persons infected with influenza viruses may develop symptoms, it is better to evaluate vaccine by measuring total infection. As all the individuals' serologic data were available before and after the 2007-2008 influenza

epidemic season, vaccine efficacy was evaluated by percentages of positive influenza infection supported by serological HI antibody at least 4-fold serotiter increase between vaccinated and unvaccinated groups from 1-month post-vaccination to 4-month post-vaccination**.** Among 498 vaccinated and 92 unvaccinated schoolchildren with their three serum samples collected at three different times (Table 7A), vaccine efficacy for A/Solomon Islands/3/2006 (H1N1) virus was -29.32% [1.41% (7/498) vs 1.09% (1/92), p=1.00]. The vaccine efficacy for A/Wisconsin/67/2005 (H3N2) virus was 86.14% [0.60% (3/498) vs. 4.35% (4/92), 95% CI= 39.11~96.85, p=0.01], the highest among the three viruses. At last, the vaccine efficacy for B/Malaysia/2506/2004 virus was 38.42% [2.01% (10/498) vs. 3.26% (3/92), 95% CI= -119.47~82.82, p=0.44]. In other words, the one dose inactivated trivalent influenza vaccine prevented the highest infection of A/H3N2 virus but minimized the lowest infection of A/H1N1 virus.

### 5.2.2 Influenza-like Illness Reduced

Next step, we evaluated the effectiveness of influenza vaccine by measuring the systematic and respiratory symptoms and signs (s/s) that were filled out by children's parents/guardians from 1-month post-vaccination (December, 2007~January, 2008) to the ending of the 2007-2008 influenza season (March, 2008). Among total 547 vaccinated and 97 unvaccinated schoolchildren with their signs and symptoms that had been recorded, the vaccine effectiveness in preventing any one of four systematic s/s (fever, chills, myalgia/ joint pain, and tiredness) plus any one of four respiratory s/s was 31.60% [19.74% (108/547) vs. 28.87% (28/97), 95% CI= 2.43~52.05, p<0.05 ] (Table 7B).

### 5.2.3 Absenteeism and Hospitalization Percentages Decreased

The third dimension is to evaluate influenza vaccine by measuring severe sickness from children's school absenteeism and hospitalization due to respiratory illness during the influenza epidemic season that were retrospectively recorded by questionnaire filled out by schoolchildren's parents/guardians. The vaccine effectiveness in decreasing absenteeism related to respiratory illness was 43.89% [16.61% (90/542) vs. 29.59% (29/98), 95% CI= 19.65~60.81, $p<0.05$], and in declining hospitalization related to respiratory illness was 45.76% [1.11% (6/542) vs. 2.04% (2/98), 95% CI= -164.88~88.89, $p=0.35$) (Table 7C).

### 5.2.4 Vaccine Efficacy among Influenza Naturally Infected Children

#### A. Influenza-like Illness (ILI) among the Human Influenza Virus Infected Schoolchildren

After we evaluated the vaccine efficacy and vaccine effectiveness in the vaccinated and unvaccinated groups, we decided to obtain the best information on the naturally infected schoolchildren by measuring their anti-influenza HI antibody at least 4-fold serotiter rises during the epidemic season because this study involved the time points before and after the influenza epidemic season. From 1-month to 4-month post-vaccination, 27 schoolchildren presented their serotiters with 4-fold rise against the testing influenza virus vaccinated strains (see Materials and Methods) and they were defined as "human influenza virus infected children". The vaccine effectiveness (VE) in preventing the presence of influenza-like illness (ILI) was calculated and the definition of ILI was described previously (Section 4.4 in the Section of METHODS). Since one unvaccinated child did not record ILI information, among the final 26 human infected children with available information, 57.14% (4/7) of influenza unvaccinated and 26.32% (5/19) of vaccinated showed ILI throughout the influenza season since 1-month

post-vaccination, and the VE was 53.95% (95% CI= -23.79~82.87, p=0.1881) (Table 8A).

**B. Absenteeism and Hospitalization**

Vaccine effectiveness in reducing the percentage of absenteeism and hospitalization due to respiratory illness during the influenza season among the 25 and 26 infected children with available information, respectively, was showed at Table 8B. The VE between the vaccinated and unvaccinated influenza cases in reducing absenteeism was 41.67% [50.00% (9/18) vs. 85.71% (6/7), 95% CI= -1.32~66.42, p=0.1794)]. The VE between the two groups in reducing schoolchildren's hospitalization was even higher to reach to 63.16% [5.26% (1/19) vs. 14.29% (1/7), 95% CI= -412.60~97.35, p=0.4738].

**C. ILI among Household Members**

The impact of influenza vaccine in reducing influenza virus transmission was further evaluated through the closest contacts in household members. Of all the 141 household members from the 27 anti-influenza seroincidence positive children, 106 (75.2%, 106/141) contacts provided their systematic and respiratory signs or symptoms during the influenza epidemic season. To understand the indirect protection from the vaccinated schoolchildren to their household contacts, the incidence of ILI among the household contacts of the 27 influenza-sero-incidence-positive children was calculated and stratified by age groups between vaccinated and unvaccinated schoolchildren (Table 8C). When using one systematic and one local s/s as the definition of ILI, the presence of ILI was higher in household contacts of unvaccinated children than in those of vaccinated children for most age groups, except for the household contacts of 13~18 years of age. For all household contacts, the VE in reducing household ILI between

vaccinated and unvaccinated influenza infected cases was 42.28% [15.28% (11/72) vs. 26.47% (9/34), 95% CI=-26.01~73.56, p=0.1692], which was quite close to the VE of schoolchildren's absenteeism rate at schools.

## 5.3 Serological Evaluation of Influenza HI Antibodies among Vaccinated and Unvaccinated Children

### 5.3.1 Percentage of Sero-protection

First of all, we examined the sero-protection rates of unvaccinated children at the baseline time point (T1) before vaccination and found that A/H3N2 virus induced the highest but B virus ranked the lowest of the three viruses. Comparing with the vaccinated children, influenza B virus was also the only virus showing apparent differences with almost 13% higher sero-protection in vaccinated than unvaccinated schoolchildren, but the rest of the two vaccine strains with miner or no differences between the two groups at this baseline. At 1-month post-vaccination, all the vaccinated children had significantly elevated sero-protection rates against the three influenza types/subtypes of the vaccine viruses (A/H1N1: from 74.13% to 94.06%, A/H3N2: from 86.19% to 97.73%, B: from 38.81% to 69.06%). The differences in sero-proteion rates between vaccinated versus unvaccinated was the highest for B virus with almost 37% increase (69.06% vs. 31.43%, $p<0.05$), followed by A/H1N1 virus with about 20% increase (94.06% vs. 75.24%, $p<0.05$), and then A/H3N2 virus with 12% increase (97.73% vs. 85.71%, $p<0.05$). At 4-month post-vaccination, the patterns of higher elevated sero-protection rates in vaccine recipients still maintained for A/H1N1 and A/H3N2 viruses and A/H3N2 virus even reached higher than 1-month post-vaccination [from 97.73% (559/572) to 97.99% (488/498)]. However, sero-protection rate for B

virus in vaccinated schoolchildren at this time point after almost the ending of influenza season dropped strikingly even during about only three months. Overall, schoolchildren had the highest sero-protection rates (serotiter $\geqq$1:40) against the vaccine A/H3N2 virus (T1: 85.67%, T2: 95.86%, T3: 96.27%), and followed by the second highest sero-protection rates against the vaccine A/H1N1 virus (T1:74.15%, T2: 91.14%, T3:89.15%), and the lowest sero-protection rates against the vaccine B virus (T1: 36.73%, T2: 63.22%, T3: 51.69%) (Table 9).

### 5.3.2 Four-fold Serotiter Rises of Anti-influenza HI Antibodies

To understand the dynamic changes of serotiters over time, percentages of anti-influenza HI antibodies with at least 4-fold serotier rise between two different time points were analyzed. Using this measurement during the time interval from pre- to 1-month post-vaccination may reflect predominantly immunologic memory boosting effect but during the time period from 1-month to 4-month post-vaccination can provide better information on sero-incidence rate of influenza virus infection. In addition, the 4-fold serotiter rise during the time span from pre-vaccination to the 4-month post-vaccination may involve an integrated outcome including immunologic boosting effect, possibly the acquired influenza infection, and antibody waning. In the aspect of natural infection, unvaccinated schoolchildren had much higher sero-incidence percentage than vaccinated schoolchildren from 1-month to 4-month post-vaccination for both A/H3N2 and B viruses but not for A/H1N1 virus (Table 10). However, examining the immunogenicity boosting effect over time, vaccinated schoolchildren had significantly higher percentages of anti-influenza HI antibody 4-fold serotiter rises against the three human influenza vaccine strains than unvaccinated schoolchildren either at 1-month post-vaccination or at 4-month post-vaccination (Table 10). The

percentages of such 4-fold serotiter rises were generally higher in evaluating T1 to T2 than in comparing T1 to T3 for the three human influenza vaccine strains in vaccinated schoolchildren. Interestingly, about 20% drops in the percentages of these 4-fold antibody rises occurred from 1-month post-vaccination to 4-month post-vaccination compared to the same antibody baseline levels at pre-vaccination for all three vaccine component influenza viruses (A/H3N2: 70.80% at T2/T1 and 49.60% at T3/T1; A/H1N1: 53.67% at T2/T1 and 34.73% at T3/T1; B: 34.44% at T2/T1 and 14.06% at T3/T1). Overall, vaccinated schoolchildren had the highest percentage of 4-fold HI antibody serotiter rise against A/H3N2 virus, but the lowest percentage of such HI antibody rise against B virus.

## 5.4 Distribution of Anti-influenza HI Serotiters and GMTs of the Three 2007 Vaccine Component Strains at Three Study Areas

### 5.4.1 Taipei City vs. Yilan Rural Area vs. Isolated Kinmen Islet

To investigate whether higher population density may influence the spreading of influenza, we then analyzed the distribution and GMT of anti-influenza HI antibody among 114, 381, and 182 schoolchildren in Taipei metropolitan City, Yilan rural area and isolated Kinmen islet, respectively (Table 11). At pre-vaccination, two important features were observed regardless of vaccination status in 2007. One is that the schoolchildren in Kinmen had the highest GMTs against A/H1N1 virus. Another finding is that the trends showed that the GMTs of A/H3N2 and B among schoolchildren in Taipei City were much higher than those in Yilan and whose GMTs of these two viruses were more elevated than those in Kinmen. After vaccination, the patterns of trends in higher GMT values of children's anti-H3N2 HI antibody correlated very well with their

schools located in areas with greater population density. In addition, vaccinated schoolchildren in Taipei City revealed the highest GMTs against A/H3N2, A/H1N1 and B all three viruses, even though the GMT against A/H1N1 in Taipei City at pre-vaccination was quite low.

To verify more clearly on the geographical area variation in immunogenicity without interference of past vaccination, 267 vaccinated schoolchildren without history of human influenza vaccination in 2005-06 and 2006-07, composed of 59, 149, and 59 children in Taipei, Yilan and Kinmen, respectively, were analyzed (Table 12). At pre-vaccination, children in Taipei had the highest GMTs against the vaccine A/H3N2 and B viruses, whereas schoolchildren in Kinmen presented the lowest GMT against these two viruses but the highest GMT against the vaccine A/H1N1 ($p<0.05$). Most interestingly, Taipei's schoolchildren had the lowest GMT value for the vaccine A/H1N1 at pre-vaccination but converted to the highest GMT value at 1-month post-vaccination.

At 1-month and 4-month post-vaccination, schoolchildren in Taipei showed the highest GMTs against the vaccine A/H1N1, A/H3N2 and B viruses. By contrast, schoolchildren in Yilan and in Kinmen exhibited the lowest GMTs against the vaccine A/H1N1 and A/H3N2 viruses, respectively ($p<0.05$). Comparing GMTs of schoolchildren in these three areas, the patterns of GMTs in the three different geographical areas and in the all three component vaccine viruses at the 4-month post-vaccination were quite similar to those at the 1-month post-vaccination. The boosted serotiter levels by vaccination remained relatively high or low throughout the influenza season, depending on the serotiters at 1-month post-vaccination, although antibody waning at the last time point did occur. In conclusion, the geographical variations in GMTs excluding the past vaccination were still parallel to the population

density for A/H3N2 and B viruses, similar to the above-mentioned findings without considering history of past influenza vaccination. Such patterns were quite different for A/H1N1 which is going to be discussed in more details in the Section of Discussion.

In comparison of influenza vaccine types/subtypes excluding the past history of human influenza vaccine, A/H3N2 viruses still offered the schoolchildren to have the highest GMTs whereas B viruses remained the lowest GMTs at all the three time points (Table 12).

### 5.4.2 GMT Variations at the Three Yilan Schools

Because the spread of influenza virus frequently occurred as outbreaks at shools (Cauchemez S, et al. 2008), we further analzyed the school variations with or without influena vaccination in details. Only the measurement of GMT among vaccinated children were used for comparison because GMTs are calcualted based on the distribtions of serotiters. Regarding to the population density, YL-LT area had the highest population density than YL-LZ and YL-SS areas in 2007 [6538.06 (74173/11.34) vs. 973.09 (37821/38.87) vs. 147.01 (21203/144.22) persons/$km^2$, respectively]. In the aspect of school body and environment, the YL-LT school, located in the center of township, was the largest in size compared to the YL-SS school, located away from the center of townshp with many duck poultry farms, was the smallest. The YL-LZ school, located also away from the cneter of township, has more natural habitat for migrating birds clsoe to school campus. The 2007 influenza vaccination coverage rates were 72.1%, 83.5%, and 63.75% for YL-LT, YL-LT and YL-SS schools, respectively, and the students' participation rates in our study were 57.81% for YL-LT , 60.05% for YL-LZ and 51.25% for YL-SS (Table 2).

In unvaccnated schoolchildren, the trends clearly showed that the GMTs of A/H3N2 and B viruses ranked the highest in YL-LT where population density and student density were also the highest. Consistently, the GMTs of these two viruses were the lowest in YL-SS where population and students were the least crowdy. Even for A/H1N1 virus, YL-LT school had more numbers of children with higher anti-influenza HA antibody serotiters $\geq$ 1:320 at pre-vaccination than the those of the other two schools. In addition, several unvaccinated children at the YL-SS school even increased their numbers in higher serotiters from 1-month to 4-month post-vaccination.

After influenza vaccination, children at YL-LT school had the highest GMTs against all the three vaccine viruses at 1-month and 4-month post-vaccination. However, children at the YL-LZ school decreased to the lowest GMTs against the three viruses at 4-month post-vaccination (Table 11).

To examine the changes of GMTs after vaccination among the three schools in Yilan area, we found that the vaccinated children at the YL-LT school had the most rise in GMT against the vaccine A/H1N1 virus, but the least rise in GMT against the vaccine A/H3N2 and B viruses, respectively (A/H1N1: 142.21/40.00= 3.56, A/H3N2: 338.25/84.56= 4.00, B: 63.64/48.91= 1.30). Conversely, the YL-SS school had the least rise in GMT against the vaccine H1N1 virus, but the most rise in GMT against the vaccine A/H3N2 and B viruses, respectively (A/H1N1: 100.35/50.88= 1.97, A/H3N2: 285.81/42.93= 6.66, and B: 37.79/13.09= 2.89) (Table 11).

### 5.4.3 GMT Variations at the Two Kimen Schools

Kinmen residents may face more challenges of influenza viruses coming from

China than any other areas in Taiwan due to direct transportation channels between China and Kinmen since 2001. Therefore, we analyzed the schools' GMT variations in scholchildren in Kinmen. Both KM-JJ and KM-JH schools are located in Larger-KM islet areas where 74007 populaiton are living in 134.453 $km^2$ areas of 2007 but KM-JJ school is much closer to the harbor of Kinmen with ships to go to Xiamen of mainland China. Comparing the two schools, KM-JJ school was larger and human influenza vaccination coverage rate in grade 1 and 2 students was 50.10% and student particiapation rate in our study was 40.47% whereas the KM-JH schol was smaller (Table 2) and the influenza vaccine coverage and participation rates were 46.61% and 40.56%, respectively.

To find out possible past influenza virus infection might be present in such an important islet as the sentinel sites for Taiwan in facing challenges of influenza viruses coming from China, higher anti-influenza HI antiobdy serotiters, particularly at pre-vaccinaiton were examined. We found that the KM-JH schoolchildren had higher GMTs for both A/H1N1 and B viruses at this initial time point regardless of vaccination or unvaccination groups. Furthermore, such higher GMTs than those at KM-JJ schools remained till the time at 4-month post-vaccination. On the other hand, the KM-JJ schoolchildren showed higer GMTs with more percentage of anti-influenza higher serotiters for A/H3N2 virus at pre-vaccination (data not shown), implying the larger schools were more likley to acquire the A/H3N2 virus infection in the past (Table 11).

Influenza natural infection can be further demonstrated from the inreases of higher serotiters among unvaccinated children in later than earlier time points during the study period and also keeping or even increasing in higher serotiters among vacccinated children from 1-month to 4-month post-vaccination without antibody waning. Based on

this rationale, we can clearly find out that KM-JJ schoolchildren had acquired A/H3N2 and B influenza and KM-JH schoolchildren had acquired influenza B in unvaccinated group (Table 11, and the distribution of serotiter raw data not shown).

The effect of immunogenicity can be further evaluted by comparing the changes of GMTs at later to earlier time points among vaccine recipeints. Between the two schools in Kinmen area, children at the KM-JJ school had higher rise in GMT against all the three human influenza vaccine strains than those children at the KM-JH school from pre-vaccination to 1-month post-vaccination (H1N1: 186.36/42.57= 4.38, A/H3N2: 298.57/34.58= 8.63, and B: 37.58/12.83=2.93) (Table 11).

## 5.5 Reactogenicity among Schoolchildren

The reactogenicity of the 468 schoolchildren during the first three days right after receiving influenza vaccination was recorded retrospectively through questionnaire filled out by schoolchildren's parents/guardians (Table 13). The reactogenicity recorded listed in order from the highest included: (1) pain at injection site (n=71, 15.2%), (2) arm pain (n=37, 7.9%), and (3) runny nose (n=33, 7.1%). The presence of reactogenicity was not often that 335 (71.6%) schoolchildren reported no any reactogenicity, 90 (19.2%) children reported only one reactogenicity, and 31 (6.6%) children reported two reactions, respectively (data not shown).

# Chapter 6 Discussion

Children attending day-care centers or going to schools are spreaders of influenza to bring the virus back home and then transmit the virus to healthy household members (Hurwitz ES, et al. 2000). With the mass-vaccination of influenza during the 2007-2008 influenza season for grade one and two elementary schoolchildren, this study is the first time to evaluate vaccine effectiveness, immunogenicity, and reactogenicity among those grade one and two schoolchildren by seroepidemiological study design. We enrolled the schoolchildren from three different areas, Taipei (a metropolitan), Yilan (a rural area), and Kinmen (an islet close to China), with different potential of exposures to influenza virus. To measure the immunogenicity against the three human influenza virus vaccine strains, longitudinal serum collection of the three time-points was designed at pre-vaccination, 1-month and 4-month post-vaccination. There are five major findings of this study. First, vaccinated grade one and two elementary schoolchildren developed good immunogenicity against the human influenza vaccine viruses and their reactogenicity of the vaccine did not show safety concerns. Second, the vaccine effectiveness was good not only to prevent ILI of the recipients and their household members but also to decrease the schoolchildren's absenteeism and hospitalization rates. Third, vaccinated and unvaccinated schoolchildren in three areas exhibited different anti-influenza antibody patterns against the three human influenza strains of 2007-08 vaccine. Fourth, the vaccine given to grade one and two schoolchildren was more effective for human influenza vaccine A/H3N2 and B than A/H1N1 viruses. Fifth, the antibody serotiters against influenza B virus among schoolchildren were the lowest, implying the needs for future improvement in influenza B vaccine.

## 6.1 Vaccine Efficacy and Vaccine Effectiveness among Grade 1-2 Elementary Schoolchildren

### 6.1.1 Low Vaccine Efficacy for A/H1N1 Virus

The vaccine efficacy (ve) using the definition of 4-fold HI antibody (Ab) serotiter rise found that schoolchildren was not effective in preventing A/H1N1 infection from 1-month to 4-month post-vaccination, in spite of A/H1N1 was the predominant subtype during the 2007-2008 influenza season in Taiwan. There were three possible reasons for the poor vaccine efficacy against A/H1N1 virus. First, the wild-type circulating strain of A/H1N1 virus during the 2007-08 influenza season was A/Brisbane/59/2007 (H1N1) virus, which did not match to the vaccine strain of A/Solomon Islands/3/2006 (H1N1) virus that we tested. Second, several A/H1N1 infected children that would occur from the pre-vaccination to 1-month post-vaccination [estimated as 3.81% (4/105) among unvaccinated schoolchildren] but were not included in the calculation of ve. Third, the sample size of unvaccinated schoolchildren was too small to be comparable with that of vaccination children. The ve in preventing A/H3N2 and B infection were 84.9% and 54.7% (Sugaya N, et al. 1994; Neuzil KM, et al. 2000), respectively, which were comparable to our data (86.14% for A/H3N2 virus and 38.42% for B virus) in this study.

### 6.1.2 Household Protection

In a previous study, the VE was most effective in preventing ILI among household members who were aged 5~17 years (Hurwitz ES, et al. 2000). In this study, we tried to analyze the household protection provided from the vaccinated schoolchildren to specific age groups of household members who were young children or elderly, and the indirect protection can be demonstrated among those who acquired the influenza virus

infection in our study. The VE was effective for all age groups of household contacts, except for age group 13~18 without statistical significance.

The most important public health concern of vaccination is to minimize the transmission among the interesting populations. In this study, we used five end-points to measure vaccine efficacy and vaccine effectiveness of schoolchildren, which were naturally infected children with anti-influenza HI Ab 4-fold serotiter rises from 1-month to 4-month post-vaccination, ILI of schoolchildren and household members, and schoolchildren's absenteeism, and hospitalization. The last three measurements were attempted to reflect "herd immunity" provided from the program of vaccinating schoolchildren. However, "herd immunity" was not easy to fully describe from our calculation, because the studied children could have complex social network with other people. For instance, they not only contact with their family members at home, but also play or talk with their classmates at school. Moreover, they can contact with other children at post-school activities or travel to other places. The frequency of contacts with influenza patients determines the potential of children to be infected (Mikolajczyk RT, et al. 2008). On the other hand, the level of immunity of children also plays a role in developing influenza disease. In addition, the severity of the epidemic, the subtype of circulating strain, and the duration of observation are also important factors to affect the long-term herd immunity. Above all, many factors are needed to be considered for herd immunity, and they are crucial to understand the transmission of influenza viruses from susceptible groups.

## 6.2 Immunogenicity and Safety of 2007-2008 Trivalent Influenza Vaccines Given to Grade 1-2 Elementary Schoolchildren

### 6.2.1 At What Levels of Antibody of HI Were Protective?

To prevent influenza virus infection, the protective levels of the HI antibody in vaccinated or unvaccinated schoolchildren are very interesting to know. The best measure is to find out from those newly infected schoolchildren participated in this study. In this study, **eight** unvaccinated children acquired the influenza infection presenting 4-fold anti-influenza HI Ab serotiter rises from pre-vaccination to 1-month post-vaccination and another **eight** unvaccinated children from 1-month to 4-month post-vaccination, and 20 vaccinated schoolchildren demonstrated with such 4-fold serotiter rises from 1-month to 4-month post-vaccination. In eight unvaccinated and infected children from pre- to 1-month post-vaccination, four A/H1N1 infected children anti-influenza HI Ab serotiters at 1:40 and lower, and the rest four children had A/H3N2 infection with their anti-influenza HI Ab serotiters at 1:80 at the first time-point. Furthermore, among the eight newly influenza infected children in unvaccinated group from 1-month to 4-month post-vaccination, one case with A/H1N1 virus infection had anti-influenza HI Ab serotiter at 1:20, four cases with A/H3N2 virus infection had anti-influenza HI Ab serotiters at 1:20 and even lower, and three children with influenza B infection had anti-influenza HI Ab serotiters at 1:40 and lower at 1-month post-vaccination. In 20 newly influenza infected children in vaccinated group from 1-month to 4-month post-vaccination, seven and three children with A/H1N1 and A/H3N2 virus infection, respectively, had their anti-influenza HI Ab serotiters at 1:40 and lower, and 10 children with B infection had their serotiters at 1:80 and lower at 1-month post-vaccination. From those serotiter data, children with low anti-influenza HI Ab serotiters, regardless of receiving influenza vaccine or not, did acquire the infection.

### 6.2.2 Implication of Geographical Variations

**A. Reasons for Geographical Variations**

The serotiters against the three vaccine viruses were different between vaccinated and unvaccinated children in Taipei, Yilan, and Kinmen and even different school places in Yilan and Kinmen were also observed. First, the GMTs against A/H1N1 virus and the other two viruses prior of vaccination were highest in Kinmen and Taipei, respectively. In fact, A/H1N1 epidemic occurred in Kinmen during 2005-06 (Lin CY. 2007), and the higher GMT of Kinmen schoolchildren could be explained. However, schoolchildren in metropolitan Taipei could have most frequent contacts with the influenza infected persons due to highest population density, resulting in higher GMTs against A/H3N2 and B viruses at pre-vaccination. Schoolchildren in Taipei and Kinmen had the highest and the lowest GMTs against the A/H3N2 and B viruses at 1-month post-vaccination, respectively. However, the highest GMT at KM-JH school implies that the schoolchildren there should encounter the A/H1N1 outbreak even before 2005-06. Above all, schoolchildren at different geographical areas showing different antibody variations could be due to prior influenza epidemic or other geographically related factors.

Geographical variation in anti-influenza antibody patterns was also observed at different schools within the same county. The GMTs against the same testing virus at the same time-points were even different within schools in Yilan or in Kinmen. For example, in Yilan, GMTs against A/H1N1 virus were the highest among schoolchildren at YL-SS school and the lowest at YL-LT school at pre-vaccination. For A/H3N2 and B viruses, children at YL-LT school and YL-SS school had the highest and the lowest GMTs at pre-vaccination, respectively. For GMTs of vaccinated children at 1-month and 4-month post-vaccination, variations in GMTs were also observed at three schools in

Yilan. For two schools in Kinmen, the GMT differences were still shown among all children at pre-vaccination or among all the vaccinated children at 1-month and 4-month post-vaccination. In summary, geographical variations of the anti-influenza HI Ab patterns at each school in the study may indicate the variations in epidemiology of this virus even at micro-levels.

**B. Geographical Variations in Antibody Waning**

GMTs against the three 2007-08 vaccine viruses were also used to observe antibody waning among vaccinated children at different geographical areas during the 2007-08 season. At 1-month post-vaccination, the levels of anti-influenza HI Ab serotiters of vaccinated children were elevated differently at various geographical areas. Schoolchildren in Taipei and Yilan had the highest and the lowest GMTs against the A/H1N1 virus at 1-month post-vaccination. Moreover, geographical variations in antibody waning showed that GMTs at 1-month post-vaccination among vaccinated children seemed to determine the serotiter levels at 4-month post-vaccination. Interestingly, schoolchildren in the area with higher GMT at the second time-point than the other areas remained their high GMT till the third time-point—almost the ending of influenza season.

### 6.2.3 Impact of Prior Infection and Levels of Immunogenicity

The influence of prior influenza virus infection can be observed from the higher levels of GMTs at pre-vaccination and then to be sustained at sufficient levels throughout the season. For A/H1N1 virus, KM-JH school had the highest GMT against the A/H1N1 virus among the six schools at pre-vaccination, indicating that there was an A/H1N1 epidemic before. At 1-month and 4-month post-vaccination, after the

intervention of vaccination made vaccinated schoolchildren at this school developed the second highest GMT among the six schools. For B virus, schoolchildren at TP-GT and YL-LT schools had relatively higher GMTs against this virus than the other four schools at pre-vaccination. Although the past season of 2006-07 had nationwide epidemic of the influenza B, our data of GMT against this virus at pre-vaccination may have two implications. It is very likely that the children at these two schools encountered an influenza B outbreak in the past prior to 2006-07 influenza season. Alternatively, the children at these two schools experienced a large-scale influenza B outbreak in the 2006-07 influenza season. The GMTs of vaccinated children at these two schools also ranked the highest two among the six schools at 1-month and 4-month post-vaccination, indicating the prior infection would provide booster effect on immunogenicity after the vaccination.

Natural infection offers all the viral antigens to be recognized by immune system. Based on this study, vaccination of the prior infection again elevated the antibody levels due to immunologic memory (Sasaki S, et al. 2008). Children whose HI antibody levels ≧1:320 for B and A/H1N1 viruses and ≧1:1280 for A/H3N2 virus at pre-vaccination had 0.30% (2/677), 1.03% (7/677), and 1.03% (7/677) of them acquired the new infection, respectively, from pre-vaccination to 1-month post-vaccination. In addition, unvaccinated children whose HI antibody levels ≧1:320 for B and A/H1N1 viruses and ≧1:1280 for A/H3N2 virus at 1-month post-vaccination had 0.00% (0/97), 1.03% (1/97), and 2.06% (2/97) of them acquired the new infection, respectively, from 1-month to 4-month post-vaccination. Furthermore, their percentages against the three vaccine viruses for developing ILI were all zero. Taken together, vaccination of the natural infection might still offer protection from the antigenically drifted viruses, while

antibody levels are differently high.

## 6.3 Social Impact of 2007-2008 Influenza Vaccine Given to Grade 1-2 Elementary Schoolchildren

Compare the epi-curves of both the 2006-07 and the 2007-08 influenza seasons, after the outbreak of influenza B virus and the mass-vaccination for grade 1-2 elementary schoolchildren, the influenza epidemic during 2007-08 was relatively mild. Moreover, the vaccination program might provide indirect protection for age groups of schoolchildren and thus reduced percentage of susceptible children to influenza infection. One more interesting phenomenon was that three subtypes of influenza viruses were all circulating during the 2007-08 season. Carefully viewing the virologic surveillance data, we found three stages. First, the subtype A/H3N2 virus appeared at the end of 2006, became dominant in the summer of 2007 and then still maintained its activity there and even resulted more cases of A/H3N2 infection prior to the 2007-2008 influenza season. Then, at the beginning of the influenza season, the 2005-06 year dominated subtype, A/H1N1 virus, maintained over the other two viruses. Finally, at the beginning of year 2008, B virus dominated over the ending period of the season.

## 6.4 Limitations

This study aimed to compare the antibody response and ILI related outcomes between vaccinated and unvaccinated schoolchildren and their associated important factors. However, several limitations were present. First, the willingness to participate the study depends on parents' choice so that the number of unvaccinated children with willingness to provide their serum samples for the three time-points was relatively less

than the number of vaccinated children in the study. Second, the schedule of receiving the 2007-2008 influenza vaccine was different among the six schools. Although the influenza mass-vaccination program intended to give vaccine shots during Oct~Nov., 2007, some schoolchildren received the influenza vaccine quite late would have more chances of exposure to influenza virus when the influenza epidemic started. Third, the risk and protective factors related to influenza and the clinical manifestation of influenza-like illness from the schoolchildren and their household members were collected by questionnaire. Fourth, the reluctance of participants' providing respiratory specimens for influenza virus identification may underestimate the true incidence of the influenza infection in the study. Fifth, the vaccine efficacy and vaccine effectiveness should be also evaluated by the antibody patterns against the wild-type circulating A/H1N1, A/H3N2, and B viruses during the 2007-08 influenza season. Such research work is under progress.

## 6.5 Recommendations and Future Perspectives

This was the first mass-vaccination policy provided to grade one and two elementary schoolchildren in Taiwan during the 2007-2008 influenza season. For grade one and two schoolchildren, who mostly did not receive the seasonal human influenza vaccine before, their antibody profiles against A/H1N1 and A/H3N2 viruses were much better than B virus. The influenza B virus in the vaccine showed difficulty to reach protective level. Further advance research was needed to improve the immunogenicity of B virus. During the 2007-2008 influenza season, although the major subtype caused influenza epidemic was A/H1N1, the epidemic was relatively milder than the previous year of 2005~2006. Future multi-year longitudinal study will provide important information to precisely

measure the long-term effect of human influenza vaccine among elementary schoolchildren combined with the different circulating strains, influenza-related disease morbidity and mortality plus the magnitude of epidemic in each year. The influenza mass-vaccination for elementary schoolchildren in Taiwan and elsewhere will be valuable experience for better public health policies after analyzing their impact at community levels, particularly important risk groups.

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**Figure 1. Geographical Location of the Three Studied Areas Where the Six Elementary Schools (1. TP-GT, 2. YL-SS, 3. YL-LT, 4. YL-LZ, 5. KM-JJ, 6. KM-JH) Are Selected.**

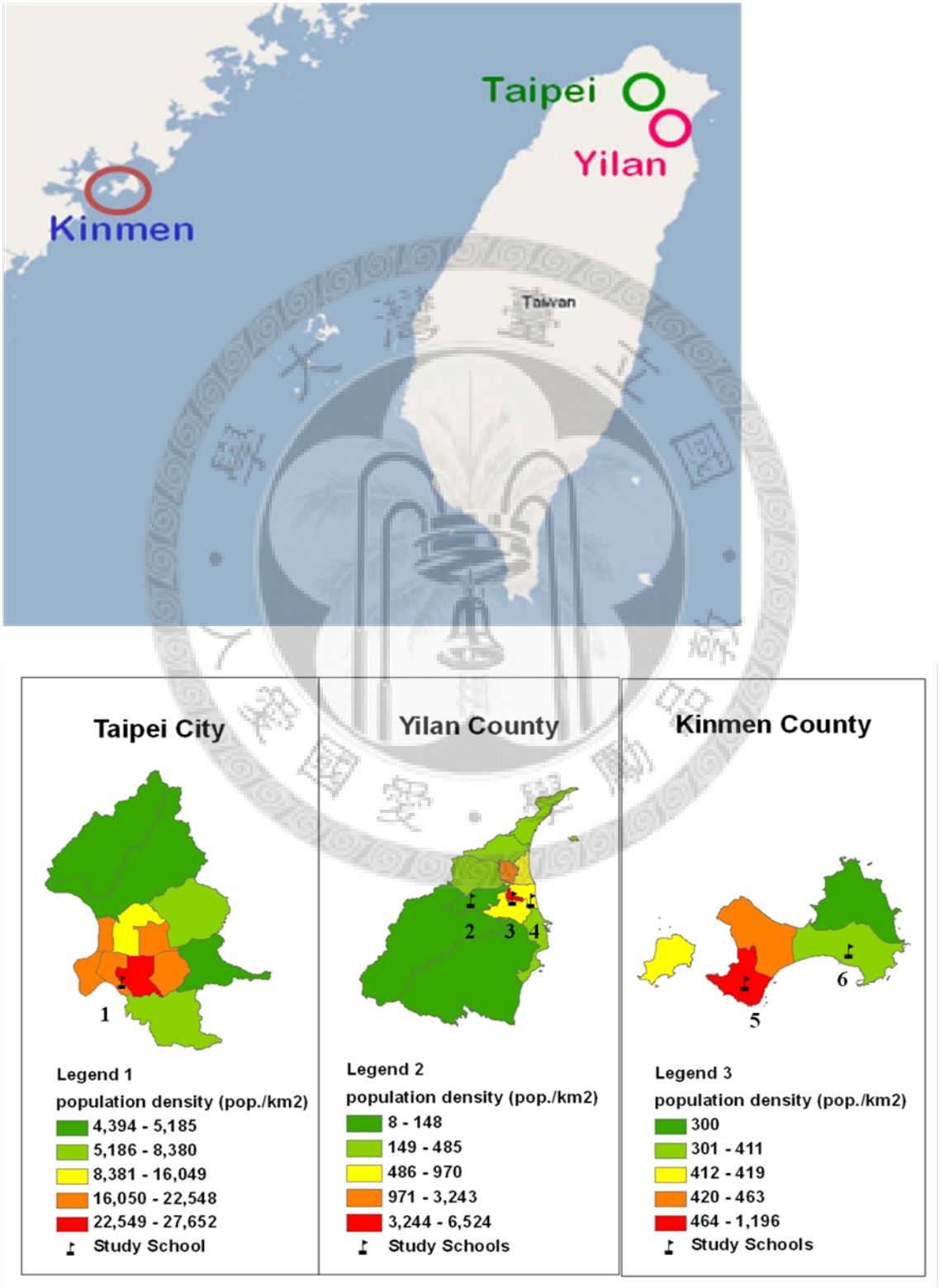
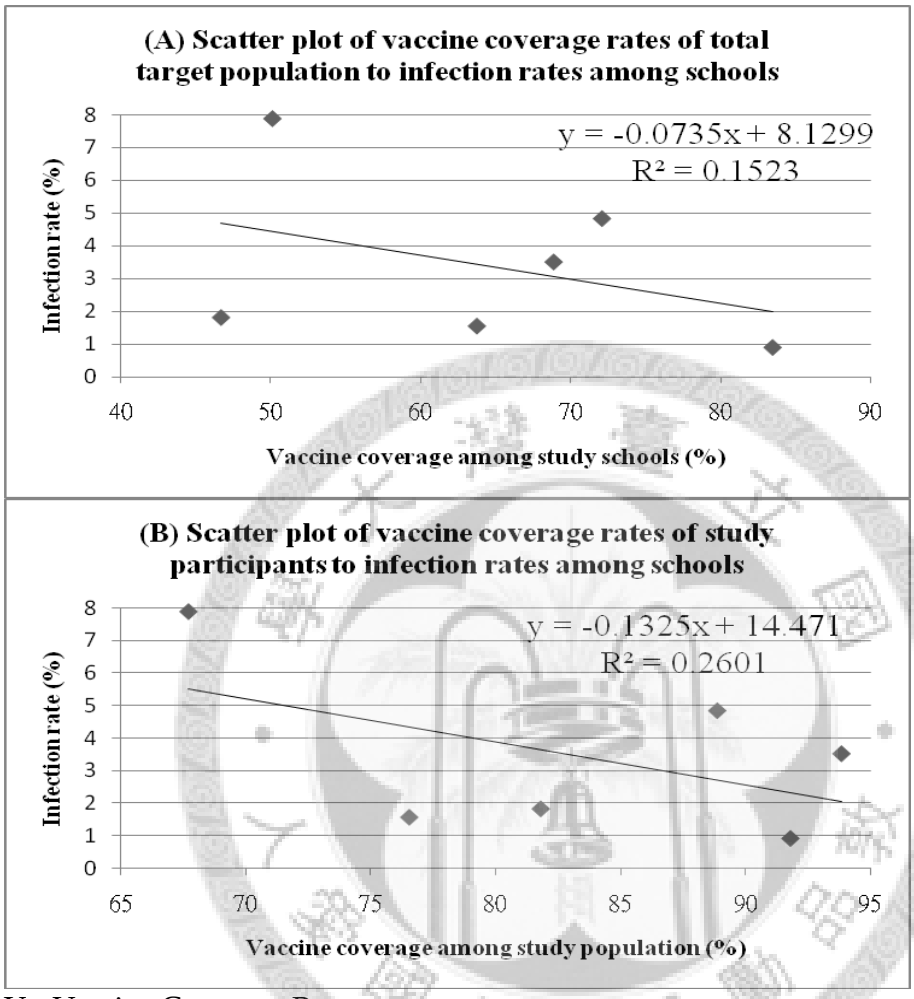


**Figure 2A~B. The Relationship between the Vaccine Coverage Rates of (A) Total Target Population and of (B) Study Participants and the Influenza Virus Infection Rates among the Six Schools, Oct, 2007-Apr, 2008.**




Y= Vaccine Coverage Rates

X= Influenza Virus Infection Rates shown by anti-influenza HI antibody 4-fold serotiter rise against the three vaccine strains of 2008~2008 human inactivated influenza vaccine [A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004]

Each point represents each school of the six selected schools in this study. The regression line was obtained by linear regression model and $R^2$ was adjusted Pearson correlationcorrelation coefficient.

**Figure 3A~F. Comparison of the Cumulative Percentages of HI Serotiters against the Three Human Influenza Virus Strains: (A) A/H1N1 Virus, (B) A/H3N2 Virus, and (C) B Virus of Vaccinated Children and (D) A/H1N1 Virus, (E) A/H3N2 Virus, and (F) B Virus of Unvaccinated Children at the Three Time-points, Oct, 2007-Apr, 2008.**

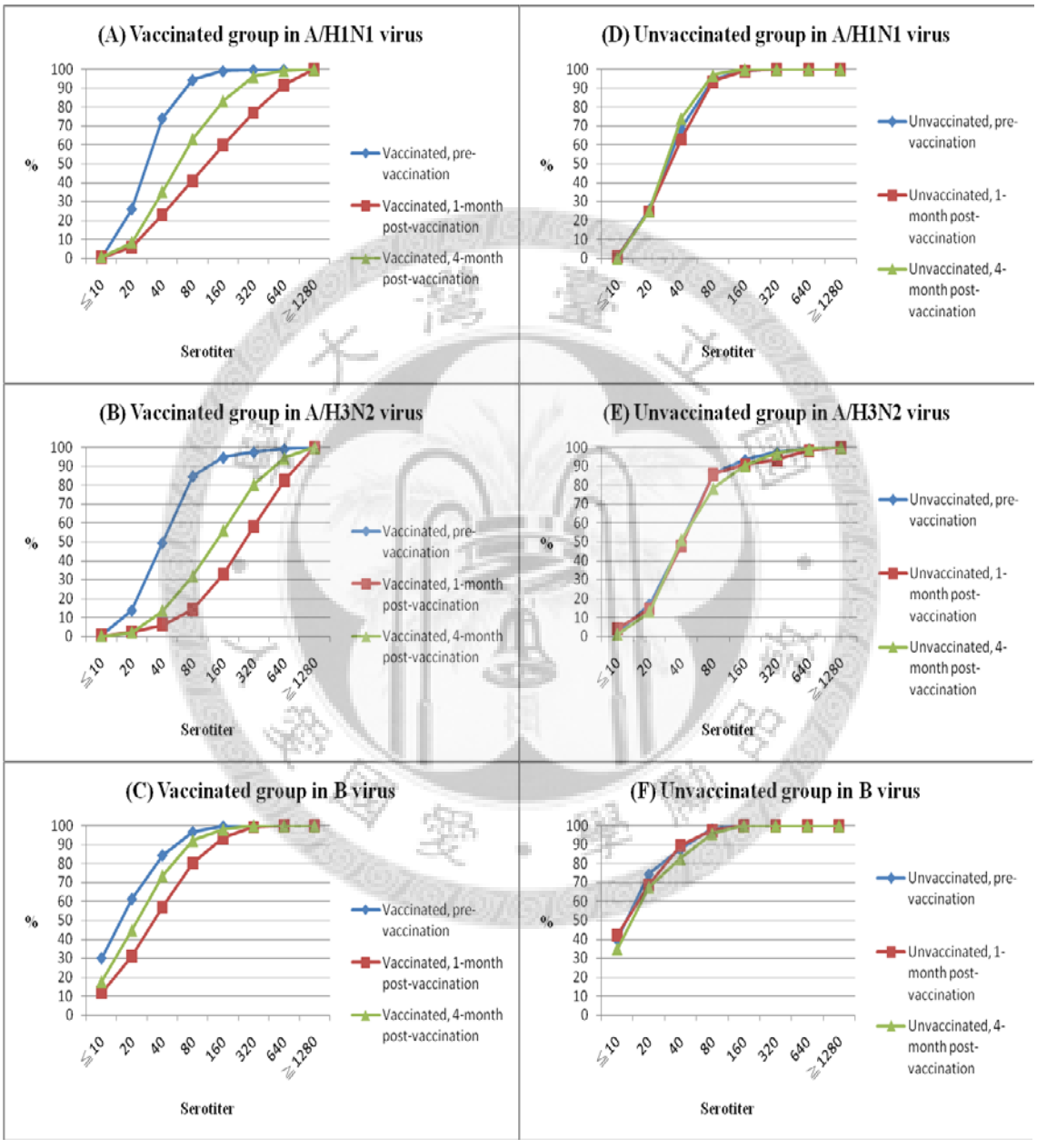


**Table 1. Dates of the Three Time-points That Serum Samples Were Collected from the Six Elementary Schools in Taiwan during Oct, 2007-Apr, 2008.**

| Schools | 1st Serum[a] | Dates of vaccination | 2nd Serum[b] | | 3rd Serum[c] | |
|---|---|---|---|---|---|---|
| | Taken Dates | | Taken Dates | Days Post-vaccination | Taken Dates | Days Post-vaccination |
| **TP-GT** | Oct-30-2007 | Grade 1: Nov-09-2007<br>Grade 2: Nov-12-2007 | Dec-13-2007 | 31~34 | Mar-19-2008 | 128~131 |
| **YL-LT** | Nov-06-2007 | Nov-15-2007 | Dec-26-2007 | 41 | Apr-03-2008 | 140 |
| **YL-LZ** | Nov-07-2007 | Nov-16-2007 | Dec-17-2007 | 31 | Mar-25-2008 | 129 |
| **YL-SS** | Nov-06-2007 | Nov-19-2007 | Dec-09-2007 | 30 | Apr-02-2008 | 135 |
| **KM-JJ** | Nov-09-2007 | Nov-12, 29, 26-2007<br>Dec-03, 10, 17-2007 | Jan-04-2008 | 18~53 | Mar-31-2008 | 105~140 |
| **KM-JH** | Nov-12-2007 | Dec-04-2007 | Jan-03-2008 | 30 | Apr-01-2008 | 119 |
| **Mean±SD** | | | | 33.3±4.3 | | 129.2±7.7 |

[a] The 1st serum samples of schoolchildren were collected **before** the vaccination.
[b] The 2nd serum samples of schoolchildren were collected at approximately **one month after** the vaccination.
[c] The 3rd serum samples of schoolchildren were collected at approximately **four months after** the vaccination.

**Table 2. Participation and Follow-up Rates of the Serum Collected from the Children of the Six Elementary Schools in Taiwan at the Three Time-points (Pre-vaccination, 1-month and 4-month Post-vaccination) during Oct, 2007-Apr, 2008.**

| Schools | Target Population (N) | 2007-2008 Influenza Vaccine | | 1st Serum (Pre-vaccination) | | 2nd Serum (1-month post-vaccination) | | | 3rd Serum (4-month post-vaccination) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | Coverage Rate (n/N) | $n_1$ | Participation Rate$_1$ ($n_1$/N) | $n_2$ | Follow-up Rate$_1$ ($n_2$/$n_1$) | Participation Rate$_2$ ($n_2$/N) | $n_3$ | Follow-up Rate$_2$ ($n_3$/$n_1$) | Participation Rate$_3$ ($n_3$/N) |
| **TP-GT** | 500 | 343 | 68.88%[a] | 177 | 35.40% | 117 | 66.10% | 23.40% | 102 | 57.63% | 20.40% |
| **YL-LT** | 448 | 323 | 72.10% | 259 | 57.81% | 210 | 81.08% | 46.88% | 185 | 71.43% | 41.29% |
| **YL-LZ** | 206 | 172 | 83.50% | 134 | 65.05% | 110 | 82.09% | 53.40% | 98 | 73.13% | 47.57% |
| **YL-SS** | 160 | 102 | 63.75% | 82 | 51.25% | 66 | 80.49% | 41.25% | 57 | 69.51% | 35.63% |
| **KM-JJ** | 509 | 255 | 50.10% | 206 | 40.47% | 130 | 63.11% | 25.54% | 114 | 55.34% | 22.40% |
| **KM-JH** | 180 | 84 | 46.67% | 73 | 40.56% | 55 | 75.34% | 30.56% | 42 | 57.53% | 23.33% |
| **Total** | 2003 | 1279 | 63.92% | 931 | 46.50% | 688 | 73.90% | 34.35% | 598 | 64.23% | 29.86% |

$n_1$: Numbers of participants at 1st serum collection.
$n_2$: Numbers of students participated at 2nd serum collection.
$n_3$: Numbers of students participated at 3rd serum collection.
[a] Only 498 students at school TP-GT provided history of immunization records for the 2007-2008 influenza vaccine.

**Table 3. Vaccine Coverage Rates and Human Influenza Infection Rates among the Six Selected Elementary Schools.**

| Schools | Vaccine Coverage Rates of Total Target Population (%) | Vaccine Coverage Rates of Study Participants (%) | Human Influenza Infection Rates (%) |
|---|---|---|---|
| **TP-GT** | 68.88 | 93.86 | 3.51 |
| **YL-LT** | 72.10 | 88.89 | 4.83 |
| **YL-LZ** | 83.50 | 91.82 | 0.91 |
| **YL-SS** | 63.75 | 76.56 | 1.56 |
| **KM-JJ** | 50.10 | 67.72 | 7.87 |
| **KM-JH** | 46.67 | 81.82 | 1.82 |

Influenza Virus Infection Rates shown by anti-influenza HI antibody 4-fold serotiter rise against the three vaccine strains of 2007-2008 human inactivated influenza vaccine [A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004].

**Table 4. Demographic Analysis of Vaccinated and Unvaccinated Groups of the 677 Studied Participants at the Six Elementary Schools in Taiwan during the 2007-2008 Influenza Season, Oct, 2007-Apr, 2008.**

| Characteristics | Vaccinated Group n=572 (84.5%) | Unvaccinated Group n=105 (15.5%) | Total n=677 (100%) | p-value[a] |
|---|---|---|---|---|
| **Age (mean±SD)** | 7.1 ± 0.6 | 7.2 ± 0.5 | 7.1 ± 0.6 | 0.3085 |
| **missing** | 8 | 1 | 9 | |
| **Gender** | | | | |
| **Males** | 305 (53.3) | 47 (44.8) | 352 (52.0) | 0.1066 |
| **Females** | 267 (46.7) | 58 (55.2) | 325 (48.0) | |
| **School** | | | | **<0.0001** |
| **TP-GT** | 107 **(18.7)** | 7 (6.7) | 114 (16.8) | |
| **YL-LT** | 184 **(32.2)** | 23 (21.9) | 207 (30.6) | |
| **YL-LZ** | 101 **(17.7)** | 9 (8.6) | 110 (16.3) | |
| **YL-SS** | 49 (8.6) | 15 **(14.3)** | 64 (9.5) | |
| **KM-JJ** | 86 (15.0) | 41 **(39.1)** | 127 (18.8) | |
| **KM-JH** | 45 (7.9) | 10 **(9.5)** | 55 (8.1) | |
| **Grade** | | | | |
| **1** | 273 (47.7) | 45 (42.9) | 318 (47.0) | 0.3580 |
| **2** | 299 (52.3) | 60 (57.1) | 359 (53.0) | |
| **Hakka ethnicity** | | | | 1.0000 |
| **Yes** | 27 (5.1) | 5 (5.3) | 31 (5.1) | |
| **No** | 502 (94.9) | 90 (94.7) | 592 (94.9) | |
| **missing** | 43 | 10 | 53 | |
| **Aboriginal ethnicity** | | | | 0.3119 |
| **Yes** | 25 (4.7) | 7 (7.3) | 32 (5.1) | |
| **No** | 504 (95.3) | 89 (92.7) | 593 (94.9) | |
| **missing** | 43 | 9 | 52 | |
| **BMI (mean±SD)** | 16.7 ± 2.8 | 17.2 ± 3.0 | 17.0 ± 2.8 | 0.5248 |
| **missing** | 9 | 1 | 10 | |
| **Blood type** | | | | |
| **O** | 161 (47.1) | 26 (49.1) | 187 (47.3) | 0.7769 |
| **A** | 87 (25.4) | 11 (20.8) | 98 (24.8) | |
| **B** | 67 (19.6) | 10 (18.9) | 77 (19.5) | |
| **AB** | 27 (7.9) | 6 (11.3) | 33 (8.4) | |
| **unknown** | 230 | 52 | 282 | |

[a] Student t test was used for continuous data, and $\chi^2$ test was used for categorical data.

To test for statistical difference among subgroups of that specific variable, $\alpha$=0.05.

**Table 5. Risk Factor Analyses of Vaccinated and Unvaccinated Groups of the Studied Participants at the Six Elementary Schools in Taiwan during the 2007-2008 Influenza Season, Oct, 2007-Apr, 2008.**

| Characteristics | Vaccinated Group n=572 (84.5%) | Unvaccinated Group n=105 (15.5%) | Total n=677 (100%) | p-value[a] |
|---|---|---|---|---|
| **No of Family Members [b, d]** | 5.2 ± 1.8 | 5.3 ± 1.7 | 5.2 ± 1.8 | 0.4582 |
| **missing** | 16 | 4 | 20 | |
| **No of Sibling [c, d]** | 1.4 ± 1.0 | 1.4 ± 1.0 | 1.4 ± 1.0 | 0.9493 |
| **missing** | 11 | 2 | 13 | |
| **Caretaker spent for child (hr/day) [d]** | 11.5 ±6.5 | 11.7 ± 6.3 | 11.5 ± 6.5 | 0.8070 |
| **missing** | 119 | 26 | 145 | |
| **Sleep (hr/day) [b]** | **8.5 ± 0.9** | **8.7 ± 0.8** | 8.5 ± 0.9 | **0.0194** |
| **missing** | 56 | 14 | 70 | |
| **Sport (hr/wk) [b]** | 4.8 ± 4.7 | 5.3 ± 4.9 | 4.9 ± 4.7 | 0.4780 |
| **missing** | 194 | 33 | 227 | |
| **Post-school activity** | | | | |
| **Yes** | 396 (**72.1**) | 58 (**59.8**) | 454 (70.3) | **0.0142** |
| **No** | 153 (27.9) | 39 (40.2) | 192 (29.7) | |
| **missing** | 23 | 8 | 31 | |
| **Outdoor activity (hr/wk) [b]** | 5.9 ± 8.6 | 5.7 ± 10.6 | 5.9 ± 8.9 | 0.8264 |
| **missing** | 148 | 29 | 177 | |
| **Indoor activity (hr/week) [b]** | 2.2 ± 3.9 | 1.7 ± 4.0 | 2.1 ± 3.9 | 0.3168 |
| **missing** | 253 | 43 | 296 | |
| **2006-7 flu vaccine** | | | | |
| **Yes** | 85 (**21.3**) | 8 (**11.1**) | 93 (19.7) | **0.0455** |
| **No** | 314 (78.7) | 64 (88.9) | 378 (80.3) | |
| **missing** | 173 | 33 | 206 | |
| **2005-6 flu vaccine** | | | | |
| **Yes** | 77 (21.8) | 8 (13.3) | 85 (20.5) | 0.1355 |
| **No** | 277 (78.2) | 52 (86.7) | 329 (79.5) | |
| **missing** | 218 | 45 | 263 | |
| ***Strep. pneuminia* vaccine** | | | | |
| **Yes** | 30 (7.7) | 1 (1.7) | 31 (6.9) | 0.1022 |
| **No** | 361 (92.3) | 59 (98.3) | 420 (93.1) | |
| **missing** | 181 | 45 | 226 | |
| **Guardian** | | | | |
| **Parents** | 494 (92.2) | 90 (94.7) | 584 (92.3) | 0.6425 |

| | | | |
|---|---|---|---|
| **Grand parents** | 18 (3.4) | 3 (3.2) | 21 (3.3) | |
| **Others** | 24 (4.5) | 2 (2.1) | 26 (4.1) | |
| **missing** | 36 | 10 | 46 | |
| **Education of guardian** | | | | **0.0044** |
| **Elementary or lower** | 25 (4.6) | 13 (**13.1**) | 38 (5.9) | |
| **Junior high** | 97 (17.9) | 15 (15.2) | 112 (17.5) | |
| **High** | 217 (40.0) | 44 (44.4) | 261 (40.7) | |
| **Undergraduate** | 148 (**27.3**) | 16 (16.2) | 164 (25.6) | |
| **Graduate or higher** | 55 (10.2) | 11 (11.1) | 66 (10.3) | |
| **missing** | 30 | 6 | 36 | |
| **Vitamin/ Healthy food** | | | | 0.4559 |
| **Yes** | 166 (30.6) | 26 (26.8) | 192 (30.0) | |
| **No** | 377 (69.4) | 71 (73.2) | 448 (70.0) | |
| **missing** | 29 | 8 | 37 | |
| **Milk-drinking** | | | | 0.3906 |
| **Yes** | 441 (79.8) | 81 (83.5) | 522 (80.3) | |
| **No** | 112 (20.3) | 16 (16.5) | 128 (19.7) | |
| **missing** | 19 | 8 | 27 | |
| **Healthy status** | | | | 0.5598 |
| **Week** | 99 (18.0) | 18 (18.6) | 117 (18.1) | |
| **Moderate** | 406 (73.7) | 74 (76.3) | 480 (74.1) | |
| **Good** | 46 (8.4) | 5 (5.2) | 51 (7.9) | |
| **missing** | 21 | 8 | 29 | |
| **Disease history** | | | | **0.0798** |
| **Yes** | 241 (**48.1**) | 48 (**58.5**) | 289 (49.6) | |
| **No** | 260 (51.9) | 34 (41.5) | 294 (50.4) | |
| **missing** | 71 | 23 | 94 | |
| **Absenteeism** | | | | **0.0009** |
| **Yes** | 120 (**22.1**) | 37 (**37.8**) | 157 (24.5) | |
| **No** | 422 (77.9) | 61 (62.2) | 483 (75.5) | |
| **missing** | 30 | 7 | 37 | |
| **Hospitalization** | | | | 0.7011 |
| **Yes** | 21 (3.9) | 3 (3.1) | 24 (3.7) | |
| **No** | 523 (96.1) | 95 (96.9) | 618 (96.3) | |
| **missing** | 28 | 7 | 35 | |

[a] Student t test was used for continuous data, and $\chi^2$ test was used for categorical data. To test for statistical difference among subgroups of that specific variable, $\alpha$=0.05.

[b] Family number was the total number of the family, including the schoolchild.

[c] Sibling number was the total number of the sibling of the schoolchild, excluding the schoolchild.

[d] It was showed as mean±SD.

**Table 6. Distribution of HI Antibody Serotiters against the Three Human Influenza Virus Vaccine Strains at Three Time-points Stratified by Vaccination Status in Taiwan, Oct, 2007-Apr, 2008.**

| Group | Sero-titer | A/Solomon Islands/3/2006 (H1N1) | | | A/Wisconsin/67/2005 (H3N2) | | | B/Malaysia/2506/2004 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccine (%) | 1-month post-vaccine (%) | 4-month post-Vaccine (%) | Pre-vaccine (%) | 1-month post-vaccine(%) | 4-month post-vaccine (%) | Pre-vaccine (%) | 1-month post-vaccine(%) | 4-monthpost-vaccine(%) |
| Vaccinated Group[a] | **≦10** | 1 (0.2) | 3 (0.5) | 3 (0.6) | 3 (0.5) | 1 (0.2) | 1 (0.2) | 167 (29.2) | 64 (11.2) | 85 (17.1) |
| | **20** | 147 (25.7) | 31 (5.4) | 38 (7.6) | 76 (13.3) | 12 (2.1) | 9 (1.8) | 183 (32.0) | 113 (19.8) | 138 (27.7) |
| | **40** | 275 **(48.1)** | 98 (17.1) | 134 (26.9) | 204 **(35.7)** | 21 (3.7) | 58 (11.7) | 132 (23.1) | 149 (26.1) | 143 (28.7) |
| | **80** | 116 (20.3) | 103 (18.0) | 139 (27.9) | 201 **(35.1)** | 47 (8.2) | 91 (18.3) | 71 (12.4) | 132 **(23.1)** | 93 (18.7) |
| | **160** | 27 (4.7) | 108 **(18.9)** | 100 **(20.1)** | 57 (10.0) | 106 **(18.5)** | 120 **(24.1)** | 17 **(3.0)** | 76 **(13.3)** | 29 **(5.8)** |
| | **320** | 4 (0.7) | 98 **(17.1)** | 64 **(12.9)** | 16 (2.8) | 145 **(25.4)** | 121 **(24.3)** | 1 **(0.2)** | 34 **(5.9)** | 10 **(2.0)** |
| | **640** | 1 (0.2) | 84 **(14.7)** | 16 **(3.2)** | 9 (1.6) | 140 **(24.5)** | 69 **(13.9)** | 1 **(0.2)** | 4 **(0.7)** | 0 (0.0) |
| | **≧1280** | 1 (0.2) | 47 **(8.2)** | 4 **(0.8)** | 6 (1.1) | 100 **(17.5)** | 29 **(5.8)** | **0** (0.0) | 0 (0.0) | 0 (0.0) |
| | **GMT** | 41.99 | **161.11** | **88.77** | 60.63 | **329.00** | **186.36** | 24.45 | 48.57 | 33.40 |
| Unvaccinated Group[a] | **≦10** | 1 (1.0) | 1 (1.0) | 0 (0.0) | 2 (1.9) | 4 (3.8) | 1 (1.1) | 42 (40.0) | 44 (41.9) | 31 (33.7) |
| | **20** | 26 (24.8) | 25 (23.8) | 23 (25.0) | 16 (15.2) | 11 (10.5) | 11 (12.0) | 36 (34.3) | 28 (26.7) | 31 (33.7) |
| | **40** | 45 **(42.9)** | 40 (38.1) | 45 (48.9) | 34 (32.4) | 35 (33.3) | 35 (38.0) | 14 (13.3) | 22 **(21.0)** | 14 (15.2) |
| | **80** | 27 (25.7) | 32 (30.5) | 21 (22.8) | 38 (36.2) | 40 (38.1) | 25 (27.2) | 11 (10.5) | 8 (7.6) | 12 (13.0) |
| | **160** | 5 **(4.8)** | 6 **(5.7)** | 3 **(3.3)** | 8 **(7.6)** | 5 **(4.8)** | 11 **(12.0)** | 2 **(1.9)** | 3 **(2.9)** | 4 **(4.4)** |
| | **320** | 1 (1.0) | 1 **(1.0)** | **0** (0.0) | 4 (3.8) | 3 **(2.9)** | 6 **(6.5)** | **0** (0.0) | 0 (0.0) | 0 (0.0) |
| | **640** | 0 (0.0) | 0 (0.0) | **0** (0.0) | 2 (1.9) | 5 **(4.8)** | 2 **(2.2)** | **0** (0.0) | 0 (0.0) | 0 (0.0) |
| | **≧1280** | 0 (0.0) | 0 (0.0) | **0** (0.0) | 1 (1.0) | 2 **(1.9)** | 1 **(1.1)** | **0** (0.0) | 0 (0.0) | 0 (0.0) |
| | **GMT** | 43.17 | **45.63** | **41.12** | 58.97 | **63.64** | **65.43** | 20.00 | 20.42 | 23.13 |

[a] There were 572 vaccinated children and 105 unvaccinated children with paired serum samples at pre-vaccination and 1-month post-vaccination. At 4-month post-vaccination, there were 498 vaccinated and 92 unvaccinated schoolchildren with triple serum samples.

**Table 7A. Vaccine Efficacy in Preventing Human Influenza Virus Infection Evaluated by their 4-fold HI Serotiter Rise against the Three Strains of Human Influenza Vaccine Viruses among Schoolchildren in Taiwan, from 1-month Post-vaccination to 4-month Post-vaccination during the 2007-2008 Influenza Season.**

| | Vaccinated Group | | | Unvaccinated Group | | | Vaccine Efficacy in Preventing Human Flu Virus Infection | | |
|---|---|---|---|---|---|---|---|---|---|
| **Virus** | **N** | **n** | **%** | **N** | **n** | **%** | **%** | **95% CI** | **p-value** |
| **A/H1N1** | 498 | 7 | 1.41 | 92 | 1 | 1.09 | **-29.32** | - | 1.0000 |
| **A/H3N2** | 498 | 3 | 0.60 | 92 | 4 | 4.35 | **86.14** | (39.11, 96.85) | 0.0134 |
| **B** | 498 | 10 | 2.01 | 92 | 3 | 3.26 | **38.42** | (-119.47, 82.82) | 0.4379 |

N=total schoolchildren in vaccinated or unvaccinated group, respectively, n=schoolchildren with 4-fold HI serotiter rise, and p-values were calculated by $\chi^2$ test.

**Table 7B. Vaccine Effectiveness in Reducing Influenza-like Illness (ILI) between Vaccinated and Unvaccinated Schoolchildren in Taiwan from 1-month Post-vaccination to the Ending Period of the 2007-2008 Influenza Season.**

| Syndrome Group Used in ILI | Vaccinated Group | | | Unvaccinated Group | | | Vaccine Effectiveness against ILI | | |
|---|---|---|---|---|---|---|---|---|---|
| **Definition**[a] | **N** | **n** | **%** | **N** | **n** | **%** | **%** | **95% CI** | **p-value** |
| **One Systematic + One local s/s** | 547 | 108 | 19.74 | 97 | 28 | 28.87 | **31.60** | (2.43, 52.05) | 0.0425 |

N=total schoolchildren in vaccinated or unvaccinated group, respectively, n=schoolchildren with 4-fold HI serotiter rise, and p-values were calculated by $\chi^2$ test.

[a] Four systematic s/s (fever, chills, myalgia/ joint pain, and tiredness) plus any one of the four respiratory s/s (sore throat, rhinorrhea or nasal congestion, cough, and raucous).

**Table 7C. Vaccine Effectiveness in Decreasing the Absenteeism and Hospitalization Rates Related to Respiratory Illness among Schoolchildren during the 2007-2008 Influenza Season.**

| | Vaccinated Group | | | Unvaccinated Group | | | Vaccine Effectiveness Related to Abnormal Activities | | |
|---|---|---|---|---|---|---|---|---|---|
| **Factor** | **N** | **n** | **%** | **N** | **n** | **%** | **%** | **95% CI** | **p-value** |
| **Absenteeism** | 542 | 90 | 16.61 | 98 | 29 | 29.59 | **43.89** | (19.65, 60.81) | 0.0024 |
| **Hospitalization** | 542 | 6 | 1.11 | 98 | 2 | 2.04 | **45.76** | (-164.88, 88.89) | 0.3529 |

N=total schoolchildren in vaccinated or unvaccinated group, respectively, n=schoolchildren with 4-fold HI serotiter rise, and p-values were calculated by $\chi^2$ test.

**Table 8A. Vaccine Effectiveness in Acquiring Influenza-like Illness (ILI) between the Vaccinated and Unvaccinated Schoolchildren with Human Influenza Virus Infection[a] from 1-month Post-vaccination through the 2007-2008 Influenza Season in April, 2008.**

| Syndrome Group Used in ILI Definition[b] | Vaccinated Group | | | Unvaccinated Group[c] | | | Vaccine Effectiveness against ILI | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | n | % | N[a] | n | % | % | 95% CI | p-value |
| **One Systematic + One local s/s** | 19 | 5 | 26.32 | 7 | 4 | 57.14 | **53.95** | (-23.79, 82.87) | 0.1881 |

[a] Measured by the 4-fold anti-influenza HI Antibody with at least 4-fold Serotiter Rise.
[b] Four systematic s/s (fever, chills, myalgia/ joint pain, and tiredness) plus any one of the four respiratory s/s (sore throat, rhinorrhea or nasal congestion, cough, and raucous).
[c] One unvaccinated child did not provide ILI information.

**Table 8B. Vaccine Effectiveness in the Rates of Absenteeism and Hospitalization Related to Respiratory Illness between the Vaccinated and Unvaccinated Schoolchildren with Human Influenza Virus Infection[a] during the 2007-2008 Influenza Season.**

| | Vaccinated Group[b] | | | Unvaccinated Group[b] | | | Vaccine Effectiveness Related to Abnormal Activities | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | N[a] | n | % | N[a] | n | % | % | 95% CI | p-value |
| **Absenteeism** | 18 | 9 | 50.00 | 7 | 6 | 85.71 | **41.67** | (-1.32, 66.42) | 0.1794 |
| **Hospitalization** | 19 | 1 | 5.26 | 7 | 1 | 14.29 | **63.16** | (-412.60, 97.35) | 0.4738 |

[a] Measured by the 4-fold anti-influenza HI Antibody with at least 4-fold Serotiter Rise.
[b] Data of absenteeism were missing for one vaccinated and one unvaccinated child, respectively. Data of hospitalization were missing for one unvaccinated child.

**Table 8C. Vaccine Effectiveness in ILI[a] of the Household Members between the Vaccinated and Unvaccinated Schoolchildren with Human Influenza Virus Infection[b] during the 2007-2008 Influenza Season.**

| Age | Vaccinated Group | | | Unvaccinated Group | | | Vaccine Effectiveness of Houshold's ILI | | |
|---|---|---|---|---|---|---|---|---|---|
| **Groups** | **N** | **N** | **%** | **N** | **n** | **%** | **%** | **95% CI** | **p-value** |
| **0~6** | 10 | 0 | 0.00 | 3 | 1 | 33.33 | 100.00 | - | - |
| **7~12** | 11 | 1 | 9.09 | 5 | 1 | 20.00 | 54.55 | (-489.35, 96.41) | 1.0000 |
| **13~18** | 2 | 1 | 50.00 | 7 | 2 | 28.57 | -75.01 | - | 1.0000 |
| **19~64** | 40 | 8 | 20.00 | 17 | 4 | 23.53 | 15.00 | (-144.76, 70.48) | 0.7371 |
| **≧65** | 9 | 1 | 11.11 | 2 | 1 | 50.00 | 77.78 | (-123.84, 97.79) | 0.3455 |
| **Total** | 72 | 11 | 15.28 | 34 | 9 | 26.47 | 42.28 | (-26.01, 73.56) | **0.1692** |
| **missing** | 25 | | | 10 | | | | | |

[a] Four systematic s/s (fever, chills, myalgia/ joint pain, and tiredness) plus any one of the four respiratory s/s (sore throat, rhinorrhea or nasal congestion, cough, and raucous).

[b] Measured by the 4-fold anti-influenza HI Antibody with at least 4-fold Serotiter Rise.

**Table 9. Sero-protection Rates of anti-influenza HI Antibodies between the Vaccinated and Unvaccinated Children for the Three Human Influenza Virus Vaccine Strains[a] at Three Time-points, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Sero-protection Rates for A/Solomon Islands/3/2006 (H1N1) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | 1-month Post-vaccination* | | | | 4-month Post-vaccination* | | |
| | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | 572 | 424 | **74.13** | (70.54, 77.72) | 538 | **94.06** | (92.12, 96.00) | 498 | 457 | **91.77** | (89.36, 94.18) |
| **Unvaccinated** | 105 | 78 | **74.29** | (65.93, 82.65) | 79 | 75.24 | (66.98, 83.50) | 92 | 69 | 75.00 | (66.15, 83.85) |
| **Total** | 677 | 502 | 74.15 | (70.85, 77.45) | 617 | 91.14 | (89.00, 93.28) | 590 | 526 | 89.15 | (86.64, 91.66) |

| Human Flu Vaccination Status | Sero-protection Rates for A/Wisconsin/67/2005 (H3N2) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | 1-month Post-vaccination* | | | | 4-month Post-vaccination* | | |
| | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | 572 | 493 | **86.19** | (83.36, 89.02) | 559 | **97.73** | (96.51, 98.95) | 498 | 488 | **97.99** | (96.76, 99.22) |
| **Unvaccinated** | 105 | 87 | **82.86** | (75.65, 90.07) | 90 | 85.71 | (79.02, 92.40) | 92 | 80 | 86.96 | (80.08, 93.84) |
| **Total** | 677 | 580 | 85.67 | (83.03, 88.31) | 649 | 95.86 | (94.36, 97.36) | 590 | 568 | 96.27 | (94.74, 97.80) |

| Human Flu Vaccination Status | Sero-protection Rates for B/Malaysia/2506/2004 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination* | | | 1-month Post-vaccination* | | | | 4-month Post-vaccination* | | |
| | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | 572 | 222 | **38.81** | (34.82, 42.80) | 395 | **69.06** | (65.27, 72.85) | 498 | 275 | **55.22** | (50.85, 59.59) |
| **Unvaccinated** | 105 | 27 | **25.71** | (17.35, 34.07) | 33 | 31.43 | (22.5, 40.31) | 92 | 30 | 32.61 | (23.03, 42.19) |
| **Total** | 677 | 249 | 36.73 | (33.10, 40.36) | 428 | 63.22 | (59.59, 66.85) | 590 | 305 | 51.69 | (47.66, 55.72) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

* Comparison between vaccinated and unvaccinated children reached statistical significance with p-value <0.05.

[a] The 2007-08 influenza vaccine strains, A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 viruses were recommended by the WHO for northern hemisphere.

**Table 10. The Percentages of the Four-fold Serotiter rise of Anti-influenza HI Antibodies against the Three Human Influenza Vaccine Viruses[a] between Vaccinated and Unvaccinated Schoolchildren in Taiwan from Pre-vaccination to 1-month and from 1-month to 4-month Post-vaccination, Oct, 2007-Apr, 2008**

| Human Flu Vaccination Status | 4-fold Sero-titer Rise and Seroincidence for A/Solomon Islands/3/2006 (H1N1) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | From Pre- to 1-month Post-vaccination | | | | From Pre- to 4-month Post-vaccination | | | | Seroincidence of Infection (from 1- to 4-month Post-vaccination) | | | |
| | N | n | % | 95% CI | N | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | 572 | 307 | **53.67** | (49.58, 57.76) | 498 | 173 | **34.73** | (30.55, 38.91) | 498 | 7 | **1.41** | (0.37, 2.45) |
| **Unvaccinated** | 105 | 4 | 3.81 | (0.15, 7.47) | 92 | 5 | 5.43 | (0.80, 10.06) | 92 | 1 | **1.09** | (-1.03, 3.21) |
| **Total** | 677 | 311 | 45.94 | (42.19, 49.69) | 590 | 178 | 30.17 | (26.47, 33.87) | 590 | 8 | 1.36 | (0.43, 2.29) |

| Human Flu Vaccination Status | 4-fold Sero-titer Rise and Seroincidence for A/Wisconsin/67/2005 (H3N2) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-month post-vaccination | | | | 4-month post-vaccination | | | | Seroincidence of Infection (from 1- to 4-month Post-vaccination) | | | |
| | N | n | % | 95% CI | N | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | 572 | 405 | **70.80** | (67.03, 74.53) | 498 | 247 | **49.60** | (45.21, 53.99) | 498 | 3 | **0.60** | (-0.08, 1.28) |
| **Unvaccinated** | 105 | 4 | 3.81 | (0.15, 7.47) | 92 | 6 | 6.52 | (1.48, 11.56) | 92 | 4 | **4.35** | (0.18, 8.52) |
| **Total** | 677 | 409 | 60.41 | (56.73, 64.09) | 590 | 253 | 42.88 | (38.89, 46.87) | 590 | 7 | 1.19 | (0.32, 2.06) |

| Human Flu Vaccination Status | 4-fold Sero-titer Rise and Seroincidence for B/Malaysia/2506/2004 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-month post-vaccination | | | | 4-month post-vaccination | | | | Seroincidence of Infection (from 1- to 4-month Post-vaccination) | | | |
| | N | n | % | 95% CI | N | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | 572 | 197 | **34.44** | (30.55, 38.33) | 498 | 70 | **14.06** | (11.00, 17.11) | 498 | 10 | **2.01** | (0.78, 3.24) |
| **Unvaccinated** | 105 | 0 | 0.00 | - | 92 | 3 | 3.26 | (-0.37, 6.89) | 92 | 3 | **3.26** | (-0.37, 6.89) |
| **Total** | 677 | 197 | 29.10 | (25.68, 32.52) | 590 | 73 | 12.37 | (9.71, 15.03) | 590 | 13 | 2.20 | (1.02, 3.38) |

N: total number of children, n: number of children with 4-fold Sero-titer Rise, %: n/N, 95% CI: 95% confidence interval.

[a] The 2007-08 influenza vaccine strains, A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 viruses were recommended by the WHO for northern hemisphere.

**Table 11 Geographical and School Variations in Geometric Mean Titers (GMT) of HI Antibodies to the 2007 Three Human Influenza Virus Vaccine Strains among Schoolchildren in the Three Areas at Pre-, 1-month Post- and 4-month Post- vaccination, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Area/ School | | (A) GMTs of HI Ab to A/Solomon Islands/3/2006 (H1N1) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N | Pre-vaccination | 1-month Post-vaccination | N | 4-month Post-vaccination |
| **Vaccinated** | **Taipei** | **GT** | 107 | **34.92** | **245.39*** | 92 | **133.52*** |
| | **Yilan** | | 334 | **41.99** | **129.96** | 294 | **74.64** |
| | | **LT** | 184 | 40.00 | 142.21 | 161 | 85.15 |
| | | **LZ** | 101 | 41.41 | 125.01 | 90 | 60.63 |
| | | **SS** | 49 | 50.88 | 100.35 | 43 | 72.60 |
| | **Kinmen** | | 131 | **49.59** | **194.27** | 112 | **97.81** |
| | | **JJ** | 86 | 42.57 | 186.36 | 78 | 93.83 |
| | | **JH** | 45 | 66.35* | 211.12 | 34 | 108.53 |
| **Unvaccinated** | **Taipei** | **GT** | 7 | **32.81** | 40.00 | 7 | 44.17 |
| | **Yilan** | | 47 | **43.17** | 49.25 | 43 | 43.47 |
| | | **LT** | 23 | 38.91 | 42.57 | 22 | 41.41 |
| | | **LZ** | 9 | 50.39 | 58.51 | 8 | 40.00 |
| | | **SS** | 15 | 45.95 | 55.29 | 13 | 49.52 |
| | **Kinmen** | | 51 | **45.32** | 43.47 | 42 | 38.64 |
| | | **JJ** | 41 | 41.41 | 39.45 | 34 | 33.87 |
| | | **JH** | 10 | 64.98* | 64.98* | 8 | 67.27* |
| **Total** | **Taipei** | **GT** | 114 | 34.77 | 219.48 | 99 | 123.46 |
| | **Yilan** | | 381 | 42.02 | 115.35 | 337 | 69.84 |
| | | **LT** | 207 | 39.86 | 124.49 | 183 | 77.92 |
| | | **LZ** | 110 | 42.08 | 117.53 | 98 | 58.60 |
| | | **SS** | 64 | 49.69 | 87.24 | 56 | 66.44 |
| | **Kinmen** | | 182 | 48.40 | 127.82 | 154 | 76.16 |
| | | **JJ** | 127 | 42.25 | 112.82 | 112 | 68.97 |
| | | **JH** | 55 | 66.21 | 170.42 | 42 | 99.18 |

N: total number of children at that specific time point

GMTs with bold phase numbers represent the highest values in geographical comparison in Taipei, Yilan and Kinmen at pre-, 1-month post- and 4-month post-vaccination.

GMTs with Star (*) signs indicate the highest values at school comparison among the six schools at pre-, 1-month post- and 4-month post-vaccination.

**Table 11. (cont.)**

| Human Flu Vaccination Status | Area/ School | | (B) GMTs of HI Ab to A/Wisconsin/67/2005 (H3N2) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N | Pre-vaccination | 1-month Post-vaccination | N | 4-month Post-vaccination |
| **Vaccinated** | **Taipei** | **GT** | 107 | **82.65** | **540.79*** | 92 | **281.49*** |
| | **Yilan** | | 334 | **64.53** | **294.46** | 294 | **172.68** |
| | | **LT** | 184 | 84.56* | 338.25 | 161 | 220.09 |
| | | **LZ** | 101 | 47.80 | 234.90 | 90 | 115.75 |
| | | **SS** | 49 | 42.93 | 285.81 | 43 | 160.00 |
| | **Kinmen** | | 131 | **41.12** | **290.41** | 112 | **161.11** |
| | | **JJ** | 86 | 34.58 | 298.57 | 78 | 160.00 |
| | | **JH** | 45 | 56.96 | 278.58 | 34 | 163.36 |
| **Unvaccinated** | **Taipei** | **GT** | 7 | **80.00** | 80.00 | 7 | 72.45 |
| | **Yilan** | | 47 | **69.16** | 83.40 | 43 | 78.90 |
| | | **LT** | 23 | 90.00* | 122.1* | 22 | 96.46* |
| | | **LZ** | 9 | 58.81 | 58.81 | 8 | 56.57 |
| | | **SS** | 15 | 50.39 | 57.88 | 13 | 68.16 |
| | **Kinmen** | | 51 | **48.91** | 47.57 | 42 | 52.78 |
| | | **JJ** | 41 | 48.23 | 46.59 | 34 | 52.05 |
| | | **JH** | 10 | 52.78 | 52.78 | 8 | 56.57 |
| **Total** | **Taipei** | **GT** | 114 | 82.48 | 481.01 | 99 | 255.81 |
| | **Yilan** | | 381 | 65.03 | 252.64 | 337 | 156.06 |
| | | **LT** | 207 | 85.27 | 301.27 | 183 | 199.32 |
| | | **LZ** | 110 | 48.64 | 209.81 | 98 | 109.21 |
| | | **SS** | 64 | 44.57 | 196.57 | 56 | 131.23 |
| | **Kinmen** | | 182 | 43.17 | 175.33 | 154 | 118.84 |
| | | **JJ** | 127 | 38.50 | 163.48 | 112 | 113.85 |
| | | **JH** | 55 | 56.22 | 205.92 | 42 | 133.43 |

N: total number of children at that specific time point

GMTs with bold phase numbers represent the highest values in geographical comparison in Taipei, Yilan and Kinmen at pre-, 1-month post- and 4-month post-vaccination.

GMTs with Star (*) signs indicate the highest values at school comparison among the six schools at pre-, 1-month post- and 4-month post-vaccination.

**Table 11. (cont.)**

| Human Flu Vaccination Status | Area/ School | | (C) GMTs of HI Ab to B/Malaysia/2506/2004 | | | | |
|---|---|---|---|---|---|---|---|
| | | | N | Pre-vaccination | 1-month Post-vaccination | N | 4-month Post-vaccination |
| **Vaccinated** | **Taipei** | **GT** | 107 | **31.08** | **61.73** | 92 | **41.84** |
| | **Yilan** | | 334 | **27.70** | **47.90** | 294 | **34.58** |
| | | **LT** | 184 | 48.91* | 63.64* | 161 | 58.56* |
| | | **LZ** | 101 | 14.29 | 31.89 | 90 | 17.81 |
| | | **SS** | 49 | 13.09 | 37.79 | 43 | 19.68 |
| | **Kinmen** | | 131 | **14.64** | **41.70** | 112 | **25.85** |
| | | **JJ** | 86 | 12.83 | 37.58 | 78 | 22.82 |
| | | **JH** | 45 | 18.79 | 51.34 | 34 | 33.40 |
| **Unvaccinated** | **Taipei** | **GT** | 7 | **32.81** | 36.23 | 7 | 32.81 |
| | **Yilan** | | 47 | **25.67** | 26.39 | 43 | 31.82 |
| | | **LT** | 23 | 49.25* | 50.98* | 22 | 62.33* |
| | | **LZ** | 9 | 18.52 | 20.00 | 8 | 20.00 |
| | | **SS** | 15 | 11.49 | 11.49 | 13 | 13.77 |
| | **Kinmen** | | 51 | **14.85** | 14.85 | 42 | 15.58 |
| | | **JJ** | 41 | 14.04 | 13.29 | 34 | 15.05 |
| | | **JH** | 10 | 18.66 | 22.97 | 8 | 18.40 |
| **Total** | **Taipei** | **GT** | 114 | 31.17 | 59.75 | 99 | 41.12 |
| | **Yilan** | | 381 | 27.49 | 44.44 | 337 | 34.27 |
| | | **LT** | 207 | 48.91 | 62.03 | 183 | 58.85 |
| | | **LZ** | 110 | 14.59 | 30.69 | 98 | 17.99 |
| | | **SS** | 64 | 12.68 | 28.60 | 56 | 18.11 |
| | **Kinmen** | | 182 | 14.69 | 31.23 | 154 | 22.38 |
| | | **JJ** | 127 | 13.21 | 26.85 | 112 | 20.13 |
| | | **JH** | 55 | 18.78 | 44.23 | 42 | 29.71 |

N: total number of children at that specific time point

GMTs with bold phase numbers represent the highest values in geographical comparison in Taipei, Yilan and Kinmen at pre-, 1-month post- and 4-month post-vaccination.

GMTs with Star (*) signs indicate the highest values at school comparison among the six schools at pre-, 1-month post- and 4-month post-vaccination.

**Table 12. Geographical Variations in Geometric Mean Titers (GMT) of HI Antibodies to the 2007 Three Human Influenza Virus Vaccine Strains among the 267 Vaccinated Schoolchildren without Influenza Vaccination in 2005 and 2006 in the Three Study Areas at Pre-, 1-month Post- and 4-month Post-vaccination, Oct, 2007-Apr, 2008.**

| | (A) GMTs of HI Ab to A/Solomon Islands/3/2006 (H1N1) | | | | |
|---|---|---|---|---|---|
| **Area** | **N** | **Pre-vaccination** | **1-month Post-vaccination** | **N** | **4-month Post-vaccination** |
| **Taipei** | 59 | 33.15 | **271.52** | 47 | **142.21** |
| **Yilan** | 149 | 41.70 | 143.10 | 131 | 85.68 |
| **Kinmen** | 59 | **46.59** | 241.34 | 51 | 107.85 |
| | **(B) GMTs of HI Ab to A/Wisconsin/67/2005 (H3N2)** | | | | |
| **Area** | **N** | **Pre-vaccination** | **1-month Post-vaccination** | **N** | **4-month Post-vaccination** |
| **Taipei** | 59 | **71.95** | **597.14** | 47 | **301.69** |
| **Yilan** | 149 | 67.97 | 369.63 | 131 | 199.87 |
| **Kinmen** | 59 | 41.93 | 312.55 | 51 | 155.73 |
| | **(C) GMTs of HI Ab to B/Malaysia/2506/2004** | | | | |
| **Area** | **N** | **Pre-vaccination** | **1-month Post-vaccination** | **N** | **4-month Post-vaccination** |
| **Taipei** | 59 | **30.89** | **75.42** | 47 | **45.00** |
| **Yilan** | 149 | 29.28 | 53.11 | 131 | 36.76 |
| **Kinmen** | 59 | 14.56 | 46.04 | 51 | 28.86 |
| | **Total** | | | | |
| **Virus** | **N** | **Pre-vaccination** | **1-month Post-vaccination** | **N** | **4-month Post-vaccination** |
| **A/H1N1** | 267 | 40.61 | 185.07 | 229 | 100.07 |
| **A/H3N2** | 267 | **61.86** | **395.88** | 229 | **205.63** |
| **B** | 267 | 25.40 | 55.64 | 229 | 36.30 |

GMTs with bold phase numbers represent the highest values at pre-, 1-month post- and 4-month post-vaccination, either in area comparison or in the three 2007 WHO recommended vaccine component comparison.

**Table 13. Reactogenicity during Three Days after Receiving the Influenza Vaccination in 2007 Recorded from Parents or Guardians of the Total 468 Participated Schoolchildren in Taiwan during Nov-Dec, 2007.**

| | Yes | No |
|---|---|---|
| **Reactions** | **n (%)** | **n (%)** |
| **Pain at injection site** | 71 (15.2) | 397 (84.8) |
| **Arm pain** | 37 (7.9) | 431 (92.1) |
| **Runny nose** | 33 (7.1) | 435 (92.9) |
| **Redness at injection site** | 19 (4.1) | 449 (95.9) |
| **Swelling at injection site** | 8 (1.7) | 460 (98.3) |
| **Fever** | 6 (1.3) | 462 (98.7) |
| **Headache** | 6 (1.3) | 462 (98.7) |
| **Tiredness** | 4 (0.9) | 464 (99.1) |
| **Nausea/Vomiting** | 3 (0.6) | 465 (99.4) |
| **Otitis Media** | 3 (0.6) | 465 (99.4) |
| **Body myalgia** | 2 (0.4) | 466 (99.6) |
| **Skin rash** | 2 (0.4) | 466 (99.6) |
| **Decrease appetite** | 1 (0.2) | 467 (99.8) |
| **Others** | 12 (2.6) | 456 (97.4) |

n: number of schoolchildren with that specific clinical symptoms/signs

**Appendix A.1 Sero-protection Rates of HI Antibodies against the Three Human Influenza Vaccine Viruses among Schoolchildren in the Three Study Areas (ie. Six Schools) Stratified by Their Vaccination Status at Three Time-points, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Area/ School | | A/Solomon Islands/3/2006 (H1N1) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pre-vaccination | | | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI | |
| **Vaccinated** | **Taipei** | **GT** | 107 | 64 | 59.81 | (50.52, 69.10) | 97 | 90.65 | (85.13, 96.17) | 92 | 86 | 93.48 | (88.44, | 98.52) |
| | **Yilan** | | 334 | 253 | 75.75 | (71.15, 80.35) | 313 | 93.71 | (91.11, 96.31) | 294 | 261 | 88.78 | (85.17, | 92.39) |
| | | **LT** | 184 | 134 | 72.83 | (66.40, 79.26) | 172 | 93.48 | (89.91, 97.04) | 161 | 143 | 88.82 | (83.95, | 93.69) |
| | | **LZ** | 101 | 74 | 73.27 | (64.64, 81.90) | 93 | 92.08 | (86.81, 97.35) | 90 | 76 | 84.44 | (76.95, | 91.93) |
| | | **SS** | 49 | 45 | 91.84 | (84.18, 99.51) | 48 | 97.96 | (94.06, 101.86) | 43 | 42 | 97.67 | (93.16, | 102.18) |
| | **Kinmen** | | 131 | 107 | 81.68 | (75.06, 88.30) | 128 | 97.71 | (95.15, 100.27) | 112 | 110 | 98.21 | (95.75, | 100.67) |
| | | **JJ** | 86 | 65 | 75.58 | (66.51, 84.66) | 83 | 96.51 | (92.63, 100.39) | 78 | 76 | 97.44 | (93.93, | 100.95) |
| | | **JH** | 45 | 42 | 93.33 | (86.04, 100.62) | 45 | 100.00 | - | 34 | 34 | 100.00 | - | |
| **Unvaccinated** | **Taipei** | **GT** | 7 | 4 | 57.14 | (20.48, 93.80) | 5 | 71.43 | (37.96, 104.90) | 7 | 5 | 71.43 | (37.96, | 104.90) |
| | **Yilan** | | 47 | 37 | 78.72 | (67.02, 90.42) | 39 | 82.98 | (72.24, 93.72) | 43 | 37 | 86.05 | (75.69, | 96.41) |
| | | **LT** | 23 | 16 | 69.57 | (50.77, 88.37) | 17 | 73.91 | (55.96, 91.86) | 22 | 18 | 81.82 | (65.70, | 97.94) |
| | | **LZ** | 9 | 7 | 77.78 | (50.62, 104.94) | 7 | 77.78 | (50.62, 104.94) | 8 | 6 | 75.00 | (44.99, | 105.01) |
| | | **SS** | 15 | 14 | 93.33 | (80.70, 105.96) | 15 | 100.00 | - | 13 | 13 | 100.00 | - | |
| | **Kinmen** | | 51 | 37 | 72.55 | (60.30, 84.80) | 35 | 68.63 | (55.90, 81.36) | 42 | 27 | 64.29 | (49.80, | 78.78) |
| | | **JJ** | 41 | 27 | 65.85 | (1.33, 80.37) | 25 | 60.98 | (46.05, 75.91) | 34 | 19 | 55.88 | (39.19, | 72.57) |
| | | **JH** | 10 | 10 | 100.00 | - | 10 | 100.00 | - | 8 | 8 | 100.00 | - | |
| **Total** | **Taipei** | **GT** | 114 | 68 | 59.65 | (50.65, 68.66) | 102 | 89.47 | (83.51, 95.43) | 99 | 91 | 91.92 | (86.55, | 97.29) |
| | **Yilan** | | 381 | 290 | 76.12 | (71.84, 80.40) | 352 | 92.39 | (89.62, 95.16) | 337 | 298 | 88.43 | (85.02, | 91.85) |
| | | **LT** | 207 | 150 | 72.46 | (66.38, 78.55) | 189 | 91.30 | (87.46, 95.14) | 183 | 161 | 87.98 | (83.27, | 92.69) |
| | | **LZ** | 110 | 81 | 73.64 | (65.41, 81.87) | 100 | 90.91 | (85.54, 96.28) | 98 | 82 | 83.67 | (76.35, | 90.99) |
| | | **SS** | 64 | 59 | 92.19 | (85.62, 98.76) | 63 | 98.44 | (95.40, 101.48) | 56 | 55 | 98.21 | (94.74, | 101.68) |
| | **Kinmen** | | 182 | 144 | 79.12 | (73.22, 85.03) | 163 | 89.56 | (84.87, 94.25) | 154 | 137 | 88.96 | (84.01, | 93.91) |
| | | **JJ** | 127 | 92 | 72.44 | (64.67, 80.21) | 108 | 85.04 | (78.84, 91.24) | 112 | 95 | 84.82 | (78.17, | 91.47) |
| | | **JH** | 55 | 52 | 94.55 | (88.55, 100.55) | 55 | 100.00 | - | 42 | 42 | 100.00 | - | |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix A.1 (cont.)**

| Human Flu Vaccination Status | Area/ School | | A/Wisconsin/67/2005 (H3N2) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pre-vaccination | | | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI | |
| **Vaccinated** | **Taipei** | **GT** | 107 | 107 | 100.00 | - | 107 | 100.00 | - | 92 | 92 | 100.00 | - | |
| | **Yilan** | | 334 | 293 | 87.72 | (84.20, 91.24) | 326 | 97.60 | (95.96, 99.24) | 294 | 287 | 97.62 | (95.88, | 99.36) |
| | | **LT** | 184 | 175 | 95.11 | (91.99, 98.23) | 181 | 98.37 | (96.54, 100.20) | 161 | 161 | 100.00 | - | |
| | | **LZ** | 101 | 83 | 82.18 | (74.72, 89.64) | 99 | 98.02 | (95.30, 100.74) | 90 | 85 | 94.44 | (89.71, | 99.17) |
| | | **SS** | 49 | 35 | 71.43 | (58.78, 84.08) | 46 | 93.88 | (87.17, 100.59) | 43 | 41 | 95.35 | (89.06, | 101.64) |
| | **Kinmen** | | 131 | 93 | 70.99 | (63.22, 78.76) | 126 | 96.18 | (92.90, 99.46) | 112 | 109 | 97.32 | (94.33, | 100.31) |
| | | **JJ** | 86 | 49 | 56.98 | (46.52, 67.44) | 81 | 94.19 | (89.25, 99.13) | 78 | 75 | 96.15 | (91.88, | 100.42) |
| | | **JH** | 45 | 44 | 97.78 | (93.48, 102.08) | 45 | 100.00 | - | 34 | 34 | 100.00 | - | |
| **Unvaccinated** | **Taipei** | **GT** | 7 | 7 | 100.00 | - | 7 | 100.00 | - | 7 | 7 | 100.00 | - | |
| | **Yilan** | | 47 | 42 | 89.36 | (80.54, 98.18) | 45 | 95.74 | (89.97, 101.51) | 43 | 40 | 93.02 | (85.40, | 100.64) |
| | | **LT** | 23 | 23 | 100.00 | - | 23 | 100.00 | - | 22 | 21 | 95.45 | (86.74, | 104.16) |
| | | **LZ** | 9 | 8 | 88.89 | (68.36, 109.42) | 9 | 100.00 | - | 8 | 7 | 87.50 | (64.58, | 110.42) |
| | | **SS** | 15 | 11 | 73.33 | (50.95, 95.71) | 13 | 86.67 | (69.47, 103.87) | 13 | 12 | 92.31 | (77.83, | 106.79) |
| | **Kinmen** | | 51 | 38 | 74.51 | (62.55, 86.47) | 38 | 74.51 | (62.55, 86.47) | 42 | 33 | 78.57 | (66.16, | 90.98) |
| | | **JJ** | 41 | 28 | 68.29 | (54.05, 82.53) | 28 | 68.29 | (54.05, 82.53) | 34 | 25 | 73.53 | (58.70, | 88.36) |
| | | **JH** | 10 | 10 | 100.00 | - | 10 | 100.00 | - | 8 | 8 | 100.00 | - | |
| **Total** | **Taipei** | **GT** | 114 | 114 | 100.00 | - | 114 | 100.00 | - | 99 | 99 | 100.00 | - | |
| | **Yilan** | | 381 | 335 | 87.93 | (84.66, 91.20) | 371 | 97.38 | (95.78, 98.98) | 337 | 327 | 97.03 | (95.22, | 98.84) |
| | | **LT** | 207 | 198 | 95.65 | (92.87, 98.43) | 204 | 98.55 | (96.92, 100.18) | 183 | 182 | 99.45 | (98.38, | 100.52) |
| | | **LZ** | 110 | 91 | 82.73 | (75.67, 89.79) | 108 | 98.18 | (95.73, 100.63) | 98 | 92 | 93.88 | (89.13, | 98.63) |
| | | **SS** | 64 | 46 | 71.88 | (60.87, 82.89) | 59 | 92.19 | (85.62, 98.76) | 56 | 53 | 94.64 | (88.74, | 100.54) |
| | **Kinmen** | | 182 | 131 | 71.98 | (65.46, 78,51) | 164 | 90.11 | (85.77, 94.45) | 154 | 142 | 92.21 | (87.98, | 96.44) |
| | | **JJ** | 127 | 77 | 60.63 | (52.13, 69.13) | 109 | 85.83 | (82.54, 89.12) | 112 | 100 | 89.29 | (83.56, | 95.02) |
| | | **JH** | 55 | 54 | 98.18 | (89.18, 107.18) | 55 | 100.00 | - | 42 | 42 | 100.00 | - | |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix A.1 (cont.)**

| Human Flu Vaccination Status | Area/ School | | B/Malaysia/2506/2004 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pre-vaccination | | | | 1-month post-vaccination | | | 4-month post-vaccination | | | |
| | | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | **Taipei** | **GT** | 107 | 56 | 52.34 | (42.88, 61.80) | 88 | 82.24 | (75.00, 89.48) | 92 | 65 | 70.65 | (61.34, 79.96) |
| | **Yilan** | | 334 | 161 | 48.20 | (42.84, 53.56) | 232 | 69.46 | (64.52, 74.40) | 294 | 171 | 58.16 | (52.52, 63.80) |
| | | **LT** | 184 | 146 | 79.35 | (42.88, 61.80) | 158 | 85.87 | (80.84, 90.90) | 161 | 145 | 90.06 | (85.44, 94.68) |
| | | **LZ** | 101 | 12 | 11.88 | (5.57, 18.19) | 50 | 49.50 | (39.75, 59.25) | 90 | 16 | 17.78 | (9.88, 25.68) |
| | | **SS** | 49 | 3 | 8.69 | (0.80, 16.58) | 24 | 48.98 | (34.98, 62.98) | 43 | 10 | 23.26 | (10.63, 35.89) |
| | **Kinmen** | | 131 | 5 | 3.82 | (0.54, 7.10) | 75 | 57.25 | (48.78, 65.72) | 112 | 39 | 34.82 | (26.00, 43.64) |
| | | **JJ** | 86 | 2 | 2.33 | (-0.86, 5.52) | 48 | 55.81 | (45.31, 66.31) | 78 | 24 | 30.77 | (20.53, 41.01) |
| | | **JH** | 45 | 3 | 6.67 | (-0.62, 13.96) | 27 | 60.00 | (45.69, 74.31) | 34 | 15 | 44.12 | (27.43, 60.81) |
| **Unvaccinated** | **Taipei** | **GT** | 7 | 4 | 57.14 | (20.48, 93.80) | 5 | 71.43 | (37.96, 104.90) | 7 | 4 | 57.14 | (20.48, 93.80) |
| | **Yilan** | | 47 | 21 | 44.68 | (30.47, 58.89) | 23 | 48.94 | (34.65, 63.23) | 43 | 23 | 53.49 | (38.58, 68.40) |
| | | **LT** | 23 | 18 | 78.26 | (61.40, 95.12) | 20 | 86.96 | (73.20, 100.72) | 22 | 20 | 90.91 | (78.90, 102.92) |
| | | **LZ** | 9 | 3 | 33.33 | (2.53, 64.13) | 3 | 33.33 | (2.53, 64.13) | 8 | 3 | 37.50 | (3.95, 71.05) |
| | | **SS** | 15 | 0 | 0.00 | - | 0 | 0.00 | - | 13 | 0 | 0.00 | - |
| | **Kinmen** | | 51 | 2 | 3.92 | (-1.41, 9.25) | 5 | 9.80 | (1.64, 17.96) | 42 | 3 | 7.14 | (-0.65, 14.93) |
| | | **JJ** | 41 | 1 | 2.44 | (-2.28, 7.16) | 3 | 7.32 | (-0.65, 15.29) | 34 | 3 | 8.82 | (-0.71, 18.35) |
| | | **JH** | 10 | 1 | 10.00 | (-8.59, 28.59) | 2 | 20.00 | (-4.79, 44.79) | 8 | 0 | 0.00 | - |
| **Total** | **Taipei** | **GT** | 114 | 60 | 52.63 | (43.46, 61.80) | 93 | 81.58 | (74.46, 88.70) | 99 | 69 | 69.70 | (60.65, 78.75) |
| | **Yilan** | | 381 | 182 | 47.77 | (42.76, 52.79) | 255 | 66.93 | (62.21, 71.65) | 337 | 194 | 57.57 | (52.29, 62.85) |
| | | **LT** | 207 | 164 | 79.23 | (73.70, 84.76) | 178 | 85.99 | (81.26, 90.72) | 183 | 165 | 90.16 | (85.84, 94.48) |
| | | **LZ** | 110 | 15 | 13.64 | (7.23, 20.05) | 53 | 48.18 | (38.84, 57.52) | 98 | 19 | 19.39 | (11.56, 27.22) |
| | | **SS** | 64 | 3 | 4.69 | (-0.49, 9.87) | 24 | 37.50 | (25.54, 49.46) | 56 | 10 | 17.86 | (7.83, 27.89) |
| | **Kinmen** | | 182 | 7 | 3.85 | (1.06, 6.65) | 80 | 43.96 | (36.75, 51.17) | 154 | 42 | 27.27 | (20.24, 34.30) |
| | | **JJ** | 127 | 3 | 2.36 | (-0.28, 5.00) | 51 | 40.16 | (31.64, 48.69) | 112 | 27 | 24.11 | (16.19, 32.03) |
| | | **JH** | 55 | 4 | 7.27 | (0.41, 14.13) | 29 | 52.73 | (39.54, 65.93) | 42 | 15 | 35.71 | (21.22, 50.20) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix A.2 Sero-protection Rates of HI Antibodies against the Three Human Influenza Vaccine Viruses between Grade One and Two Schoolchildren Stratified by Their Vaccination Status at Three Time-points, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Grade | A/Solomon Islands/3/2006 (H1N1) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | | 1-month post-vaccination | | | 4-month post-vaccination | | | |
| | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | 1 | 273 | 224 | 82.05 | (77.50, 86.60) | 264 | 96.70 | (94.58, 98.82) | 236 | 221 | 93.64 | (90.53, 96.75) |
| | 2 | 299 | 200 | 66.89 | (61.56, 72.22) | 274 | 91.64 | (88.50, 94.78) | 262 | 236 | 90.08 | (86.46, 93.70) |
| Unvaccinated | 1 | 45 | 33 | 73.33 | (60.41, 86.25) | 35 | 77.78 | (65.63, 89.93) | 40 | 28 | 70.00 | (55.80, 84.20) |
| | 2 | 60 | 45 | 75.00 | (64.04, 85.96) | 44 | 73.33 | (62.14, 84.52) | 52 | 41 | 78.85 | (67.75, 89.95) |
| Total | 1 | 318 | 257 | 80.82 | (76.49, 85.15) | 299 | 94.03 | (91.43, 96.63) | 276 | 249 | 90.22 | (86.72, 93.72) |
| | 2 | 359 | 345 | 68.25 | (63.43, 73.07) | 318 | 88.58 | (85.29, 91.87) | 314 | 277 | 88.22 | (84.65, 91.79) |

| Human Flu Vaccination Status | Grade | A/Wisconsin/67/2005 (H3N2) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | | 1-month post-vaccination | | | 4-month post-vaccination | | | |
| | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | 1 | 273 | 237 | 86.81 | (82.80, 90.82) | 268 | 98.17 | (96.58, 99.76) | 236 | 233 | 98.73 | (97.30, 100.16) |
| | 2 | 299 | 256 | 85.62 | (81.64, 89.60) | 291 | 97.32 | (95.49, 99.15) | 262 | 255 | 97.33 | (95.38, 99.28) |
| Unvaccinated | 1 | 45 | 34 | 75.56 | (63.00, 88.12) | 36 | 80.00 | (68.31, 91.69) | 40 | 36 | 90.00 | (80.70, 99.30) |
| | 2 | 60 | 53 | 88.33 | (80.21, 96.45) | 54 | 90.00 | (82.41, 97.59) | 52 | 44 | 84.62 | (74.81, 94.43) |
| Total | 1 | 318 | 271 | 85.22 | (81.32, 89.12) | 304 | 95.60 | (93.35, 97.85) | 276 | 269 | 97.46 | (95.60, 99.32) |
| | 2 | 359 | 309 | 86.07 | (82.49, 89.65) | 345 | 96.10 | (94.10, 98.10) | 314 | 299 | 95.22 | (92.86, 97.58) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix A.2 (cont.)**

| Human Flu Vaccination Status | Grade | B/Malaysia/2506/2004 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pre-vaccination | | | 1-month post-vaccination | | | | 4-month post-vaccination | |
| | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | **1** | 273 | 102 | 37.36 | (31.62, 43.10) | 183 | 67.03 | (61.45, 72.60) | 236 | 130 | 55.08 | (48.73, 61.43) |
| | **2** | 299 | 120 | 40.13 | (34.57, 45.69) | 212 | 70.90 | (65.75, 76.05) | 262 | 145 | 55.34 | (49.32, 61.36) |
| **Unvaccinated** | **1** | 45 | 10 | 22.22 | (10.07, 34.37) | 13 | 28.89 | (15.65, 42.13) | 40 | 13 | 32.50 | (17.98, 47.02) |
| | **2** | 60 | 17 | 28.33 | (16.93, 39.73) | 20 | 33.33 | (21.40, 45.26) | 52 | 17 | 32.69 | (19.94, 45.44) |
| **Total** | **1** | 318 | 112 | 35.22 | (29.97, 40.47) | 196 | 61.64 | (56.30, 66.98) | 276 | 143 | 51.81 | (45.91, 57.71) |
| | **2** | 359 | 137 | 38.16 | (33.13, 43.19) | 232 | 64.62 | (59.67, 69.57) | 314 | 162 | 51.59 | (46.06, 57.12) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix A.3 Sero-protection Rates of HI Antibodies against the Three Human Influenza Viruses between Male and Female Schoolchildren Stratified by Their Vaccination Status at Three Time-points, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Gender | A/Solomon Islands/3/2006 (H1N1) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | | 1-month post-vaccination | | | 4-month post-vaccination | | | |
| | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | Males | 305 | 225 | 73.77 | (68.83, 78.71) | 286 | 93.77 | (91.06, 96.48) | 262 | 239 | 91.22 | (87.79, 94.65) |
| | Females | 267 | 199 | 74.53 | (69.30, 79.76) | 252 | 94.38 | (91.62, 97.14) | 236 | 218 | 92.37 | (88.98, 95.76) |
| Unvaccinated | Males | 47 | 36 | 76.60 | (64.50, 88.70) | 34 | 72.34 | (59.55, 85.13) | 40 | 28 | 70.00 | (55.80, 84.20) |
| | Females | 58 | 42 | 72.41 | (60.91, 83.91) | 45 | 77.59 | (66.86, 88.32) | 52 | 41 | 78.85 | (67.75, 89.95) |
| Total | Males | 352 | 261 | 74.15 | (69.58, 78.72) | 320 | 90.91 | (87.91, 93.91) | 302 | 267 | 88.41 | (84.80, 92.02) |
| | Females | 325 | 241 | 74.15 | (69.39, 78.91) | 297 | 91.38 | (88.33, 94.43) | 288 | 259 | 89.93 | (86.45, 93.41) |

| Human Flu Vaccination Status | Gender | A/Wisconsin/67/2005 (H3N2) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | | 1-month post-vaccination | | | 4-month post-vaccination | | | |
| | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | Males | 305 | 266 | 87.21 | (83.46, 90.96) | 299 | 98.03 | (96.47, 99.59) | 262 | 254 | 96.95 | (94.87, 99.03) |
| | Females | 267 | 227 | 85.02 | (80.73, 89.30) | 260 | 97.38 | (95.46, 99.30) | 236 | 234 | 99.15 | (97.98, 100.32) |
| Unvaccinated | Males | 47 | 37 | 78.72 | (67.02, 90.42) | 38 | 80.85 | (69.60, 92.10) | 40 | 33 | 82.50 | (70.72, 94.28) |
| | Females | 58 | 50 | 86.21 | (77.34, 95.08) | 52 | 89.66 | (81.82, 97.50) | 52 | 47 | 90.38 | (82.37, 98.39) |
| Total | Males | 352 | 303 | 86.08 | (82.46, 89.70) | 337 | 95.74 | (93.63, 97.85) | 302 | 287 | 95.03 | (92.58, 97.48) |
| | Females | 325 | 277 | 85.23 | (81.37, 89.09) | 302 | 96.00 | (93.87, 98.13) | 288 | 281 | 97.57 | (95.79, 99.35) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix A.3 (cont.)**

| Human Flu Vaccination Status | Gender | B/Malaysia/2506/2004 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-vaccination | | | | 1-month post-vaccination | | | 4-month post-vaccination | | |
| | | N | n | % | 95% CI | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | **Males** | 305 | 120 | 39.34 | (33.86, 44.82) | 206 | 67.54 | (62.29, 72.79) | 262 | 133 | 50.76 | (44.71, 56.81) |
| | **Females** | 267 | 102 | 38.20 | (32.37, 44.03) | 189 | 70.79 | (65.34, 76.24) | 236 | 142 | 60.17 | (53.92, 66.42) |
| **Unvaccinated** | **Males** | 47 | 11 | 23.40 | (11.30, 35.50) | 14 | 29.79 | (16.71, 42.87) | 40 | 9 | 22.50 | (9.56, 35.44) |
| | **Females** | 58 | 16 | 27.59 | (16.09, 39.09) | 19 | 32.76 | (20.68, 44.84) | 52 | 21 | 40.38 | (27.04, 53.72) |
| **Total** | **Males** | 352 | 131 | 37.22 | (32.17, 42.27) | 220 | 62.50 | (57.44, 67.56) | 302 | 142 | 47.02 | (41.39, 52.65) |
| | **Females** | 325 | 118 | 36.31 | (31.08, 41.54) | 208 | 64.00 | (58.78, 69.22) | 288 | 163 | 56.60 | (50.88, 62.32) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix B.1 Geometric Mean Titers (GMT) of HI Antibodies against the Three Human Influenza Viruses among Grade One and Two Schoolchildren Stratified by Their Vaccination Status at Three Time-points, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Grade | A/Solomon Islands/3/2006 (H1N1) | | | | |
|---|---|---|---|---|---|---|
| | | N | Pre-vaccination | 1-month post-vaccination | N | 4-month post-vaccination |
| **Vaccinated** | **1** | 273 | 46.59 | 144.20 | 236 | 81.68 |
| | **2** | 299 | 38.37 | 177.53 | 262 | 95.14 |
| **Unvaccinated** | **1** | 45 | 43.77 | 61.48 | 40 | 38.11 |
| | **2** | 60 | 42.87 | 45.95 | 52 | 43.77 |
| **Total** | **1** | 318 | 46.20 | 122.10 | 276 | 73.26 |
| | **2** | 359 | 39.40 | 141.72 | 314 | 83.63 |

| Human Flu Vaccination Status | Grade | A/Wisconsin/67/2005 (H3N2) | | | | |
|---|---|---|---|---|---|---|
| | | N | Pre-vaccination | 1-month post-vaccination | N | 4-month post-vaccination |
| **Vaccinated** | **1** | 273 | 61.05 | 315.59 | 236 | 182.52 |
| | **2** | 299 | 60.63 | 342.97 | 262 | 188.96 |
| **Unvaccinated** | **1** | 45 | 55.40 | 55.40 | 40 | 71.11 |
| | **2** | 60 | 61.90 | 70.62 | 52 | 61.48 |
| **Total** | **1** | 318 | 60.25 | 246.92 | 276 | 159.23 |
| | **2** | 359 | 60.92 | 262.82 | 314 | 156.82 |

| Human Flu Vaccination Status | Grade | B/Malaysia/2506/2004 | | | | |
|---|---|---|---|---|---|---|
| | | N | Pre-vaccination | 1-month post-vaccination | N | 4-month post-vaccination |
| **Vaccinated** | **1** | 273 | 24.97 | 46.27 | 236 | 34.11 |
| | **2** | 299 | 24.12 | 52.05 | 262 | 32.94 |
| **Unvaccinated** | **1** | 45 | 18.53 | 19.05 | 40 | 19.72 |
| | **2** | 60 | 21.14 | 21.44 | 52 | 26.03 |
| **Total** | **1** | 318 | 23.87 | 40.78 | 276 | 31.51 |
| | **2** | 359 | 23.62 | 44.05 | 314 | 31.73 |

N: total number of children

**Appendix B.2 Geometric Mean Titers (GMT) of HI Antibodies against the Three Human Influenza Viruses between Male and Female Schoolchildren Stratified by Their Vaccination Status at Three Time-points, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | Gender | A/Solomon Islands/3/2006 (H1N1) | | | | |
|---|---|---|---|---|---|---|
| | | N | Pre-vaccination | 1-month post-vaccination | N | 4-month post-vaccination |
| Vaccinated | Males | 305 | 41.99 | 162.23 | 262 | 85.74 |
| | Females | 267 | 41.99 | 158.89 | 236 | 91.90 |
| Unvaccinated | Males | 47 | 41.70 | 45.63 | 40 | 37.32 |
| | Females | 58 | 44.69 | 45.63 | 52 | 44.38 |
| Total | Males | 352 | 42.02 | 136.71 | 302 | 76.58 |
| | Females | 325 | 42.55 | 127.36 | 288 | 80.78 |

| Human Flu Vaccination Status | Gender | A/Wisconsin/67/2005 (H3N2) | | | | |
|---|---|---|---|---|---|---|
| | | N | Pre-vaccination | 1-month post-vaccination | N | 4-month post-vaccination |
| Vaccinated | Males | 305 | 61.05 | 296.51 | 262 | 162.23 |
| | Females | 267 | 60.63 | 372.71 | 236 | 215.56 |
| Unvaccinated | Males | 47 | 49.93 | 48.57 | 40 | 48.57 |
| | Females | 58 | 70.13 | 78.90 | 52 | 82.25 |
| Total | Males | 352 | 59.55 | 232.64 | 302 | 138.42 |
| | Females | 325 | 61.82 | 282.07 | 288 | 181.39 |

| Human Flu Vaccination Status | Gender | B/Malaysia/2506/2004 | | | | |
|---|---|---|---|---|---|---|
| | | N | Pre-vaccination | 1-month post-vaccination | N | 4-month post-vaccination |
| Vaccinated | Males | 305 | 24.62 | 46.91 | 262 | 31.38 |
| | Females | 267 | 24.28 | 50.63 | 236 | 36.05 |
| Unvaccinated | Males | 47 | 17.53 | 18.79 | 40 | 17.78 |
| | Females | 58 | 29.49 | 21.73 | 52 | 28.28 |
| Total | Males | 352 | 23.55 | 41.53 | 302 | 29.08 |
| | Females | 325 | 23.92 | 43.56 | 288 | 34.46 |

N: total number of children

**Appendix C.1 The Percentage of Schoolchildren with Four-fold Serotiter Rises of HI Antibodies against the Three Human Influenza Vaccine Viruses in the Three Study Areas (ie. Six Schools) Stratified by Their Vaccination Status from Pre-vaccination to 1-month and 4-month Post-vaccination, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | | | A/Solomon Islands/3/2006 (H1N1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Area/ School | | N | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | **Taipei** | **GT** | 107 | 71 | 66.36 | (57.41, 75.31) | 92 | 48 | 52.17 | (41.96, 62.38) |
| | **Yilan** | | 334 | 161 | 48.20 | (42.84, 53.56) | 294 | 82 | 27.89 | (22.76, 33.02) |
| | | **LT** | 184 | 92 | 50.00 | (42.78, 57.22) | 161 | 55 | 34.16 | (26.83, 41.49) |
| | | **LZ** | 101 | 55 | 54.46 | (44.75, 64.17) | 90 | 20 | 22.22 | (13.63, 30.81) |
| | | **SS** | 49 | 14 | 28.57 | (15.92, 41.22) | 43 | 7 | 16.28 | (5.25, 27.31) |
| | **Kinmen** | | 131 | 75 | 57.25 | (48.78, 65.72) | 112 | 43 | 38.39 | (29.38, 47.40) |
| | | **JJ** | 86 | 53 | 61.63 | (51.35, 71.91) | 78 | 37 | 47.44 | (36.36, 58.52) |
| | | **JH** | 45 | 22 | 48.89 | (34.28, 63.50) | 34 | 6 | 17.65 | (4.83, 30.47) |
| **Unvaccinated** | **Taipei** | **GT** | 7 | 0 | 0.00 | - | 7 | 0 | 0.00 | - |
| | **Yilan** | | 47 | 3 | 6.38 | (-0.61, 13.37) | 43 | 3 | 6.98 | (-0.64, 14.60) |
| | | **LT** | 23 | 2 | 8.7 | (-2.82, 20.22) | 22 | 2 | 9.09 | (-2.92, 21.10) |
| | | **LZ** | 9 | 0 | 0.00 | - | 8 | 0 | 0.00 | - |
| | | **SS** | 15 | 1 | 6.67 | (-5.96, 19.30) | 13 | 1 | 7.69 | (-6.79, 22.17) |
| | **Kinmen** | | 51 | 1 | 1.96 | (-1.84, 5.76) | 42 | 2 | 4.76 | (-1.68, 11.20) |
| | | **JJ** | 41 | 1 | 2.44 | (-2.28, 7.16) | 34 | 2 | 5.88 | (-2.03, 13.79) |
| | | **JH** | 10 | 0 | 0.00 | - | 8 | 0 | 0.00 | - |
| **Total** | **Taipei** | **GT** | 114 | 71 | 62.28 | (53.38, 71.18) | 99 | 48 | 48.48 | (38.64, 58.33) |
| | **Yilan** | | 381 | 164 | 43.04 | (38.07, 48.01) | 337 | 85 | 25.22 | (20.58, 29.86) |
| | | **LT** | 207 | 94 | 45.41 | (38.63, 52.19) | 183 | 57 | 31.15 | (24.44, 37.86) |
| | | **LZ** | 110 | 55 | 50.00 | (40.66, 59.34) | 98 | 20 | 20.41 | (12.43, 28.39) |
| | | **SS** | 64 | 15 | 23.44 | (13.06, 33.82) | 56 | 8 | 14.29 | (5.12, 23.46) |
| | **Kinmen** | | 182 | 76 | 41.76 | (34.60, 48.92) | 154 | 45 | 29.22 | (22.04, 36.40) |
| | | **JJ** | 127 | 54 | 42.52 | (33.92, 51.12) | 112 | 39 | 34.82 | (26.00, 43.64) |
| | | **JH** | 55 | 22 | 40.00 | (27.05, 52.95) | 42 | 6 | 14.29 | (3.71, 24.87) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix C.1 (cont.)**

| Human Flu Vaccination Status | Area/ School | A/Wisconsin/67/2005 (H3N2) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | | N | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | **Taipei GT** | 107 | 85 | 79.44 | (71.78, 87.10) | 92 | 51 | 55.43 | (45.27, 65.59) |
| | **Yilan** | 334 | 217 | 64.97 | (59.85, 70.09) | 294 | 128 | 43.54 | (37.87, 49.21) |
| | **LT** | 184 | 109 | 59.24 | (52.14, 66.34) | 161 | 65 | 40.37 | (32.79, 47.95) |
| | **LZ** | 101 | 74 | 73.27 | (64.64, 81.90) | 90 | 39 | 43.33 | (33.09, 53.57) |
| | **SS** | 49 | 34 | 69.39 | (56.49, 82.29) | 43 | 24 | 55.81 | (40.97, 70.65) |
| | **Kinmen** | 131 | 103 | 78.63 | (71.61, 85.65) | 112 | 68 | 60.71 | (51.66, 69.76) |
| | **JJ** | 86 | 73 | 84.88 | (77.31, 92.45) | 78 | 51 | 65.38 | (54.82, 75.93) |
| | **JH** | 45 | 30 | 66.67 | (52.90, 80.44) | 34 | 17 | 50.00 | (33.19, 66.81) |
| **Unvaccinated** | **Taipei GT** | 7 | 0 | 0.00 | - | 7 | 0 | 0.00 | - |
| | **Yilan** | 47 | 4 | 8.51 | (0.53, 16.49) | 43 | 2 | 4.65 | (-1.64, 10.94) |
| | **LT** | 23 | 4 | 17.39 | (1.90, 32.88) | 22 | 1 | 4.55 | (-4.16, 13.26) |
| | **LZ** | 9 | 0 | 0.00 | - | 8 | 0 | 0.00 | - |
| | **SS** | 15 | 0 | 0.00 | - | 13 | 1 | 7.69 | (-6.79, 22.17) |
| | **Kinmen** | 51 | 0 | 0.00 | - | 42 | 4 | 9.52 | (0.64, 18.40) |
| | **JJ** | 41 | 0 | 0.00 | - | 34 | 4 | 11.76 | (0.93, 22.59) |
| | **JH** | 10 | 0 | 0.00 | - | 8 | 0 | 0.00 | - |
| **Total** | **Taipei GT** | 114 | 85 | 74.56 | (66.57, 82.56) | 99 | 51 | 51.52 | (41.68, 61.37) |
| | **Yilan** | 381 | 221 | 58.01 | (53.05, 62.97) | 337 | 130 | 38.58 | (33.83, 43.78) |
| | **LT** | 207 | 113 | 54.59 | (47.81, 61.37) | 183 | 66 | 36.07 | (29.11, 43.03) |
| | **LZ** | 110 | 74 | 67.27 | (58.50, 76.04) | 98 | 39 | 39.80 | (30.11, 49.49) |
| | **SS** | 64 | 34 | 53.13 | (40.90, 65.36) | 56 | 25 | 44.64 | (31.62, 57.66) |
| | **Kinmen** | 182 | 103 | 56.59 | (49.39, 63.79) | 154 | 72 | 46.75 | (38.87, 54.63) |
| | **JJ** | 127 | 73 | 57.48 | (48.88, 66.08) | 112 | 55 | 49.11 | (39.85, 58.37) |
| | **JH** | 55 | 30 | 54.55 | (41.39, 67.71) | 42 | 17 | 40.48 | (25.63, 55.33) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix C.1 (cont.)**

| Human Flu Vaccination Status | Area/ School | B/Malaysia/2506/2004 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | | N | n | % | 95% CI | N | n | % | 95% CI |
| **Vaccinated** | **Taipei GT** | 107 | 37 | 34.58 | (25.57, 43.59) | 92 | 10 | 10.87 | (4.51, 17.23) |
| | **Yilan** | 334 | 97 | 29.04 | (24.17, 33.91) | 294 | 29 | 9.86 | (6.4, 13.27) |
| | **LT** | 184 | 41 | 22.28 | (16.27, 28.29) | 161 | 16 | 9.94 | (5.32, 14.56) |
| | **LZ** | 101 | 34 | 33.66 | (24.44, 42.88) | 90 | 6 | 6.67 | (1.52, 11.82) |
| | **SS** | 49 | 22 | 44.90 | (30.97, 58.83) | 43 | 7 | 16.28 | (5.25, 27.31) |
| | **Kinmen** | 131 | 63 | 48.09 | (39.53, 56.65) | 112 | 31 | 27.68 | (19.39, 35.97) |
| | **JJ** | 86 | 45 | 52.33 | (41.77, 62.89) | 78 | 21 | 26.92 | (17.08, 36.76) |
| | **JH** | 45 | 18 | 40.00 | (25.69, 54.31) | 34 | 10 | 29.41 | (14.09, 44.73) |
| **Unvaccinated** | **Taipei GT** | 7 | 0 | 0.00 | - | 7 | 0 | 0.00 | - |
| | **Yilan** | 47 | 0 | 0.00 | - | 43 | 1 | 2.33 | (-2.18, 6.84) |
| | **LT** | 23 | 0 | 0.00 | - | 22 | 1 | 4.55 | (-4.16, 13.26) |
| | **LZ** | 9 | 0 | 0.00 | - | 8 | 0 | 0.00 | - |
| | **SS** | 15 | 0 | 0.00 | - | 13 | 0 | 0.00 | - |
| | **Kinmen** | 51 | 0 | 0.00 | - | 42 | 2 | 4.76 | (-1.68, 11.20) |
| | **JJ** | 41 | 0 | 0.00 | - | 34 | 2 | 5.88 | (-2.03, 13.79) |
| | **JH** | 10 | 0 | 0.00 | - | 8 | 0 | 0.00 | - |
| **Total** | **Taipei GT** | 114 | 37 | 32.46 | (23.87, 41.06) | 99 | 10 | 10.10 | (4.16, 16.04) |
| | **Yilan** | 381 | 97 | 25.46 | (21.09, 29.84) | 337 | 30 | 8.90 | (5.86, 11.94) |
| | **LT** | 207 | 41 | 19.81 | (14.38, 25.24) | 183 | 17 | 9.29 | (5.08, 13.50) |
| | **LZ** | 110 | 34 | 30.91 | (22.27, 39.55) | 98 | 6 | 6.12 | (1.37, 10.87) |
| | **SS** | 64 | 22 | 34.38 | (22.74, 46.02) | 56 | 7 | 12.50 | (3.84, 21.16) |
| | **Kinmen** | 182 | 63 | 34.62 | (27.71, 41.53) | 154 | 33 | 21.43 | (14.95, 27.91) |
| | **JJ** | 127 | 45 | 35.43 | (27.11, 43.75) | 112 | 23 | 20.54 | (13.06, 28.02) |
| | **JH** | 55 | 18 | 32.73 | (20.33, 45.13) | 42 | 10 | 23.81 | (10.93, 36.69) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix C.2 The Percentages of the Four-fold Serotiter Rise of HI Antibodies against the Three Human Influenza Vaccine Viruses between Grade 1 and 2 Schoolchildren Stratified by Their Vaccination Status from Pre-vaccination to 1-month and 4-month Post-vaccination, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | | A/Solomon Islands/3/2006 (H1N1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Grade | N | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | 1 | 273 | 129 | 47.25 | (41.32, 53.17) | 236 | 63 | 26.69 | (21.05, 32.33) |
| | 2 | 299 | 178 | 59.53 | (53.97, 65.09) | 262 | 110 | 41.98 | (36.00, 47.96) |
| Unvaccinated | 1 | 45 | 2 | 4.44 | (-1.58, 10.46) | 40 | 1 | 2.50 | (-2.34, 7.34) |
| | 2 | 60 | 2 | 3.33 | (-1.21, 7.87) | 52 | 4 | 7.69 | (0.45, 14.93) |
| Total | 1 | 318 | 131 | 41.19 | (35.78, 46.60) | 276 | 64 | 23.19 | (18.21, 28.17) |
| | 2 | 359 | 180 | 50.14 | (44.97, 55.31) | 314 | 114 | 36.31 | (30.99, 41.63) |

| Human Flu Vaccination Status | | A/Wisconsin/67/2005 (H3N2) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Grade | N | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | 1 | 273 | 181 | 66.30 | (60.69, 71.91) | 236 | 109 | 46.19 | (39.83, 52.55) |
| | 2 | 299 | 224 | 74.92 | (70.01, 79.83) | 262 | 138 | 52.67 | (46.62, 58.72) |
| Unvaccinated | 1 | 45 | 0 | 0.00 | - | 40 | 4 | 10.00 | (0.70, 19.30) |
| | 2 | 60 | 4 | 6.67 | (0.36, 12.98) | 52 | 2 | 3.85 | (-1.38, 9.08) |
| Total | 1 | 318 | 181 | 56.92 | (51.48, 62.36) | 276 | 113 | 40.94 | (35.14, 46.74) |
| | 2 | 359 | 228 | 63.51 | (58.53, 68.49) | 314 | 140 | 44.59 | (39.09, 50.09) |

| Human Flu Vaccination Status | | A/Wisconsin/67/2005 (H3N2) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Grade | N | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | 1 | 273 | 181 | 66.30 | (60.69, 71.91) | 236 | 109 | 46.19 | (39.83, 52.55) |
| | 2 | 299 | 224 | 74.92 | (70.01, 79.83) | 262 | 138 | 52.67 | (46.62, 58.72) |
| Unvaccinated | 1 | 45 | 0 | 0.00 | - | 40 | 4 | 10.00 | (0.70, 19.30) |
| | 2 | 60 | 4 | 6.67 | (0.36, 12.98) | 52 | 2 | 3.85 | (-1.38, 9.08) |
| Total | 1 | 318 | 181 | 56.92 | (51.48, 62.36) | 276 | 113 | 40.94 | (35.14, 46.74) |
| | 2 | 359 | 228 | 63.51 | (58.53, 68.49) | 314 | 140 | 44.59 | (39.09, 50.09) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

**Appendix C.3 The Percentage of the Four-fold Serotiter Rise of HI Antibodies against the Three Human Influenza Vaccine Viruses between Male and Female Schoolchildren Stratified by Their Vaccination Status from Pre-vaccination to 1-month and 4-month Post-vaccination, Oct, 2007-Apr, 2008.**

| Human Flu Vaccination Status | | A/Solomon Islands/3/2006 (H1N1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Gender | N | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | Males | 305 | 164 | 53.77 | (48.17, 59.37) | 262 | 86 | 32.82 | (27.13, 38.51) |
| | Females | 267 | 143 | 53.56 | (47.58, 59.54) | 236 | 87 | 26.86 | (21.21, 32.51) |
| Unvaccinated | Males | 47 | 1 | 2.13 | (-2.00, 6.26) | 40 | 1 | 2.50 | (-2.34, 7.34) |
| | Females | 58 | 3 | 5.17 | (-0.53, 10.87) | 52 | 4 | 7.69 | (0.45, 14.93) |
| Total | Males | 352 | 165 | 46.88 | (41.67, 52.09) | 302 | 87 | 28.81 | (23.70, 33.92) |
| | Females | 325 | 146 | 44.92 | (39.51, 50.33) | 288 | 91 | 31.60 | (26.23, 36.97) |

| Human Flu Vaccination Status | | A/Wisconsin/67/2005 (H3N2) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Gender | N | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | Males | 305 | 204 | 66.89 | (61.61, 72.17) | 262 | 114 | 43.51 | (37.51, 49.51) |
| | Females | 267 | 201 | 75.28 | (70.11, 80.45) | 236 | 133 | 56.36 | (50.03, 62.69) |
| Unvaccinated | Males | 47 | 0 | 0.00 | - | 40 | 2 | 5.00 | (-1.75, 11.75) |
| | Females | 58 | 4 | 6.90 | (0.38, 13.42) | 52 | 4 | 7.69 | (0.45, 14.93) |
| Total | Males | 352 | 204 | 57.95 | (52.79, 63.11) | 302 | 116 | 38.41 | (32.92, 43,90) |
| | Females | 325 | 205 | 63.08 | (57.83, 68.33) | 288 | 137 | 47.57 | (41.80, 53.34) |

| Human Flu Vaccination Status | | B/Malaysia/2506/2004 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-month post-vaccination | | | | 4-month post-vaccination | | | |
| | Gender | N | n | % | 95% CI | N | n | % | 95% CI |
| Vaccinated | Males | 305 | 101 | 33.11 | (27.83, 38.39) | 262 | 30 | 11.45 | (7.59, 15.31) |
| | Females | 267 | 96 | 35.96 | (30.20, 41.72) | 236 | 40 | 16.95 | (12.16, 21.74) |
| Unvaccinated | Males | 47 | 0 | 0.00 | - | 40 | 0 | 0.00 | - |
| | Females | 58 | 0 | 0.00 | - | 52 | 3 | 5.77 | (-0.57, 12.11) |
| Total | Males | 352 | 101 | 28.69 | (23.96, 33.42) | 302 | 30 | 9.93 | (6.56, 13.30) |
| | Females | 325 | 96 | 29.54 | (24.58, 34.50) | 288 | 43 | 14.93 | (10.81, 19.05) |

N: total number of children, n: number of children with sero-protection, %: n/N, 95% CI: 95% confidence interval.

# Appendix D

# Laboratory Protocols

## 1. Receptor Destroying Enzyme (RDE) Treatment for Tested Serum Samples

(1) Reconstitute the RDE powder with 20 ml normal saline.

(2) Add 3 volumes (vol.) of RDE to 1 vol of serum (300 ul RDE + 100 ul serum).

(3) Incubate overnight in a 37℃ water-bath for 18~20 hours (hr).

(4) Heat in a 56℃ water-bath for 30 minutes (min) to inactivate complement.

(5) Allow the serum to cool to room temperature. Add 6 vol (600 ul) of phosphate buffer saline (PBS, pH=7.2). The final dilution of serum is 1:10.

(6) Store at -20℃ or use for HI assay.

## 2. Identification of Non-specific Agglutinin in Treated Serum Samples

(1) Choose U-shaped 96-well microtiter plate and add 25 ul of each treated serum to each well.

(2) Add 25 ul of PBS to each well.

(3) Add 50 ul of 0.75% human type O RBCs to each well.

(4) Mix by manually agitating the plates thoroughly.

(5) Prepare negative control (NC) as (a)~(d) except replace the 25 ul of serum with 25 ul of PBS.

(6) Incubate the plate at room temperature for 1 hr by checking the negative control for complete settling of RBCs.

(7) Record the results. The serum with complete settling of RBCs is acceptable for use.

## 3. Hemagglutination Assay

(1) Choose U-shaped 96-well microtiter plates.

(2) Add 50 ul of PBS to #2 through #12 (A2-A12) wells of each lettered row.

(3) Add 100 ul of each virus to the first well (A1-G1) of the lettered rows except row H.

(4) Prepare an RBC control well in row H (H1) by adding 100 ul of PBS.

(5) Make serial twofold dilutions by transferring 50 ul from the first well of lettered rows to successive rows. Discard the final 50 ul.

(6) Add 50 ul of 0.75 % type O RBC suspension to each well on the plate.

(7) Mix by manually agitating the plates thoroughly.

(8) Incubate the plates at room temperature and check RBC control for complete settling of RBCs.

(9) Record results.

The highest dilution of the tested human influenza virus that causes complete hemagglutination is considered as the end point of the HA titration. The HA titer is calculated as the reciprocal of the dilution of virus in the last well with complete hemagglutination.

## 4. Hemagglutination Inhibition (HI) Assay

(1) Choose and label U-shaped 96-well microtiter plates. Virus antigen with 8 HA units/50 ul were prepared before using.

(2) Add 25 ul of PBS to wells B through H (B1-H12) of each numbered column.

(3) Using the RDE treated serum with final dilution 1:10, add 50 ul of each serum to the first wells A (A1-A12). Serum samples from the same person were tested on the same plates.

(4) Prepare serial twofold dilutions (1:10-1:1280) of the treated serum by transferring 25 ul from the first wells of numbered columns 1-12 to successive wells. Discard the final 25 ul after row H.

(5) Add 25 ul of virus antigen with 8 HA units/50 ul to all wells of a complete set of diluted treated serum.

(6) Mix the contents of the plates by agitating the plates manually.

(7) Cover the plates and incubate at room temperature for 15 min.

(8) Add 50 ul of 0.75% human type O RBCs to all wells.

(9) Incubate the plates and allow the RBCs to settle at 4℃ for 1 hr.

(10) Except the HI assay described above, also perform a “back titration” to verify units by performing the second HA assay using the virus antigen dilution preparation.

(11) Record the results and HI titers.

The highest dilution of serum samples that cause complete hemagglutination inhibition is considered the end point of HI titration. The HI titer is the reciprocal of the last dilution of serum that completely inhibits hemaggutination. If the HI titer is less than 1:10, we regarded as the titer 1:5.

# Appendix E.1 Questionnaire at Pre-vaccination

## ＸＸ地區國小學童流感疫苗評估之流行病學研究問卷

**一、學童個人背景**（____國小__年____班____號，學童姓名_____）

1. 民國___年___月___日出生，□(0)男生 □(1)女生，血型：□(0)O □(1)A □(2)B □(3)AB □(4)不知道
2. 過去兩年是否打過**流感疫苗**？ a.去年(95 年 9 月～96 年 4 月)：□(0)無 □(1)有 □(2)不確定
   b.前年(94 年 9 月～95 年 4 月)：□(0)無 □(1)有 □(2)不確定
3. 過去是否打過**肺炎鏈球菌疫苗**？ □(0)無
   □(1)有，在___年，施打那種肺炎鏈球菌疫苗？□(a)23 價紐蒙肺(Pneumovax)疫苗，滿兩歲以上兒童才可接種
   □(b)新型 7 價沛兒(Prevenar)疫苗，二個月以上幼兒就可接種
   □(c)不確定

**二、學童家庭背景**

1. 平時家庭中與學童**同住**(包含學童)總共____人，共有兄弟姊妹____人，家人背景請填如下：

| **關係** | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ |
|---|---|---|---|---|---|---|---|
| **民國幾年出生** | ____年 | ____年 | ____年 | ____年 | ____年 | ____年 | ____年 |
| **去年(95 年 9 月至 96 年 4 月)是否接種流感疫苗？** | □(0)無<br>□(1)有<br>□(2)不確定 | □(0)無<br>□(1)有<br>□(2)不確定 | □(0)無<br>□(1)有<br>□(2)不確定 | □(0)無<br>□(1)有<br>□(2)不確定 | □(0)無<br>□(1)有<br>□(2)不確定 | □(0)無<br>□(1)有<br>□(2)不確定 | □(0)無<br>□(1)有<br>□(2)不確定 |
| **自去年 9 月至今是否有過下列感冒症狀？請在□中打勾 複選** | | | | | | | |
| 發燒 | □ | □ | □ | □ | □ | □ | □ |
| 畏寒 | □ | □ | □ | □ | □ | □ | □ |
| 肌肉/關節酸痛 | □ | □ | □ | □ | □ | □ | □ |
| 嚴重倦怠 | □ | □ | □ | □ | □ | □ | □ |
| 喉嚨痛 | □ | □ | □ | □ | □ | □ | □ |
| 流鼻水/鼻塞 | □ | □ | □ | □ | □ | □ | □ |
| 咳嗽 | □ | □ | □ | □ | □ | □ | □ |
| 聲音沙啞 | □ | □ | □ | □ | □ | □ | □ |

2. 家中**最主要**照顧學童**生活起居**是：□(1)母 □(2)父 □(3)(外)祖母 □(4)(外)祖父 □(5)其他_____
3. 這位照顧者**每天**平均照顧____小時
4. 學童平時在家**學習、睡覺**是否與兄弟姊妹同房間？□(0)無兄弟姊妹 □(1)不同房間
   □(2)有，與___人同房間

**三、學童生活與健康資料**

1. 是否參加課外活動(課後安親班、才藝班、校隊)：
   □(0)無 □(1)有，**室內**課外活動**每週**平均_____小時，**室外**課外活動**每週**平均_____小時
2. **每週**平均運動____小時，**每天**平均睡眠____小時
3. 過去半年(96 年 4 月至今)是否出國？複選
   □(0)均無 □(1)中國大陸_____省 □(2)越南 □(3)泰國 □(4)印尼 □(5)其他_____
4. 平常是否接觸下列動物？複選 □(0)均無 □(1)豬 □(2)雞 □(3)鴨 □(4)鵝 □(5)候鳥 □(6)其他___
5. 平常是否喝牛奶？□(0)無 □(1)有，**每週**平均喝____天，每天平均_____毫升(ml/cc)
6. 平常是否服用維他命或藥物？複選
   □(0)否 □(1)維他命 C □(2)維他命 B □(3)綜合維他命 □(4)保健食品_____ □(5)藥物_____
7. 95 年 9 月至今是否有過下列**感冒症狀**？複選
   □(0)均無 □(1)發燒 □(2)畏寒 □(3)肌肉/關節酸痛 □(4)嚴重倦怠
   □(5)喉嚨痛 □(6)流鼻水/鼻塞 □(7)咳嗽 □(8)聲音沙啞
8. 整體上，您認為學童身體狀況如何？ □(1)身體弱常生病(一年看醫生 5 次以上)
   □(2)普通、不錯(一年看醫生 2~4 次) □(3)十分健康(一年看醫生 0~1 次)
9. 是否曾有下列**經醫師確診**之疾病史？複選 □(0)均無

□(1)氣喘(慢性支氣管炎) □(2)過敏性鼻炎 □(3)鼻竇炎 □(4)氣管過敏 □(5)異位性皮膚炎
□(6)肝炎 □(7)肺結核 □(8)貧血 □(9)先天性遺傳疾病_____ □(10)其他_____

λ 非常謝謝您抽空填寫此份問卷，請**再檢查一次每題都填了**，臺大流行病學研究所 敬上

# Appendix E.2　Questionnaire at 1-month Post-vaccination

## ＸＸ地區國小學童流感疫苗評估之流行病學研究問卷

**一、學童接種疫苗情況**

(____國小____年____班____號，學童姓名_____)

1. 學童在**第一次抽血(MM/DD)和學校打流感疫苗日(MM/DD)之間**是否產生下列**感冒症狀**？複選
   □(0)沒有　□(1)有：□(a)發燒　□(b)畏寒　□(c)肌肉/關節酸痛　□(d)嚴重倦怠
   □(e)喉嚨痛　□(f)流鼻水/鼻塞　□(g)咳嗽　□(h)聲音沙啞
2. 學童在今(2007)年有打**流感疫苗嗎**？　□(0)沒有**（跳到第 6 題繼續填寫）**
   □(1)有（繼續第 3 題）
3. 學童在哪裡打疫苗？　□(1)學校統一施打　□(2)自行在外施打(地點：_____，打___劑)
4. 學童什麼時候打疫苗？　□(1)學校統一接種日　□(2)其他(____月____日)
5. 學童接種後**三日內(含)**是否產生下列症狀？複選

□(0) 皆無
□(1) 注射部位疼痛　□(2) 注射部位發紅　□(3) 注射部位腫脹　□(4) 發燒
□(5) 頭痛　□(6) 流鼻水　□(7) 食慾不振　□(8) 噁心嘔吐
□(9) 全身倦怠　□(10) 全身酸痛　□(11) 注射手臂酸痛　□(12) 皮膚長疹子
□(13) 中耳炎　□(14) 其他_____

6. 學童在**學校打流感疫苗日(MM/DD)後至今**是否有下列**感冒症狀**？複選
   □(0)沒有　□(1)有：□(a)發燒　□(b)畏寒　□(c)肌肉/關節酸痛　□(d)嚴重倦怠
   □(e)喉嚨痛　□(f)流鼻水/鼻塞　□(g)咳嗽　□(h)聲音沙啞
7. 學童有沒有(表/堂)兄弟姊妹也在同個學校的一年級或二年級就讀？
   □(0)沒有
   □(1)有，在____年____班____號，姓名_____；____年____班____號，姓名_____：
   ____年____班____號，姓名_____；____年____班____號，姓名_____。

**二、家人接種疫苗情況**

1. 同住的家人在今(2007)年有打**流感疫苗嗎**？
   □(0)同住家人都沒有打流感疫苗
   □(1)有，是(a)學童的_____：(b)學童的_____：(c)學童的_____：(d)學童的_____
2. 同住的家人從**今年 10 月到現在**若有發生下列感冒症狀者，請在□中打勾：複選

| **關係** | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ | 學童的_____ |
|---|---|---|---|---|---|---|---|---|---|
| 發燒 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 畏寒 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 肌肉/關節酸痛 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 嚴重倦怠 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 喉嚨痛 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 流鼻水/鼻塞 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 咳嗽 | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| 聲音沙啞 | □ | □ | □ | □ | □ | □ | □ | □ | □ |

λ　非常謝謝您抽空填寫此份問卷，請**再檢查一次每題都填了**，臺大流行病學研究所 敬上

# Appendix E.3　Questionnaire at 4-month Post-vaccination

## ＸＸ地區國小學童流感疫苗評估流行季末問卷

____國小____年____班____號，學童姓名_____

一、學童部分

1. 請問學童是客家人嗎？　□(0)不是　□(1)是

2. 請問學童是原住民嗎？　□(0)不是　□(1)是，_____族

3. 請問學童現在居住的地方：_____縣/市_____鄉/鎮/市/區_____里

4. 請問您(幫忙填寫這份問卷者)與學童的關係？　我是學童的_____。

5. 請問您(幫忙填寫這份問卷者)的學歷：

□(1)國小以下　□(2)國中　□(3)高中　□(4)職業專科　□(5)大學以上

6. 學童在**寒假期間**是否產生下列**感冒症狀**？複選

□(0)沒有　□(1)發燒　□(2)畏寒　□(3)肌肉/關節酸痛　□(4)嚴重倦怠
□(5)喉嚨痛　□(6)流鼻水/鼻塞　□(7)咳嗽　□(8)聲音沙啞

7. 學童**這學期開學到現在**是否產生下列**感冒症狀**？複選

□(0)沒有　□(1)發燒　□(2)畏寒　□(3)肌肉/關節酸痛　□(4)嚴重倦怠
□(5)喉嚨痛　□(6)流鼻水/鼻塞　□(7)咳嗽　□(8)聲音沙啞

8. 學童上學期(去年 10 月)到現在有**請過病假**嗎？

□(1)有，請了____次，醫生說生_____病

□(0)沒有

9. 學童上學期(去年 10 月)到現在有因為生病而**住院**嗎？

□(1)有，住院____次，醫生說生_____病

□(0)沒有

10. 學童在這次流感流行季中(去年 10 月到現在)，「**最後**」有打流感疫苗嗎？

□(1)有打，總共打□(a)一劑　□(b)兩劑

□(0)沒有打

**<請翻下一面>**

二、家人部分

1. 學童家中**總共**(包括學童)有____個人住在一起。

請細心、詳細填寫下列表格，**每一行代表一位家人**

| 家人 | **學童對他的稱呼** | 出生年份 | 從**去年 10 月到現在**有打流感疫苗嗎？ | 若有打疫苗，打幾劑？ |
|---|---|---|---|---|
| 1 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 2 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 3 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 4 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 5 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 6 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 7 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 8 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 9 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |
| 10 | | _____年 | □(0)無 □(1)有 □(2)不確定 | □(1)一劑 □(2)兩劑 |

2. 家人從**去年 10 月到現在**發生過下列**感冒症狀或因生病而住院**嗎？

複選 請細心、詳細填寫下列表格，**每一直條代表一位家人**

| 家人 | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **學童對他的稱呼** | | | | | | | | | | | |
| **症狀** | 1.發燒 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 2.喉嚨痛 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 3.流鼻水或鼻塞 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 4.咳嗽 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 5.聲音沙啞 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 6.畏寒(覺得身體涼) | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 7.肌肉或關節痠痛 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| | 8.嚴重倦怠 | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| **住院** | | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| **都沒有** | | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |

3. 請問**去年 10 月到現在**有家人生重病而過世嗎？

□(1)有，____位家人，他是學童的_____，醫生說生_____病。

□(0)沒有

**<請翻下一面>**

三、學童食物喜好部分

請根據**最近一個月內**學童吃東西的情況回答問題。多種食物中，大多數喜歡或不喜歡就算

| 問題：請問學童喜不喜歡… | **喜歡** | **普通** | **不喜歡** | **沒吃過** | **不知道** |
|---|---|---|---|---|---|
| 1. 吃蔬菜？ | □ | □ | □ | □ | □ |
| 2. 吃水果或喝純果汁？ | □ | □ | □ | □ | □ |
| 3. 喝牛奶？ | □ | □ | □ | □ | □ |
| 4. 吃起司？ | □ | □ | □ | □ | □ |
| 5. 吃蛋？ | □ | □ | □ | □ | □ |
| 6. 喝豆漿？ | □ | □ | □ | □ | □ |
| 7. 吃豆腐或豆腐干？ | □ | □ | □ | □ | □ |
| 8. 喝優酪乳或吃優格？ | □ | □ | □ | □ | □ |
| 9. 喝養樂多、比菲多、或益菌多等？ | □ | □ | □ | □ | □ |
| 10. 吃肉或魚，包括雞、鴨、豬、牛、蝦子等？ | □ | □ | □ | □ | □ |
| 11. 吃雞肉或鴨肉？ | □ | □ | □ | □ | □ |
| 12. 吃豬肉？ | □ | □ | □ | □ | □ |
| 13. 吃牛肉？ | □ | □ | □ | □ | □ |
| 14. 吃豬肝、雞肝、雞心等內臟類？ | □ | □ | □ | □ | □ |
| 15. 吃魚？ | □ | □ | □ | □ | □ |
| 16. 吃蝦子、螃蟹、牡蠣、蛤？ | □ | □ | □ | □ | □ |
| 17. 吃漢堡、披薩、薯條、炸雞、鹹酥雞？ | □ | □ | □ | □ | □ |
| 18. 吃冰淇淋、聖代、雪糕？ | □ | □ | □ | □ | □ |
| 19. 吃蛋糕、派、西點麵包？ | □ | □ | □ | □ | □ |
| 20. 吃洋芋片、翠果子、蠶豆酥、蝦味先、乖乖、滿天星、金牛角等？ | □ | □ | □ | □ | □ |
| 21. 吃科學麵、王子麵、小心點等速食麵？ | □ | □ | □ | □ | □ |
| 22. 吃餅乾？ | □ | □ | □ | □ | □ |
| 23. 喝汽水、可樂、奶茶、或其他甜飲料？ | □ | □ | □ | □ | □ |
| 24. 吃冰棒、冰沙、剉冰？ | □ | □ | □ | □ | □ |
| 25. 吃糖或巧克力？ | □ | □ | □ | □ | □ |
| 26. 吃麥片或全麥麵包？ | □ | □ | □ | □ | □ |
| 27. 吃糙米或五穀？ | □ | □ | □ | □ | □ |
| 28. 吃棗子或芭樂？ | □ | □ | □ | □ | □ |
| 29. 吃草莓或桑椹？ | □ | □ | □ | □ | □ |

**〈問卷結束，謝謝您的耐心，請再檢查一次每題都填了〉**

# Appendix F

## Autobiography of Author Ms. Yun-Chin Chu

**Yun-Chin Chu**
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**EDUCATION**

-M. PH., Institute of Epidemiology, College of Public Health, National Taiwan University, 2006-2009

-B.S., Department of Public Health, College of Public Health, National Taiwan University, College of Public Health, 2002-2006

**PAST EXPERIENCES**

- Teaching assistant of medical biostatistics class, College of Public Health National Taiwan University (2006-2007)

- Epidemic investigation qualified staff, Center for Disease Control, Taiwan (2005)

- Intern at Division of Emerging Infectious Diseases, Center for Disease Control, Taiwan (2005)

- Administrator of music and club member in Orchestra of Chinese Music, National Taiwan University (2002-2004)

**RESEARCH INTERESTS**

- Public Health

- Infectious Disease Epidemiology

- International Health