

# Association Between Seasonal Influenza Vaccination in 2008–2009 and Pandemic Influenza A (H1N1) 2009 Infection Among School Students From Kobe, Japan, April–June 2009

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**We assessed the effect of seasonal trivalent inactivated influenza vaccination (TIV) on pandemic influenza 2009 (pH1N1)-related illness from April to June 2009 among 2849 students (aged 12–18 years). TIV was associated with an increase in the frequency of pH1N1-related illness among subjects (adjusted odds ratio, 1.47; 95% confidence interval, 1.14–1.89). TIV during the 2008–2009 season increased the risk of pH1N1-related illness from April to June 2009.**

In March 2009, pandemic influenza 2009 (pH1N1) virus emerged in North America [1]. On 16 May 2009, the first domestic pH1N1 infection was confirmed by reverse-transcription polymerase chain reaction (RT-PCR) in students in Kobe, Japan [2]. There were 114 laboratory-confirmed cases from 16 May to 5 June 2009 in Kobe. Approximately 72% involved students aged 15–19 years. The local government of Hyogo prefecture, including Kobe, implemented extensive school closures. The outbreak ended on 21 May 2009.

Previous laboratory studies have indicated that pH1N1 virus is antigenically and genetically distinct from seasonal influenza

viruses [3]. Vaccination with recent seasonal influenza vaccines is not likely to provide protection against pH1N1 [4]. The effect of seasonal influenza vaccination on risk of pH1N1 infection has attracted attention after a Canadian study in which vaccination was associated with increased risk of pH1N1-related illness [5]. However, the results of recent studies have been inconsistent, and few studies have been conducted in the general population. Additionally, the chance of contracting the virus has not been considered. We attempted to evaluate the association between seasonal trivalent inactivated influenza vaccine (TIV) during the 2008–2009 season and pH1N1-related illness among school students, considering the chance of contracting influenza virus.

## METHODS

### Study Design and Participants

This was an active epidemiological investigation under Article 15 of the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections in Japan. Subjects were 2963 students (aged 12–18 years) from 3 schools in Kobe, where the first Japanese case was reported. A questionnaire was distributed to all students and included questions regarding sex, age, class, after-school club activities, extracurricular/study activities, symptoms from 23 April to 18 May 2009, and vaccination status for the 2008–2009 season. Moreover, for students in 2 schools, we extended the period to 6 June 2009 using daily health records. Information on underlying conditions (eg, respiratory disorder, heart disease, and epilepsy) ( $n = 763$ ) and history of seasonal influenza infection in the 2008–2009 season ( $n = 997$ ) was also collected using school records from students whose information was available. The RT-PCR results for pH1N1 virus were obtained from the Public Health Center of Kobe and Kobe Institute of Health, although these were available only for students who visited the hospital and had positive RT-PCR results.

### Definition of Probable and Confirmed Cases

Probable cases were students who had fever  $\geq 37^{\circ}\text{C}$  and  $>1$  symptom (cough, runny or stuffy nose, or sore throat) during the period from 23 April to 6 June 2009. We excluded students with a diagnosis of influenza B, as determined by rapid antigen test. Confirmed cases were students who met the definition of probable cases and whose diagnoses were confirmed by RT-PCR. We used a cutoff of  $\geq 37^{\circ}\text{C}$  based on our observation that some confirmed cases developed mild fever ( $\geq 37^{\circ}\text{C}$  but  $< 38^{\circ}\text{C}$ ) and a previous study in China in which 64.1% patients had fever

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<38°C [6]. For analysis, we combined probable and confirmed cases. Non-cases were students who did not meet the definition of probable or confirmed cases.

### Statistical Analysis

We used TIV status as an independent variable and pH1N1-related illness as a dependent variable. School, school year, and sex were adjusted as confounding variables. We performed univariate and multivariate analyses and estimated crude odds ratios (ORs), adjusted ORs, and 95% confidence intervals (CIs). Adjusted ORs were then used to estimate vaccine effectiveness according to the following formula:  $(1 - \text{adjusted OR}) \times 100$ . Vaccine effectiveness is known to be affected by settings such as influenza or preinfluenza period and severe or mild season—that is, the chance of contracting influenza [7]. As a proxy for the chance of contracting, we used attack rate (AR) of groups (ie, classroom, club activity, and extracurricular or study activity) to which each subject belonged. AR was calculated by dividing the number of cases in each group by the total number in the group. We then assigned each subject a value for AR. If a subject belonged to multiple groups, he or she was assigned the value of the highest AR. Subsequently, we stratified the subjects into 3 categories using this AR, so that each stratum had almost the same number of subjects.

We conducted 2 sensitivity analyses. First, we additionally adjusted for underlying conditions by restricting the subjects who had information regarding underlying conditions ( $n = 763$ ). Second, we restricted the subjects for whom information about the seasonal infection in 2008–2009 was available ( $n = 997$ ). Statistical analysis was conducted using IBM SPSS Statistics software (version 19).

## RESULTS

### Subject Characteristics

A total of 2918 (98.5%) of the 2963 study questionnaires were returned. Of the 2918 students, 2849 (96.2%) provided

information regarding TIV (Supplementary data appendix 1). The numbers of vaccinated and nonvaccinated subjects were 1592 and 1257, respectively. The numbers of case and non-cases were 336 (266 probable, 70 confirmed) and 2513, respectively. The epidemic curve is shown in Supplementary data appendix 2. Among 997 students for whom we obtained information regarding seasonal influenza infection (Supplementary data appendix 1), TIV and seasonal infection were not associated (OR, 0.99; 95% CI, .53–1.85). TIV did not reduce the incidence of seasonal infection during the 2008–2009 season.

### TIV in 2008–2009 and pH1N1-Related Illness

After adjustment by school, school year, and sex, TIV significantly increased the frequency of pH1N1-related illness (OR, 1.47; 95% CI, 1.14–1.89) (Table 1). The stratified analysis by AR (as a proxy for the chance of virus contraction) showed a trend: the higher the AR, the higher the point estimates of OR.

In sensitivity analyses, among 763 students with information regarding underlying conditions, the adjusted ORs did not change substantially even if we adjusted for these conditions; they changed from 2.02 (95% CI, 1.36–3.01) to 2.02 (95% CI, 1.36–3.02). When we restricted the subjects who had information about seasonal infection ( $n = 997$ ), the adjusted OR was 1.19 (95% CI, .80–1.76). Even if we further excluded 42 students with a positive history of seasonal infection, the adjusted OR did not change (1.21; 95% CI, .81–1.80).

## DISCUSSION

Our results suggest that TIV in 2008–2009 increased the risk of pH1N1-related illness. This result is inconsistent with the previous laboratory study mentioned above, which showed no beneficial effect of TIV [4], and epidemiological studies conducted in Australia and Mexico, which also showed no or partial

**Table 1. Odds Ratios and Vaccine Effectiveness for Association Between Seasonal Trivalent Inactivated Influenza Vaccination in 2008–2009 and Pandemic Influenza A (H1N1) 2009 ( $n = 2848$ )<sup>a</sup>**

| Attack Rate, % <sup>b</sup> | Seasonal Influenza Vaccination in 2008–2009 | Cases <sup>c</sup> | Noncases  | Crude OR (95% CI) | Adjusted OR <sup>d</sup> (95% CI) | VE, % <sup>e</sup> |
|-----------------------------|---|--------------------|-----------|-------------------|-----------------------------------|--------------------|
| >17.6 ( $n = 944$ )         | Yes/No                                      | 118/111            | 310/405   | 1.39 (1.03–1.87)  | 1.86 (1.34–2.58)                  | –86.1              |
| 10.0–17.6 ( $n = 953$ )     | Yes/No                                      | 49/35              | 489/380   | 1.09 (.69–1.71)   | 1.06 (.65–1.74)                   | –6.1               |
| ≤10.0 ( $n = 951$ )         | Yes/No                                      | 13/10              | 612/316   | 0.67 (.29–1.55)   | 0.81 (.33–1.97)                   | 19.1               |
| Total ( $n = 2848$ )        | Yes/No                                      | 180/156            | 1411/1101 | 0.90 (.72–1.13)   | 1.47 (1.14–1.89)                  | –47.0              |

Abbreviation: CI, confidence interval; OR, odds ratios; VE, vaccine effectiveness.

<sup>a</sup> One subject was excluded for missing value regarding sex (Supplementary data appendix 1) ( $n = 2849$ ).

<sup>b</sup> As a proxy for the chance of contracting influenza virus, attack rate of groups (classroom, club activity, and extracurricular or study activity) was calculated by dividing the number of case in each group by the total number in the group. If a subject belonged to multiple groups, he or she was assigned the highest attack rate value.

<sup>c</sup> Cases included probable and confirmed cases (see text for definitions).

<sup>d</sup> Adjusted by school, school year, and sex.

<sup>e</sup> VE was calculated by the following formula:  $(1 - \text{adjusted OR}) \times 100$ .

beneficial effect [8, 9]. However, the result is consistent with those of studies in Canada and Hong Kong, which showed an increased risk of pH1N1-related illness [5, 10, 11]. Our sample reflected the general community, whose members were usually healthy. Our study therefore seems similar to the Canada and Hong Kong studies in terms of the target community and time between investigation and TIV vaccination in the 2008–2009 season.

Our study was stratified by the chance of contracting influenza (reflected by the value of AR). Stratified analysis showed a trend that a higher chance of contracting influenza led to higher OR. This trend may lead us not to suspect large unadjusted bias [7].

Our results indicate that TIV increased the risk of pH1N1-related illness. As possible explanations, TIV may be a proxy variable for something that increases onset, an unidentified confounder may exist, or this may be a true association. If the association is true, antibody-dependent enhancement could be an explanation, as described elsewhere for influenza A virus [12]. Antibodies for H1N1 and H3N2, subtypes of influenza A virus, induced by TIV might work as cross-reactive, non-neutralizing antibodies and enhance cell uptake of pH1N1 virus. Moreover, most students in this study were vaccinated in November–December 2008, and this outbreak occurred in mid-May 2009. The antibodies induced by TIV would be left, because antibody levels are thought to decrease by 50% within 6 months. The timing of this study and age of the subjects could have affected our results.

This study had several limitations. Because this was the first outbreak in Japan, many influenzalike illnesses were not tested by RT-PCR, even if patients consulted physicians, and we could not collect negative RT-PCR results. If health-conscious subjects were more likely to have TIV and to be tested by RT-PCR in the clinic, then a positive association between TIV and pH1N1-related illness might be observed, as in the present study. However, the probable and confirmed cases showed similar epidemic curves (Supplementary data appendix 2), validating the diagnosis of confirmed cases. Moreover, the results did not change when we used as cases only the subjects who met the definition of probable cases, identified by questionnaire alone. Thus, this undercoverage of RT-PCR testing is not likely to have affected the present findings. Next, we did not observe a positive association between TIV and the frequency of pH1N1-related illness among subjects who had information about seasonal infection. This was probably due to a small sample size and a small proportion (302/997; data not shown) in the high-AR stratum ( $>17.6$ ). More epidemiological and biological studies are necessary to confirm this unexpected association and determine the uncertain mechanisms.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online ([http://www.oxfordjournals.org/our\\_journals/cid/](http://www.oxfordjournals.org/our_journals/cid/)). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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