# Influenza surveillance and vaccination in Hong Kong children

**TF Leung *, PKS Chan, KL Hon, AM Li, FWT Cheng**

KEY MESSAGES

1. This prospective influenza surveillance study found that mild influenza infection was common among Hong Kong children aged 2 to 12 years during the influenza seasons of 2014-2015.
2. Seasonal influenza vaccination protected against influenza-like illness (ILI) but not laboratory-confirmed influenza in surveillance samples of local children. The effectiveness of influenza vaccine for ILI varied between 42.1% and 51.9%.
3. Seropositivity, defined by a haemagglutination inhibition titre of ≥1:40, was found in 92%, 91%, 68%, 49%, and 85% of participants for pandemic A/H1N1, A/H3N2, A/H3N2_Switzerland, B/Victoria, and B/Yamagata, respectively. However, neither haemagglutination inhibition titre nor seropositivity was a useful surrogate of influenza immunity in children.
4. Neither ILI nor influenza infection was associated with any demographic, environmental, or clinical factors in the children.
5. Approximately half of local preschool and primary school children had received seasonal influenza vaccination within the past 3 years.

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## Introduction

In healthy children, seasonal influenza vaccination reduces the rate of laboratory-confirmed influenza by 59% and the rate of influenza-like illness (ILI) by 36%. The efficacy of seasonal influenza vaccine has been reported. Nonetheless, there are limited post-licensure effectiveness data for Asian children. In European sentinel surveillance networks, seasonal influenza vaccine offered low-to-moderate effectiveness of 43% against influenza A(H3) in the early 2011/2012 season.[1] A similar level of vaccine effectiveness has been reported from 19 influenza surveillance sites in Guangzhou,[2] supporting the need to delineate the effectiveness of seasonal influenza vaccine in Hong Kong children.

The number of laboratory-confirmed cases underestimates the number of true cases, as surveillance focuses on more severe cases. Influenza surveillance in prospective cohorts is thus necessary to define the full spectrum of influenza. Because of viral antigenic drift, the usefulness of seasonal influenza vaccination programmes varies from year to year. Annual estimates of the effectiveness of influenza vaccine could monitor changes in the impact of such programmes. This study aimed to ascertain the full spectrum of influenza infections in local preschool and school-age children, identify risk factors for clinically significant influenza in these children, and delineate the effectiveness of influenza vaccine in preventing influenza and ILI among these children and their family members and classmates.

## Methods

The Joint Chinese University of Hong Kong – New Territories East Clinical Research Ethics Committee approved this study. This prospective cohort study recruited children aged 2 to 12 years from kindergartens and primary schools that were randomly selected using stratified (by districts) and clustered (all subjects within a class) sampling. Written informed consent was obtained from the parents of eligible students for influenza surveillance and blood testing. There was no exclusion criterion. We conducted surveillance during three consecutive influenza seasons throughout an 18-month period (January to May 2014, August 2014, and January to February 2015).

Subject demographics, pre-existing medical illnesses, and history of influenza vaccination within the past 3 years were recorded using a questionnaire completed by the parents. Having been vaccinated was defined as (1) ≥14 days post-vaccination, (2) received two doses 28 days apart if vaccinated for the first time, or (3) received at least one dose in a previous influenza season and one dose in the season under study.[2] Serial biweekly surveillance flocked nasopharyngeal swab (NPS) samples were collected from subjects during school visits regardless of the presence of respiratory symptoms. Surveillance was started within 2 weeks upon announcement of the start of influenza seasons by the Centre for Health Protection. Subjects' parents were called biweekly to enquire about symptoms of ILI (ie, fever ≥38°C plus

two of the following: sore throat, cough, rhinorrhoea, myalgia, arthralgia). Subjects were invited to report to the outpatient clinic at Prince of Wales Hospital within 48 hours of the onset of ILI.

NPS samples were collected using flocked swabs (Copan Diagnostics, Corona, CA). Each child was swabbed twice, using one swab in each nostril. Both swabs were placed in the same specimen bottle with viral transport medium. Viral RNA was extracted with the PureLink Viral RNA/DNA Mini Kit (Life Technologies). Real-time PCR was performed using the SuperScript III Platinum One-Step Quantitative RT-PCR System. Molecular typing of influenza viruses was based on the World Health Organization guidelines with slight modifications. Primers were designed to target the haemagglutinin genes specific to formerly pandemic A (H1N1) 2009 (A/H1N1 pdm), A/H3, B/Victoria, and B/Yamagata strains. Conventional PCR was performed using the AmpliTaq Gold 360 Master Mix (Life Technologies) system, and the PCR products were resolved in 2% agarose gel. Viral load was estimated by comparing the PCR results with standard curves generated by serial dilution of plasmids containing the PCR fragments. In addition, sera obtained from 2 mL of clotted blood were tested in parallel using haemagglutination inhibition (HAI) assays against A/H1N1 pdm, A/H3N2, A/H3N2_Switzerland, B/Victoria, and B/Yamagata strains.[3] An HAI antibody titre ≥1:40 was used as the cut-off for protective immunity.[4]

The association of laboratory-confirmed influenza (primary outcome) or ILI with vaccination was analysed using logistic regression adjusted for covariates, including seasonality (month of study), subject age, sex, body mass index, and comorbid medical conditions. Surveillance data from all influenza seasons were combined. The effectiveness of the influenza vaccine was estimated using a test-negative case-control design. All analyses were two-tailed. A P value of <0.05 was considered statistically significant.

TABLE 1. Distribution of numbers of nasopharyngeal swab (NPS) samples collected

| No. of NPS samples collected | Frequency |
|---|---|
| 1 | 16 |
| 2 | 102 |
| 3 | 221 |
| 4 | 23 |
| 5 | 28 |
| 6 | 116 |
| 7 | 114 |
| 8 | 3 |

## Results

A total of 630 children (322 in 2014 and 308 in 2015) with a mean age of 7.3 (standard deviation, 2.4) years from five primary schools and 10 kindergartens participated. Seven subjects withdrew consent before any NPS collection. A total of 337 (53.5%) subjects had received influenza vaccination within the past 3 years. A total of 2633 NPS samples were collected; most children recruited in 2014 provided six to seven samples, and those recruited in 2015 gave two to three samples (Table 1). Two samples and three samples were obtained from 607 (97.4%) and 505 (81.1%) of 623 subjects, respectively. Of the subjects, 99 were reported to have ILI episodes. In addition, nine illness visits were arranged for five other subjects. There was no reported ILI outbreak in the schools or transmission of influenza within the same classes and household of influenza-infected children.

Influenza A and B were detected in 27 and 30 subjects, respectively, with respective median (interquartile range) viral loads of 918 (99-14 864) copies/μL and 262 (98-324 027) copies/μL. Influenza B predominated in 2014 and influenza A in 2015 (P<0.001). Overall, 36 (11.2%) of 321 subjects had influenza A or B infection in 2014, whereas all 19 (6.3%) of the 302 subjects with influenza had influenza A infection in 2015. Six influenza A and 11 influenza B isolates were not typable. For the remaining isolates, influenza A was typed into four A/H1N1 pdm and one A/H3 in 2014 and three A/H1N1 pdm and 13 A/H3 in 2015 (P=0.025). Among the influenza B isolates, all of which were detected in 2014, nine were Yamagata and 10 were Victoria strain. All such children were not reported to be sick at the time of NPS collection. All nine illness NPS samples collected from five subjects in 2014 were negative for both influenza A and B.

ILI was not associated with demographic, environmental, or clinical factors (Table 2). Seasonal influenza vaccination at all time points was protective against ILI (P=0.022-0.002). Logistic regression confirmed such association for seasonal influenza vaccination within the past 3 years (odds ratio=0.49, 95% confidence interval=0.29-0.81, P=0.005). None of the listed factors was associated with laboratory-confirmed influenza detected by surveillance visits (data not shown).

HAI assays were successfully conducted on 181 sera samples, and data for the respective influenza type (ie A or B) of subjects whose earlier surveillance NPS samples were positive for influenza were excluded from analysis. Seropositivity rates for A/H1N1 pdm, A/H3N2, A/H3N2_Switzerland, B/Victoria, and B/Yamagata were 92%, 91%, 68%, 49%, and 85%, respectively. The mean reciprocal HAI titres for A/H1N1 pdm were significantly higher in children who had been vaccinated within the past 3

TABLE 2. Association of influenza-like illness with demographic, environmental, and allergic factors

| Factor | Influenza-like illness* | | P value |
|---|---|---|---|
| | Yes (n=99) | No (n=523) | |
| Male sex | 55 (55.6) | 272 (52.0) | 0.039 |
| Age, y | 5.8±2.2 | 7.6±2.4 | <0.001 |
| Born in Hong Kong | 87 (87.9) | 423 (80.9) | 0.097 |
| Born by normal vaginal delivery | 75 (75.8) | 340 (65.0) | 0.087 |
| Environmental exposures | | | |
| Breastfeeding ever | 60 (60.6) | 282 (53.9) | 0.220 |
| Current domestic smoking exposure | 39 (39.4) | 210 (40.2) | 0.888 |
| Current maternal smoking | 8 (8.1) | 48 (9.2) | 0.727 |
| Current dog/cat cohabitation | 10 (10.1) | 43 (8.2) | 0.539 |
| Indoor dampness or visible mould | 41 (41.1) | 180 (34.4) | 0.182 |
| Presence of older brother | 24 (24.2) | 117 (22.4) | 0.683 |
| Presence of older sister | 25 (25.3) | 103 (19.7) | 0.210 |
| Allergy phenotypes | | | |
| Wheezing ever | 18 (18.2) | 89 (17.0) | 0.778 |
| Current wheezing | 12 (12.1) | 53 (10.1) | 0.553 |
| Asthma ever | 5 (5.1) | 34 (6.5) | 0.585 |
| Use of asthma medication in past 12 months | 4 (4.0) | 13 (2.5) | 0.721 |
| Rhinitis ever | 31 (31.3) | 188 (35.9) | 0.376 |
| Eczema ever | 26 (26.3) | 137 (26.2) | 0.989 |
| History of influenza vaccination | | | |
| Within the past 3 years | 39 (39.4) | 294 (56.2) | 0.002 |
| 25-36 months prior | 28 (28.3) | 212 (40.5) | 0.022 |
| 13-24 months prior | 27 (27.3) | 229 (43.8) | 0.002 |
| Within the past 12 months | 29 (29.3) | 237 (45.3) | 0.003 |
| Ever received human swine influenza vaccine | 3 (3.0) | 21 (4.0) | 0.641 |
| Ever received pneumococcal vaccine | 50 (50.5) | 220 (42.1) | 0.120 |

* Data are presented as mean±standard deviation or No. (%) of participants

years (186 vs 106, P=0.022) and within the previous year (192 vs 112, P=0.032). Neither HAI titres nor seropositivity rates differed between subjects with and without ILI or influenza in surveillance samples.

In general, influenza vaccine was moderately protective against ILI, with vaccine effectiveness varying between 42.1% (10.5%-63.1%) and 51.9% (24.5%-70.1%) when subjects were vaccinated at different time points before this study (Table 3). Nonetheless, the effectiveness of seasonal influenza vaccine was poor in preventing laboratory-confirmed influenza in surveillance samples.

## Discussion

During influenza seasons in 2014-2015, NPS surveillance detected 104 children with ILI and 55 clinically asymptomatic children with laboratory-confirmed influenza. A significant proportion of patients with respiratory viral infections had mild symptoms only.

Some post-marketing studies have challenged the real-life effectiveness of influenza vaccine.[1,2,5] Multiple confounding factors, such as difficulty matching influenza A subtype for the vaccine with the dominant viruses, suboptimal vaccine uptake, and poor infection control practices affected the effectiveness of influenza vaccine, which can only be defined by well-designed observational studies. This prospective cohort study enabled biweekly surveillance of influenza A and B viruses by molecular methods. Our results supported seasonal influenza vaccine as an effective public health measure to prevent ILI in local children.

Among the typable influenza virus isolates, we found four A/H1N1 pdm and one A/H3 in 2014 and three A/H1N1 pdm and 13 A/H3 in 2015. These findings are consistent with local surveillance data for influenza A, which indicate that the 2013/14 winter influenza season was dominated by A/H1N1 pdm from early January 2014 to early March 2014, whereas A/H3N2 was predominant from late December 2014 to early April 2015. All of our influenza B virus-positive samples were detected in 2014 (nine B/Yamagata and 10 B/Victoria). Our typing results are also concordant with local data showing B/Yamagata dominance between early March and late April in 2014 and low activity of influenza B in the winter 2014/15 season.

During the period of this study, approximately 40% of Hong Kong children received seasonal influenza vaccination annually. Breaking barriers to seasonal influenza vaccination should be a public health priority against influenza outbreaks. The moderate effectiveness of influenza vaccine for ILI in children aged 2 to 12 years may suggest that the Government Vaccination Programme can be expanded to older children.

## Conclusion

Mild influenza was common during influenza seasons in 2014-2015 among Hong Kong children. The effectiveness of influenza vaccine for ILI varied between 42.1% and 51.9%, depending on the year of vaccination. Our findings do not support HAI titres or seropositivity as useful surrogates of influenza immunity in children.

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TABLE 3. Effectiveness of seasonal influenza vaccine in terms of laboratory-confirmed influenza (by surveillance) and influenza-like illness

| Timing of vaccination | No. (%) of participants | | Vaccine effectiveness (95% confidence interval) |
|---|---|---|---|
| | Vaccinated | Not vaccinated | |
| Laboratory-confirmed influenza by surveillance | | | |
| Within the past 3 years | n=333 | n=290 | |
| Positive | 26 (7.8) | 27 (9.3) | 17.5 (–39.0 to 51.3) |
| Negative | 307 (92.2) | 263 (90.7) | |
| Within the past 12 months | n=266 | n=357 | |
| Positive | 18 (6.8) | 35 (9.8) | 33.2 (–16.9 to 62.2) |
| Negative | 248 (93.2) | 322 (90.2) | |
| 13-24 months prior | n=256 | n=367 | |
| Positive | 21 (8.2) | 32 (8.7) | 6.4 (–58.8 to 44.9) |
| Negative | 235 (91.8) | 335 (91.3) | |
| 25-36 months prior | n=240 | n=383 | |
| Positive | 22 (9.2) | 31 (8.1) | –14.6 (–92.2 to 31.5) |
| Negative | 218 (90.8) | 352 (91.9) | |
| Influenza-like illness | | | |
| Within the past 3 years | n=333 | n=289 | |
| Yes | 39 (11.7) | 60 (20.8) | 49.4 (24.0 to 66.9) |
| No | 294 (88.3) | 229 (79.2) | |
| Within the past 12 months | n=266 | n=356 | |
| Yes | 29 (10.9) | 70 (19.7) | 50.0 (22.4 to 68.4) |
| No | 237 (89.1) | 286 (80.3) | |
| 13-24 months prior | n=256 | n=366 | |
| Yes | 27 (10.5) | 72 (19.7) | 51.9 (24.5 to 70.1) |
| No | 229 (89.5) | 294 (80.3) | |
| 25-36 months prior | n=240 | n=382 | |
| Yes | 28 (11.7) | 71 (18.6) | 42.1 (10.5 to 63.1) |
| No | 212 (88.3) | 311 (81.4) | |

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