Surveillance of human- and swine-origin influenza in Hong Kong children

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KEY MESSAGES

1. Of 49 children aged 5 years recruited from three randomly selected kindergartens in the New Territories East Cluster, 49 provided at least one nasopharyngeal swab for influenza surveillance. Of them, 44 (89.8%) provided four bi-weekly nasopharyngeal samples between early February and late March 2012. Serial nasopharyngeal sampling is a feasible approach for respiratory virus surveillance in local preschool children.
2. Of all samples, 13 from 12 (24.5%) children were influenza-positive, including those from 10 (20.4%) of 49 children under surveillance and three of 10 children who provided illness visit samples. Asymptomatic and mildly symptomatic influenza infection is common in these young children.
3. Of the 49 children, 27 (55.1%) had received influenza vaccination within 3 years, but only two (16.7%) who were infected with influenza had been vaccinated within the past 12 months. The low level of vaccine uptake is probably the main cause of influenza infection in our preschoolers.
4. Influenza infection was not associated with any personal or environmental factors, including influenza vaccination.

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Introduction

In 2009, the emergence of a triple reassortant H1N1 virus resulted in an influenza pandemic.[1] In contrast to seasonal influenza virus, this H1N1 virus caused illness primarily in younger age groups. In the United States, 60% of patients were 18 years of age or younger. The number of laboratory-confirmed cases underestimates the number of true cases, as surveillance focused on severe cases and caused bias with respect to the disease spectrum of novel influenza infection. Patients with mild infection or who were asymptomatic could not be identified because they were unlikely to seek medical treatment. This H1N1 virus is now circulating seasonally. The pandemic H1N1 vaccine is a component of seasonal influenza vaccine, which for 2012/2013 (northern hemisphere winter) comprised A/California/7/2009 (H1N1)-like virus, A/Victoria/361/2011 (H3N2)-like virus, and B/Wisconsin/1/2010-like virus.

The Centre for Health Protection recommends influenza vaccination for children aged 6 months to 5 years. Among healthy children aged 2 to 15 years, seasonal influenza vaccination has reduced laboratory-confirmed influenza and clinical influenza-like illness by 59% and 36%, respectively. Although influenza vaccination has proven efficacy in children, the data remain inconsistent on the real-life effectiveness of seasonal influenza vaccine against transmission of influenza infection within schools and among household members. Population-based surveillance studies are needed to address this public health issue. This study aimed to investigate the feasibility of serial nasopharyngeal swab (NPS) sampling and characterise the clinical features of influenza infections in Hong Kong preschool children.

Methods

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Clinical Research Ethics Committee. This prospective cohort study targeted 50 K3 students from three randomly selected kindergartens in Shatin and Ma On Shan. The parents were contacted to obtain informed written consent for their children. There was no exclusion criterion to facilitate recruitment of an unbiased cohort. This study was timed to cover the influenza season of early 2012. Influenza infection was defined as the detection of influenza virus by multiplex PCR assay. The primary outcome was the success rate of obtaining serial NPS samples from participants. The secondary outcomes included the detection rate and clinical features of influenza infections and rates of influenza-like illness and laboratory-confirmed influenza among children who received and had not received influenza vaccination.

Parents completed a questionnaire to record the participants' demographics, early-life events, environmental exposures, health status, and history of influenza vaccination within 3 years prior to recruitment. Two sets of NPS samples were obtained. For active surveillance during the influenza season, four serial NPS samples were collected from each participant during bi-weekly school visits between early February and late March in 2012. The other set of NPS samples was obtained from children with symptoms of respiratory infections throughout the

study period. We called the parents every 2 weeks to enquire about influenza-like illness and vaccination history. The parents were encouraged to inform us as soon as their children developed symptoms and then visit our hospital within 48 hours of the onset of influenza-like illness. Alternatively, home visits were provided to collect NPS. Possible clustering of influenza infection among household members and classmates was also recorded.

The NPS samples were collected using flocked swabs (Copan Diagnostics, Corona [CA], United States). Each child was swabbed two times, using one swab in each nostril. Both swabs were placed in the same specimen bottle containing virus transport medium. Total RNA and DNA were extracted together by the PureLink Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad [CA], United States). The extracted preparation was mixed with random primers and dNTPs at 65°C for 5 minutes. The solution was equilibrated at 4°C and completed with two units (U) of RNaseOUT, 4 μL of 5× First-Strand buffer, 0.5 mM DTT, and 10 U M-MLV Superscript III reverse transcriptase (Invitrogen) to a final volume of 20 μL. Reverse transcription was performed for 50 minutes at 50°C and then stopped by heating for 15 minutes at 70°C. The resulting complementary DNA products (cDNAs) were used immediately for PCR. Two sets of primers targeting influenza A and B viruses were used during PCR for both the first and second rounds. The 73- and 516-bp PCR products from influenza A and B, respectively, were identified by electrophoresis and visualised in 1.5% agarose gel pre-stained with SYBR-Safe (Invitrogen).

TABLE 1. Clinical features of 49 participants in influenza surveillance

Feature	Value*
Age, y	5.5±0.4
Male sex	27 (55.1)
History of influenza vaccination	
Received seasonal vaccine within 3 years	27 (55.1)
Received seasonal vaccine in 2009	24 (49.0)
Received seasonal vaccine in 2010	18 (36.7)
Received seasonal vaccine in 2011	9 (18.4)
Ever received human swine influenza monovalent vaccine	3 (6.1)
Physician-diagnosed influenza in past 12 months	22 (44.9)
Anti-viral treatment for influenza in past 12 months	4 (8.2)
Asthma phenotypes	
Wheezing ever	14 (28.6)
Current wheezing	8 (16.3)
Awakening due to wheezing in past 12 months	6 (12.2)
Asthma ever	4 (8.2)
Asthma medication in past 12 months	17 (34.7)
Exercise-induced wheezing	8 (16.3)
Rhinitis ever	18 (36.7)
Eczema ever	15 (30.6)
Environmental exposures	
Breastfeeding ever	31 (63.3)
Breastfeeding for 4 months and longer	17 (34.7)
Current cat/dog cohabitation	2 (4.1)
Current maternal smoking	2 (4.1)
Maternal smoking during infancy	3 (6.1)
Maternal smoking during pregnancy	2 (4.1)
Current domestic smoking exposure	18 (36.7)
Indoor dampness or visible mould	7 (14.3)

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

Results

A total of 54 children were included, but five withdrew consent before collection of the first surveillance swab (Table 1). On the second, third, and fourth visits, NPS of 48, 46, and 44 children, respectively, were collected for bi-weekly surveillance. Of the children, 27 (55.1%) had received influenza vaccination within the previous 3 years, including nine (18.4%) vaccinated in 2011. Of the 14 children reported to have respiratory symptoms, eight visited our hospital and six visited general practitioners. NPS samples were collected from 10 of these sick children.

Influenza B virus was detected in 10 children who provided at least one NPS sample. All such children were not sick at the time of NPS collection. One child had influenza A virus followed by influenza B virus detected in two consecutive NPS samples collected 2 weeks apart. She remained asymptomatic during this period. In addition, three of the 10 sick children with available NPS had detectable influenza B virus. There was no transmission of influenza within the same classrooms or households of the children infected with influenza. Only two (16.7%) of the 12 children who were infected with influenza (either under surveillance or sick) had been vaccinated within past 12 months. Influenza infection was not associated with personal, clinical, or vaccine-related factors (Table 2).

Discussion

This prospective cohort design enabled surveillance of respiratory viruses because a large proportion of the participants had mild symptoms only. They may have had mild cough and runny nose but no fever, myalgia, or arthralgia. It is unlikely that these participants would have been identified in any hospital-based study. A study that collected weekly respiratory samples from community participants reliably defined the disease severity and seasonality of picornavirus infection.[2] We applied sensitive molecular diagnostic tests to the nasopharyngeal secretions of clinically 'asymptomatic' children.

Influenza vaccination is effective against

TABLE 2. Association of influenza infection with demographic, environmental, allergic, and vaccine-related factors

Parameter	Influenza*		P value
	Yes (n=10)	No (n=39)	
Male sex	7 (70.0)	20 (51.3)	0.478
Age, y	5.3 (5.2-5.9)	5.6 (5.3-5.8)	0.524
Environmental exposures			
Breastfeeding ever	6 (60.0)	25 (64.1)	0.542
Current domestic smoking exposure	3 (30.0)	15 (38.5)	0.458
Maternal smoking during infancy	0 (0)	2 (5.1)	0.630
Current dog/cat cohabitation			
Indoor dampness or visible mould	3 (30.0)	4 (10.3)	0.140
Allergic phenotypes			
Current wheezing	1 (10.0)	7 (17.9)	1.000
Asthma ever	2 (20.0)	2 (5.1)	0.180
Asthma medication in past 12 months	4 (40.0)	13 (33.3)	0.721
Rhinitis ever	3 (30.0)	15 (38.5)	0.726
Eczema	4 (40.0)	11 (28.2)	0.470
History of seasonal influenza vaccination within 3 years	6 (60.0)	21 (53.8)	0.727
2009	5 (50.0)	19 (48.7)	
2010	5 (50.0)	13 (33.3)	
2011	2 (20.0)	7 (17.9)	
Received influenza vaccine within 12 months	2 (20.0)	7 (17.9)	0.597
Ever received human swine influenza monovalent vaccine	1 (10.0)	2 (5.1)	0.504
Received anti-viral treatments for influenza within 12 months	0 (0)	4 (10.3)	0.569
Physician-diagnosed influenza within 12 months	7 (70.0)	15 (38.5)	0.090

* Data are presented as mean (range) or No. (%) of participants

natural infection.[3] However, vaccine trials are conducted under optimal circumstances in which participants are closely monitored for outcomes and frequently reminded about personal hygiene. These findings may not be generalisable to real-life situations. There has been growing concern about the effectiveness of influenza vaccination.[4] In 2011, a systematic review showed low effectiveness (often <60%) of seasonal influenza vaccines at protection of those at the highest risk of severe disease from infection. The Influenza Monitoring Vaccine Effectiveness in Europe Project, funded by the European Centre for Disease Prevention and Control, revealed low early-season effectiveness (43%) of the 2011-12 influenza vaccine. Vaccine effectiveness can only be defined by well-designed observational studies that account for confounding factors. Nonetheless, conclusions about the real-life effectiveness of influenza vaccination in Hong Kong children cannot be made owing to the small sample size and low vaccination rate of participants. Larger population-based surveillance studies are needed to address this public health question.

The Hong Kong Government has included children aged 6 months to 5 years in the influenza vaccination subsidy scheme. Nonetheless, the vaccine uptake rate among local children has not been satisfactory over the past few years. In this study, only 56% of preschoolers had received influenza vaccination within 3 years. Only half of these young children were vaccinated following the 2009 H1N1 influenza pandemic, and the uptake rate dropped to <20% in 2011. Breaking barriers to accept seasonal influenza vaccination should be a public health priority against influenza outbreaks and an integral component of the influenza pandemic preparedness plan.

One limitation of this study was its small sample size. We originally proposed a full-scale project with over 600 preschool and school-age children. Owing to a criticism about the feasibility of serial nasopharyngeal sampling, our study was scaled down and was insufficient to evaluate the transmissibility of influenza within household and school contacts or the effectiveness of influenza vaccination. The sampling method was another limitation. Nasopharyngeal aspirate is the most accurate method for influenza detection; further, it requires suctioning and is not suitable for field studies. NPS is an acceptable alternative for the detection of respiratory viruses, including influenza.[5] Blood was not collected from participants to determine their immune status against influenza. The baseline susceptibility of participants to natural influenza infection was unknown.

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