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Original Article

Epidemiology of pediatric influenza in Hangzhou, China: 2010-2015

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Abstract: In order to analyze the epidemic features of pediatric influenza at a sentinel surveillance local hospital, and provide a scientific basis for disease prevention and control, children with influenza-like illness during 2010-2015 were included. Those who met sampling criteria were sampled with throat swab. Specimens were sent to the Center for Disease Control in Hangzhou for detection of influenza virus using real-time polymerase chain reaction (RT-PCR). During the study period, 4942 throat swab samples were collected, of which 698 samples (14.1%) were positive for influenza virus, 298 (6.0%) positive for seasonal influenza A H3; 287 (5.8%) positive for influenza B and 113 (2.3%) positive for new influenza A H1N1. Influenza A H1N1 incidence was decreased during 2010-2015 after having been epidemic in 2009. Seasonal influenza B and influenza A H3 epidemics occurred alternately. Pediatric influenza had high incidence in the winter and overall low incidence in the summer. However, a seasonal epidemic of influenza A H3 outbreak in July-August 2015 occurred. The incidence of influenza was higher among older children (> 3 years) ($P < 0.01$). We concluded that influenza virus A H3, influenza B virus and influenza H1N1 were epidemic during the monitoring period, with different strains epidemic in different years. Preschool and School-age children were found most likely to be positive.

Keywords: Epidemiology, pediatric, influenza

Introduction

Influenza is a common acute respiratory infection characterized by a short incubation period, rapid spread, and high infectivity [1]. The symptoms can be mild to severe [2]. The most common symptoms include fatigue, sudden onset of high fever, rhinorrhea, sore throat, muscle pains, headache, and cough. Complications of influenza may include viral pneumonia, secondary bacterial pneumonia, sinusitis, and exacerbation of underlying disease, including asthma and heart failure [2-4]. The disease can be fatal. Influenza spreads around the world in a yearly outbreak, resulting in 1/4-1/2 million of deaths [1, 5]. Death occurs mostly in the young, the old and those with underlying health problems. Occasional pandemics occur with particularly high penetration and/or high mortality.

Three types of influenza viruses affect humans: Type A, Type B, and Type C [6]. The high antigenic variability (particularly of Type A), and high population infectivity contribute to the annual global spread. The recurrent annual outbreak is a distinctive epidemiological feature that involves changes in the genetic and antigenic characteristics of the viral surface antigens hemagglutinin (HA) and neuraminidase (NA), which are the main targets for the human immune system [3, 5]. In 2009, a new influenza A virus-H1N1-was identified by the United States Centers for Disease Control in the US and Mexico [7, 8]. H1N1 caused the first worldwide pandemic influenza outbreak of the 21st century. H1N1 isolated from patients was found to be a triple-reassortant influenza virus, containing genes from human, swine, and avian influenza [9].

The goal of influenza surveillance is to provide timely and high-quality data to evaluate the impact and severity of influenza virus each season. Using these data, we can determine the influenza burden, monitor the evolution of influenza viruses, track antiviral susceptibility, and

Table 1. the primers and probes for the real-time PCR

Primers/probes	Genes
FluA-Forward	5'-GAC CRA TCC TGT CAC CTC TGA C-3'
FluA-Reverse	5'-GGG CAT TYT GGA CAA AKC GTC TAC G-3'
FluA-probe	5'-TGC AGT CCT CGC TCA CTG GGC ACG-3'
H1-F247	5'-AAC ATG TTA CCC AGG GCA TTT CGC-3'
H1-R361	5'-GTG GTT GGG CCA TGA GCT TTC TTT-3'
H1-P278	5'-GAG GAA CTG AGG GAG CAA TTG AGT TCA G-3'
H3-F293	5'-ACC CTC AGT GTG ATG GCT TCC AAA-3'
H3-R400	5'-TAA GGG AGG CAT AAT CCG GCA CAT-3'
H3-P384	5'-ACG CAG CAA AGC CTA CAG CAA CTG T-3'
Flu B Forward	5'-TCC TCA ACT CAC TCT TCG AGC G-3'
Flu B Reverse	5'-CGG TGC TCT TGA CCA AAT TGG-3'
Flu B probe	5'-CCA ATT CGA GCA GCT GAA ACT GCG GTG-3'
SWH1 Forward	5'-GGG TAG CCC CAT TGC AT-3'
SWH1 Reverse	5'-AGA GTG ATT CAC ACT CTG GAT TTC-3'
SWH1 probe	5'-TGG GTA AAT GTA ACA TTG CTG GCT GG-3'
AH5 Forward	5'-TGG AAA GTR TAA RAA ACG GAA CGT-3'
AH5 Reverse	5'-YGC TAG GGA RCT CGC CAC TG-3'
AH5 probe	5'-TAC CCG CAG TAT TCA GAA GAA GC-3'
AN1 Forward	5'-TAY AAC TCA AGG TTT GAG TCT GTY GCT TG-3'
AN1 Reverse	5'-ATG TTR TTC CTC CAA CTC TTG ATR GTG TC-3'
AN1 probe	5'-TCA GCR AGT GCY TGC CAT GAT GGC A-3'
AH9 Forward	5'-CAA GCT GGA ATC TGA RGG AAC TTA CA-3'
AH9 Reverse	5'-GCA TCT GCA AGA TCC ATT GGA CAT-3'
AH9 probe	5'-CCC AGA ACA RGA AGG CAG CAA ACC CCA TTG-3'
RNP-Forward	5'-AGA TTT GGA CCT GCG AGC G-3'
RNP-Reverse	5'-GAG CGG CTG TCT CCA CAA GT-3'
RNP-probe	5'-TTC TGA CCT GAA GGC TCT GCG CG-3'

provide recommendations for laboratory diagnostics, vaccines, and risk assessment, including in highly susceptible populations such as children. Influenza infection may be confirmed by sampling the throat, sputum, or nose for the virus. Rapid tests are available; however, a type of polymerase chain reaction that detects the virus's RNA is more accurate. Therefore, in this current surveillance of influenza virus from January 2010 to December 2015 in pediatric patients from an influenza surveillance sentinel hospital in Hangzhou City, we monitored influenza strain changes by RT-PCR, and the results are reported below.

Materials and methods

Patients

The study protocol and standardized data collection form were approved by the Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine. Informed consent was obtained from the patients' legal guardians. In this retrospective descriptive study, we included all children (age ranging 6 months to 14 years) who manifested with fever (≥ 38°C), accompanied by symptoms of acute respiratory infections such as cough or sore throat, who sought diagnosis and treatment at the Department of Pediatrics at an influenza surveillance sentinel hospital-Children's Hospital of Zhejiang University School of Medicine, China, from January 2010 to December 2015.

Sample collection and detection

Specimens collected with a viscose swab were obtained from the throats of patients in the outpatient clinic. The specimens were stored at 4°C and delivered within 24 hour after collection to the Center for Disease Control in Hangzhou, where influenza virus nucleic acid detection, virus isolation and viral genotyping were performed by real-time PCR referred to the protocol of TAKARA kit, and the primers and probes were list in **Table 1**.

Statistical analysis

SPSS 13.0 statistical software was used for statistical analysis. The rates were compared using χ^2 test, and Cochran-Armitage trend test was used to study the trend. All tests were two-tailed and p values below 0.05 were considered significant. 95% confidence intervals were used.

Results

From January 2010 to December 2015, a total of 4942 samples (2628 from males and 2314 from females) were collected from pediatric patients with influenza-like illness (ILI) aged 6 months to 14 years. Influenza virus tested by PCR was positive in 698 samples (14.1%). Among the 698 children with influenza infection, the median age was 4.9 years and 55% were males.

Table 2. Influenza surveillance data in children, 2010-2015

Year	Influenza-like illness	PCR Positive	Positive rate (%)
2010	870	160	18.4
2011	557	70	12.6
2012	593	95	16.0
2013	824	79	9.6
2014	1044	147	14.1
2015	1054	147	13.9
Total	4942	698	14.1

Table 3. Pathogens in pediatric influenza, 2010-2015

Year	Positive cases	Influenza A H3	Influenza A H1	Influenza B
2010	160	59	8	93
2011	70	3	38	29
2012	95	53	0	42
2013	79	57	21	1
2014	147	51	25	70
2015	127	75	21	52
Total	698	298	113	287

The positive rates for annual influenza surveillance varied, as is shown in **Table 2**. In the fall/winter of 2009, 298 cases (6.0%) were positive for seasonal influenza A H3, 287 (5.8%) positive for influenza B virus, and 113 cases (2.3%) positive for influenza A subtype H1N1- at the start of the new influenza H1N1 epidemic.

The identification of influenza virus from patients was shown in **Table 3**. After the epidemic of influenza H1N1 in the fall/winter of 2009, the influenza A H1N1 incidence decreased during 2010-2015, peaking only in January 2011 and December 2015. Seasonal influenza A H3 and influenza B were found to be epidemic alternately during this period. The influenza A H3 epidemic presented differently from that of influenza B or the new influenza A H1N1. Influenza A H3 peaked in the summer (a positive rate of 43.8% in July 2015, the highest in nearly six years), with an additional small epidemic peaks in September 2010, March and August 2012, and December 2015.

Influenza A H1N1 and influenza B reached epidemic peaks mainly in winter and spring, and very rarely in summer. Influenza B epidemic did not occur in 2013.

Table 4. PCR positivity in influenza-like illness: seasonal surveillance data of pediatric influenza, 2010-2015

Year	Spring (Mar-May)	Summer (Jun-Aug)	Fall (Sep-Nov)	Winter (Dec-Feb)
2010	43/271[a]	13/258	44/152	51/142
2011	11/144	0/149	4/129	46/129
2012	27/149	26/155	5/150	12/144
2013	12/182	0/200	21/259	94/262
2014	31/260	18/260	19/262	64/260
2015	10/265	67/269	5/260	98/264
Total	134/1271	124/1291	98/1212	365/1201

[a]: PCR positive cases/total influenza like illness cases.

Table 5. Influenza surveillance data for different age groups in 2010-2015

Age group	Influenza-like illness	RT-PCR Positive	Positive rate (%)
< 3 years	3325	341	10.3
3-6 years	1052	212	20.3
> 6 years	565	145	25.7
Total	4942	698	14.1

Cochran-Armitage trend test, $Z = 11.57$, $P < 0.01$.

The seasons in Hangzhou City are divided based on the weather: spring (March-May), summer (June-August; hottest season), fall (September-November), and winter (December-February; coldest season). Among patients with influenza-like illness, influenza virus was identified at the highest rates mainly in winter: as high as 24.6%-40.8% (**Table 4**). This percentage was greater compared to other seasons (winter *vs.* spring: $\chi^2 = 150.99$, $P < 0.01$; winter *vs.* summer: $\chi^2 = 170.45$, $P < 0.01$; winter *vs.* autumn: $\chi^2 = 193.56$, $P < 0.01$), indicating that winter/spring seasons were dominant flu seasons in Hangzhou (**Table 4**).

We also compared the positive rates of influenza virus detection in different age groups. The trend of influenza virus positive rates in all age groups were analyzed using the Cochran-Armitage trend test ($Z = 11.57$, $P < 0.01$). The results indicated that the positive rate of influenza increased with increasing age (**Table 5**). During the epidemic seasons, among pediatric patients manifesting with fever and influenza-like illness, children aged six or older have more probability of being provably infected with influenza.

Discussion

Influenza A virus is a member of the Orthomyxoviridae family with a negative-sense single-stranded RNA genome in the cytoplasm [10]. Resulting from the fact that the influenza polymerase has no proofreading activity, replication of viral genome results in a high gene mutation rate of approximately one error per replicated genome [11], and such antigenic mutations help influenza evade the host's adaptive immune recognition and clearance [12]. The mutated virus obviates herd immunity, repeatedly and effectively breaking through the immune barriers in the population, causing epidemics ranging from local outbreaks to worldwide pandemics. The epidemic outbreaks cause varying degrees of excess mortality and heavy economic burdens. Influenza is the first infectious disease target of global monitoring, allowing for some control annual influenza epidemics and providing an important basis for an influenza pandemic alarm.

Influenza epidemic is characterized by sudden outbreak, rapid and wide spreading, and seasonal fluctuation. Children are the most susceptible to respiratory infections, including influenza. Domestic surveillance data on pediatric influenza in China are rare. This 72-month continuous surveillance shows a positive detection rate of 14.1% of pediatric influenza in children in Hangzhou presenting with influenza-like illness. In the flu seasons, the positive detection rate rose as high as 40%-63.8%. Surveillance data from western countries has shown that, in the flu seasons, influenza was found in 10% to 30% of children presenting to outpatient and emergency department for influenza-like illness. Our surveillance data also demonstrates that 43.9%-79.2% of influenza-like illness in children resulted from infection with the new influenza A H1N1 when the new influenza A H1N1 was pandemic in 2009 in Hangzhou. The affected children were mostly preschool and school-age children, which may result from over-population of children at school, which therefore serves as a nexus of viral vectors from which local outbreaks are initiated.

Hangzhou is located in the northern subtropical zone, and the weather is warm and humid, with four distinct seasons, characterized by wet spring, hot and humid summer, cool autumn and dry and cold winter, a season for high incidence of respiratory diseases including influenza in children. The continuous 72 months surveillance and analysis of influenza found winter with the overall highest incidence of RT-PCR documented influenza, with positive rates of different types of influenza virus varying season-to-season. Influenza A H1N1 and influenza B epidemic mainly occurred in winter and spring, and rarely in summer. Influenza A H3 epidemic showed distinctive differences from influenza B and influenza A H1N1, as it was usually epidemic in the summer. It also showed a positive rate of 43.8% in July 2015, the highest in the six years monitored. This is possibly resulted from the relatively low average temperature in Hangzhou in July 2015 compared to previous years.

International surveillance on influenza outbreaks has demonstrated that 15% to 20% of children were infected with influenza annually. Our study found that the positive rates of children testing positive for influenza increased with age, suggesting that children at 3 years old or older (preschool and school-age) were more likely to be tested positive for influenza. After the influenza epidemic in fall/winter 2009 [13], the incidence of influenza A H1N1 decreased in 2010 through 2015, possibly resulting from the massive vaccination effort against influenza A H1N1 after the pandemic in 2009. The key strategy for preventing influenza in children is vaccination. Children above 3 years old (preschool and school-age) are at high risk of influenza, and therefore should be vaccinated before flu season. Improved childhood vaccination coverage at preschools and schools appears to be beneficial [14], and may serve to effectively reduce outbreaks and epidemics. Therefore, rational use of antiviral drugs and timely vaccination of targeted influenza vaccine for children appear to be important measures to reduce influenza morbidity and mortality. We also recommend that parents and public health agencies in the region work to enhance monitoring of influenza, augment health education, and increase prevention awareness.

In summary, we monitored epidemic influenza in Hangzhou over a six-year period and our data suggests that influenza epidemic is seasonal, and mainly in winter. Children above 3 years of age are at high risk of influenza. The main strategy for influenza prevention is to improve influenza surveillance, timely isolation and control

of the disease, and active promotion of influenza vaccination targeted to the likely epidemic strains. Influenza vaccination of children at school and daycare centers should be recommended before the schools starting in fall.

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Disclosure of conflict of interest

None.

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References

[1] From the Centers for Disease Control and Prevention. Update: influenza activity-United States, 2002-03 season. JAMA 2003; 289: 37-39.

[2] Williams L. Influenza and the flu vaccine (continuing education credit). Kans Nurse 1996; 71: 6; quiz 7.

[3] Stamboulian D, Bonvehi PE, Nacinovich FM and Cox N. Influenza. Infect Dis Clin North Am 2000; 14: 141-166.

[4] Chowell G, Bertozzi SM, Colchero MA, Lopez-Gatell H, Alpuche-Aranda C, Hernandez M and Miller MA. Severe respiratory disease concurrent with the circulation of H1N1 influenza. N Engl J Med 2009; 361: 674-679.

[5] Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, Bresee JS, Cox NS; Centers for Disease Control and Prevention (CDC); Advisory Committee on Immunization Practices (ACIP). Prevention and control of influenza: recommendations of the advisory committee on immunization practices (ACIP), 2008. MMWR Recomm Rep 2008; 57: 1-60.

[6] Dhama K, Verma AK, Rajagunalan S, Deb R, Karthik K, Kapoor S, Mahima, Tiwari R, Panwar PK and Chakraborty S. Swine flu is back again: a review. Pak J Biol Sci 2012; 15: 1001-1009.

[7] Centers for Disease Control and Prevention (CDC). Swine influenza A (H1N1) infection in two children--southern california, march-april 2009. MMWR Morb Mortal Wkly Rep 2009; 58: 400-402.

[8] Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quinones-Falconi F, Bautista E, Ramirez-Venegas A, Rojas-Serrano J, Ormsby CE, Corrales A, Higuera A, Mondragon E, Cordova-Villalobos JA; INER Working Group on Influenza. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med 2009; 361: 680-689.

[9] Centers for Disease Control and Prevention (CDC). 2009 pandemic influenza A (H1N1) virus infections-Chicago, Illinois, April-July 2009. MMWR Morb Mortal Wkly Rep 2009; 58: 913-918.

[10] Noah DL and Krug RM. Influenza virus virulence and its molecular determinants. Adv Virus Res 2005; 65: 121-145.

[11] Malpica JM, Fraile A, Moreno I, Obies CI, Drake JW and Garcia-Arenal F. The rate and character of spontaneous mutation in an RNA virus. Genetics 2002; 162: 1505-1511.

[12] Price GE, Ou R, Jiang H, Huang L and Moskophidis D. Viral escape by selection of cytotoxic T cell-resistant variants in influenza A virus pneumonia. J Exp Med 2000; 191: 1853-1867.

[13] Chen Y, Qiao H, Zhang CM, Tong M and Shang S. Risk factors for prolonged shedding of 2009 H1N1 influenza virus. Indian Pediatr 2011; 48: 961-963.

[14] van Essen GA, Palache AM, Forleo E and Fedson DS. Influenza vaccination in 2000: recommendations and vaccine use in 50 developed and rapidly developing countries. Vaccine 2003; 21: 1780-1785.