SURVEILLANCE OF INFLUENZA VIRUS DURING 2010-2012 IN CHANGSHA, CHINA

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Abstract. The objective of this study was to examine the circulating influenza viruses in Changsha, China, during 2010-2012. Nasopharyngeal specimens were collected from persons with influenza-like illness (ILI) who presented for care at two hospitals. Of 2,955 patients tested, 278/(9.4%) were positive for influenza virus: 116/(41.7%) were influenza type A(H3N2), 79/(28.4%) were type A(H1N1) pandemic 2009 (pdm09) and 83/(29.9%) were influenza type B. The rates of virus detection varied by age and sex. The highest rate was in the 5-14 year old age group and females were infected more than males. After the initial 2009 A(H1N1) pdm09 outbreak, the number of cases of this virus declined and the season become shorter. Influenza A(H3N2) and B viruses occurred mainly during the spring and summer, while influenza A(H1N1)pdm09 occurred mainly during the winter and spring. Influenza A(H1N1)pdm09 replaced the usual seasonal H1N1 virus during 2010-2012. Continuing epidemiological surveillance of influenza virus is important to monitor trends in influenza infections and to develop prevention and control measures.

Keywords: influenza virus, epidemiology, surveillance, China

INTRODUCTION

Influenza is a common respiratory disease which increases mortality. Seasonal influenza creates a heavy health care and economy burden (Simonsen, 1999; Clem and Galwankar, 2009). Influenza is the only globally monitored infectious disease (Kitler *et al*, 2002). Understanding the epidemic patterns of influenza is critical. Surveillance information is needed to determine seasonal epidemics and predict future pandemics, to help guide vaccination programs and evaluate diagnostic tests and antiviral drugs.

In late March and early April 2009, a novel influenza virus [influenza A(H1N1) pdm09] of swine origin emerged among humans in Mexico and the USA and spread rapidly worldwide, prompting the WHO to declare an influenza pandemic (Dawood *et al*, 2009; WHO, 2009). In Changsha, China, the first case of influenza A(H1N1)pdm09 was reported in May 2009. The epidemic in Changsha peaked during late October and November, then decreased rapidly (Xiao *et al*, 2011). On 10 August 2010, the WHO declared the influenza A(H1N1)pdm09 had largely run its course and the world was moving into

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the post-pandemic period (WHO, 2010). Little information is available about the epidemiology of the A(H1N1)pdm09 in Changsha; therefore, we examined the epidemiological data for influenza during 2010-2012.

MATERIALS AND METHODS

Patient selection and sample collection

Influenza-like illness (ILI) was defined as sudden onset fever (≥38ºC) and cough or sore throat, with or without other symptoms, such as headache, malaise, or muscle aches. Nasopharyngeal swab specimens were obtained from patients with ILI at two sentinel hospitals [Changsha Central Hospital (CCH) and the First Hospital of Changsha (FHC)]. The specimens were transported to the laboratory in viral transport media. Demographic data were obtained for each patient.

Viral identification

Monolayer Madin-Darby canine kidney (MDCK) cells were used to isolate virus in 2,743 specimens and cytopathic effects (CPE) were checked daily for 10 days. All cultures were screened with a hemagglutination (HA) test to detect the presence of influenza virus. Influenza virus subtypes were further identified with the HA inhibition (HI) test. The reference antisera provided by China CDC were: goat anti-A/Tianjin-Jinnan/15/2009 (H1N1) serum, goat anti-A/Fujian-Tongan/196/2009 (H3N2) serum, goat anti-A/Sichuan/SWL1/2009 serum, goat anti-B/Jiangxi-Xinjian/39/2008(Victoria) serum and goat anti-B/Shaixi- Beilin/127/2008 (Yamagata) serum used in 2010; goat anti-B/ Chongqin-Yuzhong/1384/ 2010 (Victoria) serum and goat anti-B/ Sichuan-Anyue/139/2011 (Yamagata) serum used in 2011 and 2012. A HI titer≥20 or a fourfold titer increase between the HA inhibited antiserum and other types of antisera was considered diagnostically positive for that influenza type/subtype.

Two hundred twelve severe cases were detected by one-step real-time reverse transcription-polymerase chain reaction on Applied Biosystems platform ABI 7300 following the recommended China CDC protocol/(Cui *et al*, 2012). Virus RNA extractions were performed using the QIAmp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The assay was performed using the SuperScript® Platinum®One-Step qRT-PCR kit (Invitrogen, Carlsbad, CA). Briefly, 25 µl PCR mixture containing 0.5 µl of each probe, forward and reverse primers, 1 µl enzyme mix, 12.5 µl of 2x master mix, 5 µl of nuclease-free water and 5 µl of extracted RNA was amplified under the following conditions: 1 cycle of 50ºC for 30 minutes, followed by 95ºC for 10 minutes and 45 cycles at 95ºC for 15 seconds and 55ºC for 45 seconds.

Data analysis

We classified the positive cases into different age groups. We used the χ^2 test. A p-value < 0.05 was considered statistically significant. We used the 0-4 year old age group as the reference group.

RESULTS

General findings

Samples were collected from 2,955 patients with ILI at the two sentinel hospitals during the study period of 2010-2012. Fifty-three point two percent of patients were males and 46.8% were females. The gender distribution at the two sites were similar. Patients ranged in age from 1 month to 90 years with a mean age of 25.4

Table 1
Study population at two sentinel hospitals in Changsha, China.

Characteristics of population	CCH No. (%)	FHC No. (%)	Total No. (%)
Number of cases tested	1,554	1,401	2,955
Gender			
Female	703 (45.2)	681 (48.6)	1384 (46.8)
Male	851 (54.8)	720 (51.4)	1571 (53.2)
Age			
Mean (years)	20.1 ±18.7	31.2 ± 20.9	25.4 ± 20.6
Median (range in years)	19 (1mo-90)	26 (1mo-88)	23 (1mo-90)
0-4	500 (32.2)	147 (10.5)	647 (21.2)
5-14	193 (12.4)	116 (8.3)	309 (10.5)
15-24	320 (20.6)	369 (26.3)	689 (23.3)
25-59	469 (30.2)	592 (42.3)	1,061 (35.9)
≥60	72 (4.6)	177 (12.6)	249 (8.4)

CCH, Changsha Central Hospital; FHC, the First Hospital of Changsha.

years (SD = 20.6 years) and a median age of 23 years. Thirty-five point nine percent were aged 25-59 years, 23.3% were aged 15-24 years and 21.2% were aged <5 years. Ten point five percent of patients were aged 5-14 years and 8.4% were aged ≥60 years. Patients who presented to FHC had a significantly greater mean age (31.2 ± 20.9 years) than those who presented to CCH (20.1±18.7 years) (Table 1). The virus type and infection rates varied by sex and age (Table 2). More females (10.6%) were infected than males (8.3%)(p=0.034). The virus detection rate in the 5-14 year old age group was the highest (16.5%, p<0.001) followed by the 25-59 year old age group (9.6%); the rates were less than 10% in each of the remaining age groups.

The distribution of influenza virus subtypes in the different age groups was significantly different (χ^2=44.80, p<0.001). Persons aged 25-59 years (3.7%) had the highest detection rate of influenza A(H1N1)pdm09; persons aged ≥60 years (6.4%) had the highest detection rate of influenza A(H3N2); persons aged 5-14 years (9.4%) had the highest detection rate of influenza B virus. The seasonal influenza H1N1 virus which circulated before the 2009 pandemic was not observed during the period of study.

Laboratory results

Of the 2, 955 samples obtained, 278/ (9.4%) tested positive for influenza virus (Table 2): 195 (70.1%) tested positive for influenza A and 83 (29.9%) tested positive for influenza B. Of the 195 influenza A specimens, 116 (59.5%) were seasonal influenza type A(H3N2) and 79/(40.5%) were A(H1N1)pdm09. Of the 83 influenza B specimens, 68 (81.9%) were influenza B type Victoria and 15 (18.1%) were type Yamagata.

Temporal distribution

Fig 1 shows the temporal distribution of ILI cases and the viral etiologies. During 2010, the number of ILI cases decreased gradually. It peaked in February 2010 and stayed at a relatively low level during 2011 and 2012. The prevalences of influenza virus subtypes varied by sea-

Table 2
Age distribution of patients infected with different influenza virus subtypes.

Characteristics	2,955 patients tested			Distribution of influenza subtypes					
	Positive (n=278)	Negative (n=2,677)	p-value	A (n=195)	A/(H1N1)pdm09 (n=79)	A/(H3N2) (n=116)	B (n=83)	B/(Victoria) (n=68)	B/(Yamagata) (n=15)
Gender									
Female	147 (10.6%)	1,237 (89.4%)	0.034	97 (7.0%)	46 (3.3%)	51 (3.7%)	50 (3.6%)	40 (2.9%)	10 (0.7%)
Male	131 (8.3%)	1,440 (91.7%)		98 (6.2%)	33 (2.1%)	65 (4.1%)	33 (2.1%)	28 (1.8%)	5 (0.3%)
Age (years)									
0-4	52 (8.0%)	595 (92.0%)	-	40 (6.2%)	11 (1.7%)	29 (4.5%)	12 (1.9%)	11 (1.7%)	1 (0.2%)
5-14	51 (16.5%)	258 (83.5%)	0.001	22 (7.1%)	8 (2.6%)	14 (4.5%)	29 (9.4%)	23 (7.4%)	6 (2.0%)
15-24	51 (7.4%)	638 (92.6%)	0.664	30 (4.4%)	17 (2.5%)	13 (1.9%)	21 (3.0%)	16 (2.3%)	5 (0.7%)
25-59	102 (9.6%)	959 (90.4%)	0.270	83 (7.8%)	39 (3.7%)	44 (4.1%)	19 (1.8%)	16 (1.5%)	3 (0.3%)
≥60	22 (8.8%)	227 (91.2%)	0.697	20 (8.0%)	4 (1.6%)	16 (6.4%)	2 (0.8%)	2 (0.8%)	0 (0)

χ^2 test, subjects aged 0-4 years used as reference group.

son. Influeza A(H1N1)pdm09 circulated mainly during the winter and spring: from November 2010 to March 2011; Influenza A(H3N2) circulated mainly during the spring and summer: from September to December 2010 and February to September 2012; Influenza B circulated mainly during the Spring: from January to April during 2010-2012 where types Victoria and Yamagata circulated conventionally.

DISCUSSION

Since the year 2000, the People's Republic of China has had a nationwide surveillance network for influenza, which since 2005 has been reported on the Chinese Center for Disease Control and Prevention website (www.cnic.org.cn/ch/) (Huang *et al*, 2008). The influenza laboratory for the Center for Disease Control and Prevention of Changsha (Changsha CDC) joined the national influenza monitoring network in October 2005 and was designated as an approved laboratory for diagnosing influenza virus infection in Changsha (Liu *et al*, 2006). Changsha, the capital of Hunan Province, is located in the south central part of the Chinese mainland and has a subtropical climate. Surveillance data for southern China show influenza is prevalent throughout the year; it had a peak during the summer and a less pronounced peak during the winter prior to the 2009 pandemic (Shu *et al*, 2010; Zou *et al*, 2013). To understand the activity and seasonal patterns of influenza virus in Changsha, we examined the epidemiological data collected during 2010-2012.

During 2009, influenza A(H1N1) pdm09 virus altered the pattern of seasonal influenza (Patel *et al*, 2010; Laguna-Torres *et al*, 2010). In Changsha, influenza A(H1N1)pdm09 spread throughout the

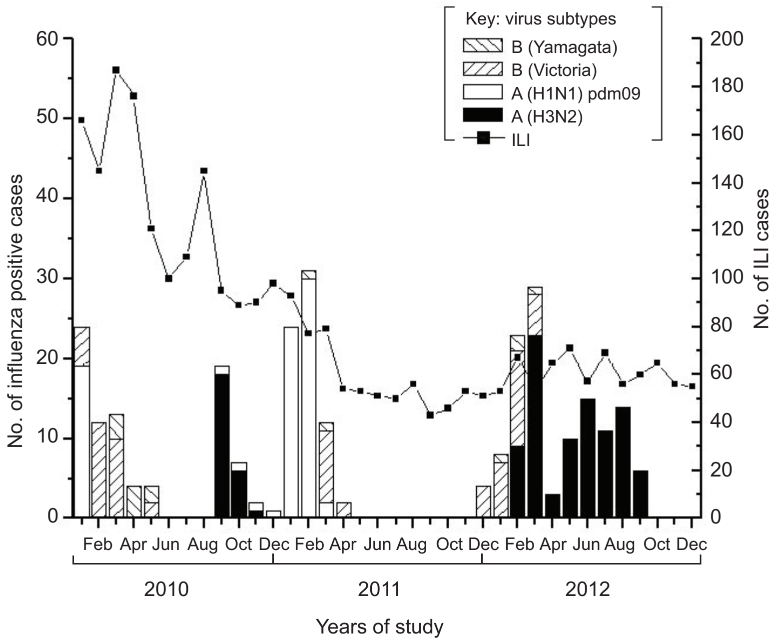

Fig 1–Monthly distribution of influenza-like illness and influenza virus positive patients.

city resulting in a large outbreak from August 2009 to January 2010 (Fig 1). The prevalence and duration of influenza A(H1N1)pdm09 during 2010-2011 was significantly lower and shorter, similar to other cities in China (Li *et al*, 2011; Yang *et al*, 2012). Interestingly, the usual seasonal influenza H1N1 virus, predominant subtype before the 2009 epidemic, disappeared completely thereafter. We conclude the influenza A(H1N1)pdm09 replaced the usual seasonal influenza H1N1 virus in Changsha, China.

During 2010-2011, influenza B and A(H3N2) circulated during the spring and summer, respectively, while A(H1N1) pdm09 recirculated during the winter and spring but then disappeared. During 2011-2012, influenza A(H3N2) became the predominant circulating strain. Influenza A(H1N1)pdm09 occurred mainly during the winter and spring, influenza A(H3N2) occurred mainly during the spring and summer and influenza B occurred mainly during the spring. Different seasonal distributions of seasonal influenza and influenza A(H1N1)pdm09 have been reported by other studies (Haddock *et al*, 2010; Brammer *et al*, 2011).

The prevalence of influenza A (6.6%) and influenza B (2.8%) in our study are similar to those seen in Korea, Thailand and Guangzhou, China (Li *et al*, 2011; Kanchana *et al*, 2012; Song *et al*, 2013). Our finding of the Victoria type of influenza B virus as the most common type (81.9%) is similar to a study from Japan (Dapat *et al*, 2012), but different from Hong Kong (Chan *et al*, 2013) and Taiwan (Lo *et al*, 2013) where Yamagata was the most common type.

The influenza virus subtypes varied by age. School age children had the highest prevalence of influenza (Table 2). Persons aged ≥60 years had the greatest prevalence of influenza A(H3N2); whereas persons aged 25-59 years had the greatest prevalence of influenza A(H1N1) pdm09. Influenza A(H1N1)pdm09 had different epidemiological characteristics when it occurred during the pandemic compared to when it occurred seasonally (Chuang *et al*, 2012; Guiomar *et al*, 2012; Borja-Aburto *et al*, 2012).

Our study had limitations worth noting. Data collection occurred at only two hospitals, so this study cannot be generalized to the whole population. The low virus detection rates might be attributable to variations in the skill of the staff who collected the specimens. Despite the aforementioned limitations, our study is important, because it documents the circulation of seasonal and pandemic influenza in Changsha during 2010-2012.

The surveillance data suggested influenza infection was less prevalent and had a shorter season after the 2009 pandemic. The influenza A(H1N1)pdm09 virus probably replaced the usual seasonal influenza H1N1. The epidemic characteristics of influenza A(H1N1)pdm09 and seasonal influenza virus were different. These need to be monitored continuously for changes in the epidemiology of influenza virus.

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