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Prevalence of Seasonal Influenza Viruses and Pandemic H1N1 Virus in Beijing from 2008 to 2012

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In northern China, influenza circulates on a seasonal and regular basis during the winter-spring season [1]. Our study was conducted in Beijing between November 2008 and March 2012, specifically from November 2008 to March 2009 (period 1), from November 2009 to March 2010 (period 2), from November 2010 to March 2011 (period 3), and from November 2011 to March 2012 (period 4), in order to evaluate the annual incidence rates of influenza and to identify the circulating viral types and subtypes for facilitating the local vaccination programs and regional influenza control.

Virological prevalence, the subject of the surveillance, was defined based on the influenza-like illnesses (ILIs) as follows: a temperature of ≥38℃, either cough or sore throat, and no laboratory-confirmed evidence of another disease in patients who presented at the Fever Outpatient Clinic Department of the sentinel hospitals. Over the 4 yr, 6,397 throat swab samples from outpatients with ILIs were collected and tested. The ages of outpatients ranged between 6 months and 91 yr (median, 32 yr; mean, 37.1 yr). Specimens were collected from both female (n=3,338; 52.18%) and male (n=3,059; 47.82%) patients. Total RNA was extracted from 100 μL of each sample using QIAmp Viral RNA Mini kit (QIAGEN, Valencia, CA, USA); subsequently, they were analyzed by real-time (RT) PCR methods for influenza viruses, as recommended by the Chinese National Influenza Center, including seasonal influenza viruses such as FluA(H1N1), FluA(H3N2), FluB, and pdmH1N1 under the same testing conditions and procedures with the exception of the respective primers and probe, i.e., FluA(H1N1)-F, AACATGTTACCCAGGGCATTTCGC; FluA(H1N1)-R, GTGGTTGGGCCATGAGCTTTCTTT; FluA(H1N1)-P, GAGGAACTGAGGGAGCAATTGAGTTCAG; FluA (H3N2)-F, ACCCTCAGTGTGATGGCTTCCAAA; FluA(H3N2)-R, TAAGGGAGGCATAATCCGGCACAT; FluA(H3N2)-P, ACGCAGCAAAGCCTACAGCAACTGT; FluB-F, TCCTCAACTCACTCTTCGAGCG; FluB-R, CGGTGCTCTTGACCAAATTGG; FluB-P, CCAATTCGAGCAGCTGAAACTGCGGTG; pdmH1N1-F, GGGTAGCCCCATTGCAT; pdmH1N1-R, AGAGTGATTCACACTCTGGATTTC; and pdmH1N1-P, TGGGTAAATGTAACATTGCTGGCTGG. Real-time (RT) PCR was performed using AgPath-ID™ One-Step RT-PCR Kit (Applied Biosystems International, Foster City, CA, USA) with an ABI Prism 7500 Taqman machine (Applied Biosystems International). The reaction was conducted at a total volume of 25 μL containing 12.5 μL of 2×RT-PCR buffer, 1 μL of 2×RT-PCR enzyme, 1.67 μL of detection enhancer, 400 nM of each primer, 200 nM of probe, 3.33 μL of double distilled water (ddH_2O), and 5 μL of template. Optimized amplification conditions were as follows: 1 cycle of 50℃ for 30 min, followed by 10 min at 95℃, and 45 cycles of 15 sec at 95℃ and 45 sec at 55℃.

Influenza viruses were detected in 6,397 clinical samples of outpatients with ILIs at peak times, with varying compositions of influenza numbers. Fluctuating trends were observed in Beijing, China, over the 4 continuous periods. The results of prevalence of common seasonal influenza are summarized in Fig. 1. From

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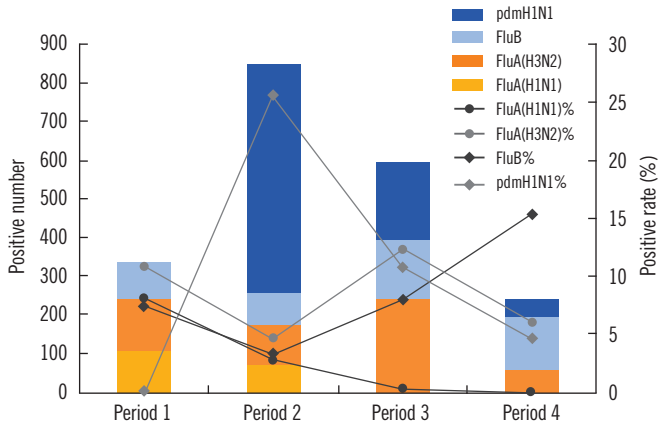

Fig. 1. Virological surveillance of influenza during the 4 periods. Bars show the positive number of different seasonal influenza viruses and lines indicate the positive rate of different seasonal influenza viruses detected in 4 periods.

period 1 to period 4, the positive prevalence rate of FluA(H1N1) decreased sharply year by year (period 1, 8.12%; period 2, 2.9%; period 3, 0.32%; and period 4, 0%), especially for period 4, where no positive case of FluA(H1N1) was recorded. Conversely, pdmH1N1 gradually replaced FluA(H1N1) from the start of the 2009 epidemics (period 1, 0%; period 2, 25.64%; period 3, 10.71%; and period 4, 4.65%). FluA(H3N2) and FluB also present fluctuating changes in the positive detection rate of the surveillance; they are the predominant viral members of seasonal influenza due to the principle of dominance by competitive circulation, whereby 1 type or subtype of seasonal influenza virus becomes the predominant form while the other types and subtypes of seasonal influenza virus play a secondary role. The predominant positive detection rates over the 4 periods were: FluA(H3N2), 10.88%; pdmH1N1, 25.64%; FluA(H3N2), 12.39%; and FluB, 15.37%. Especially in period 2, the pandemic H1N1 virus has the capacity to suppress seasonal influenza A virus by competitive circulation with multiple factors such as low preexisting immune capacity and stronger infectivity. Conversely, the seasonal peak time was recorded slightly later than the peak time of the previous period. In winter, period 1 and period 2 were the main peak time of seasonal influenza, while in spring it peaked during periods 3 and 4. There were significant differences in the positive rate of surveillance for the same seasonal influenza virus between the 4 detection periods ($P<0.01$) as well as significant differences for the positive rate of surveillance for 4 types/subtypes of seasonal influenza viruses in the same detection period ($P<0.01$).

Influenza surveillance is an important step in understanding the epidemiology and virology of influenza, while the collection of comprehensive data on the burden of influenza is vital for guiding policy decisions about prevention and control of the influenza virus [2-4]. Our findings propose that vaccination against seasonal influenza should be enhanced; local surveillance advocates the October of every year as the optimal time for influenza vaccination in Beijing in order to ensure maximum level of protection during the seasonal peak times. On the other hand, virology data from Beijing can certainly contribute to a better understanding of the evolution of seasonal influenza viruses globally and is of a major interest, especially for the control of new emerging influenza strains [5-7].

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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