

Emergency surveillance of influenza during 2009 in the Chinese city of Qingdao

Zhao-Guo Wang,^{a,b} Ying Yi,^a Ting-Ting Yang,^a Xiao-Lin Liu,^a Fa-Chun Jiang,^a Zhi-Yu Wang,^b Ji-Ming Chen^c

^aQingdao Center for Disease Control and Prevention, Qingdao, China. ^bCollege of Public Health, Shandong University, Shandong, China. ^cChina Animal Health and Epidemiology Center, Qingdao, China.

Correspondence: Zhi-Yu Wang, College of Public Health, Shandong University, Shandong, China. E-mail: zhiyu.wang@sdu.edu.cn; Ji-Ming Chen, China Animal Health and Epidemiology Center, Qingdao, China. E-mail: jmchen66@yahoo.cn

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Background In April, 2009, a new influenza pandemic caused by a swine-origin H1N1 subtype influenza virus was imminent. We thereby carried out an emergency surveillance study in a Chinese city of Qingdao.

Methods Pharyngeal swab samples were collected from four targeted groups and tested by reverse-transcription polymerase chain reaction. Each laboratory-confirmed pandemic H1N1 case or cluster was investigated, and the hemagglutinin genes of some of the viruses were sequenced and analyzed.

Results A total of 140 pandemic H1N1 cases including 92 from 7 clusters were identified in the four targeted groups. None of them developed into severe infections. Meanwhile, 103 cases of seasonal

influenza (98 H3N2 and 5 H1N1) and 10 clusters of seasonal H3N2 influenza were also identified. Among them, 38 pandemic H1N1 and two seasonal H3N2 influenza cases were air travellers, suggesting that air travel facilitates the spread of pandemic and seasonal influenza even in the northern hemisphere summer. In addition, it was found that pandemic H1N1 and seasonal H3N2 influenza viruses co-circulated in two clusters. No significant mutations were found in the hemagglutinin gene sequences of pandemic H1N1 viruses, but the seasonal H3N2 influenza viruses have become genetically distinguishable from those circulating in 2007–2008.

Keywords Epidemiology, influenza virus, pandemic, surveillance.

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Introduction

On April 24, 2009, human infections with a swine-origin H1N1 subtype influenza virus in the USA and Mexico were reported by the World Health Organization (WHO). Within a few days, the virus spread to other countries through international air travel.^{1–3} Therefore, a new influenza pandemic caused by the virus, designated as pandemic H1N1 virus, was imminent at the end of April, 2009.

To prevent the virus from spreading into China and to mitigate the severity of the coming pandemic, each Chinese city immediately carried out comprehensive prophylactic measures including emergency surveillance and isolation of confirmed cases. This report describes an emergency surveillance study carried out in Qingdao, a beach city in Shandong province of China (Figure 1). The city covers the urban, suburban areas and five county-level cities. Every year many people from South Korea, Japan and other countries visit the city for economic, cultural or other reasons.

On April 30, 2009, at the direction of the central and provincial government, the city (municipal) government established the emergency surveillance system, which consisted of the health section of the city government, the city Center for Disease Control and Prevention (CDC) and its branches at the county level, the city airport and 3 sentinel hospitals (Qingdao Haici Hospital, Qingdao Children Hospital and Jiaozhou Central Hospital). The health section of the city government was the leader, coordinator and financial supporter of the system. The city CDC was responsible for testing the samples and providing technical support for its branches. It was also responsible for monitoring the international and national pandemic situation. Its branches were responsible for collecting samples from influenza-like illness (ILI) clusters reported to them and investigating all the confirmed pandemic H1N1 cases within their jurisdiction. The sentinel hospitals were responsible for collecting a certain amount of samples each week from the patients with ILI treated in the hospitals. The health quarantine department of the city airport was responsible for

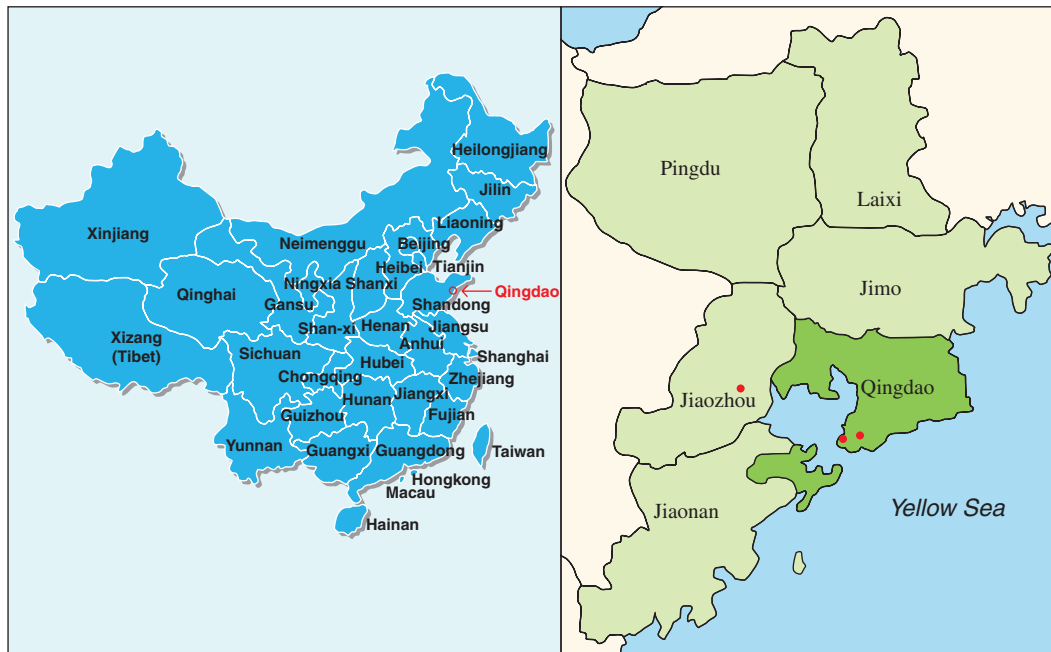


Figure 1. The map of the city of Qingdao. Left: the site of Qingdao in a map of China; right: the map of Qingdao (the area shaded in dark green is the city proper, and the area shaded in light green is the five county-level cities subordinate to Qingdao). The sites of the three sentinel hospitals are marked with red circles.

identifying travellers with fever and collecting samples. All of the samples were submitted to the city CDC for testing.

The emergency surveillance system was responsible for monitoring the international and national pandemic situation and to identify and investigate influenza cases (esp. pandemic H1N1 cases) using limited resources. Therefore, cases considered to have a high probability of influenza were targeted. In the first 5 months, all the identified pandemic H1N1 cases were isolated to minimize the rate of spread in the city.

Methods

Situation monitoring and data comparison

Raw epidemiological data about influenza A (H1N1) cases in China and in the world were collected from the official websites of WHO (<http://www.who.int>), the Ministry of Health of China (<http://www.moh.gov.cn>), China CDC (<http://www.chinacdc.cn>) and some newspapers. The national and worldwide data as well as relevant analysis were updated daily during the period of the emergency surveillance on the city CDC website (<http://www.qdcdc.org/>).

Case or cluster definition

An ILI case was defined as a person with sudden onset of fever of $>38^{\circ}\text{C}$ and cough or sore throat in the absence of other diagnoses. An ILI cluster was defined as 5 or more ILI cases emerging within a period of 7 days in the

same geographic area, such as a kindergarten, school or factory. A laboratory-confirmed influenza case was defined as one detected as positive using the real-time reverse-transcription polymerase chain reaction (RT-PCR) described in later section. A severe influenza case was defined as an influenza case with temperature $>39^{\circ}\text{C}$ for more than 3 days or symptoms such as pneumonia requiring hospital admission. These definitions were in the national pandemic influenza surveillance plan issued by the Ministry of Health of China on May 12, 2009.

Targeted groups of surveillance

Four groups were selected as the emergency surveillance targets: (i) passengers coming into Qingdao by airplane with temperature higher than 37.0°C detected by a digital infrared temperature scanner (Huazhong Digital Control, Wuhan, China), and all positives were further checked manually with a thermometer, if needed; (ii) patients with ILI treated at the three sentinel hospitals; (iii) patients of any ILI clusters which should be reported to the city CDC within 2 hours after the cluster was observed; (iv) close contacts of the confirmed pandemic H1N1 cases.

Surveillance time

The emergency surveillance was started on May 1 and stopped on September 30, but routine influenza surveillance targeting patients with ILI from the three sentinel hospitals and ILI clusters continued.

Sample collection and detection

Pharyngeal swab samples were collected from each patient within the first, third and fourth targeted groups. At each of the three sentinel hospitals, 15–20 pharyngeal swab samples were collected randomly each week from the second targeted group. All samples were tested using a commercial real-time RT-PCR kit (Jinhao, Beijing, China), which was able to detect influenza A virus of all subtypes and pandemic H1N1 virus simultaneously. Subtyping of seasonal H1N1 and H3N2 influenza viruses and detection of influenza B virus was carried out using two other real-time RT-PCR kits (Liferiver, Shanghai, China), targeting the viral hemagglutinin (HA) and matrix gene, respectively. All real-time RT-PCR detection was performed with a Light-cycler 480 (Roche, Switzerland).

Epidemiological investigation

Each RT-PCR confirmed pandemic H1N1 case or cluster was investigated. Information about the gender, ages, living places, travelling history, close contacts, symptoms and treatment was collected using a standard questionnaire.

Sequence analysis

Hemagglutinin (HA) gene sequences of the viruses isolated from some RT-PCR-positive samples were randomly selected and amplified using the universal primers as reported previously.⁴ The amplification products were purified with an agarose gel DNA extraction kit (TaKaRa, Dalian, China), cloned into a pMD18-T vector (TaKaRa, Dalian, China), and the clones were sequenced using a Perkin-Elmer model 377 XL DNA sequencer, from two senses with the M13 ± primers, respectively. The sequences were analyzed using the BLAST search function in National Center for Biotechnology Information (NCBI) and compared with some pandemic H1N1 or seasonal H3N2 influenza virus representatives using the method reported previously.⁵ Briefly, nucleotide sequences were aligned using Clustal X 1.83. Phylogenetic trees of the representatives were calculated using mega 4.0 software by the neighbor-joining method.⁶ The substitution rates among sites were set in Gamma distribution. Bootstrap values were calculated from 1000 replicates.

Results

Situation monitoring

The first pandemic H1N1 case and the first pandemic H1N1 cluster in mainland China were identified on May 11 and June 19, respectively. Up to October 30, 2009, 44981 cases were confirmed in mainland China, among which, 6 were fatal (See Supplementary Table S1). Up to October 30, 2009, worldwide there had been >441661 laboratory-confirmed cases of influenza A (H1N1) and >5712 deaths reported to WHO.

Detection of samples of air travellers

From May 1 to September 30, 50150 national or international passengers arrived in Qingdao by air travel. All of them were screened by temperature scanner, and 589 (0.12%) were found with a temperature higher than 37.0°C. Thirty-eight (6.45%) of the 589 passengers with fever were confirmed to be pandemic H1N1 cases. In addition, two cases of seasonal H3N2 subtype influenza were also identified from the 589 passengers with fever.

Detection of samples of patients with ILI

From May 1 to September 30, 1543 patients with ILI sought treatment at the three sentinel hospitals in the city. Among them, 271 patients were sampled at random for influenza, and seven pandemic H1N1 virus positives were identified. All seven positive samples were collected after August 26, 2009. In addition, 96 cases of seasonal H3N2 subtype influenza and five cases of seasonal H1N1 subtype influenza were also identified from these 271 patients with ILI.

Detection of samples of ILI clusters

From May 1 to September 30, 18 ILI clusters were reported in the city. Seven of them (1 from a primary school, 3 from middle schools and 3 from universities) were confirmed to be pandemic H1N1, with a total of 92 confirmed cases. Additionally, 10 clusters of seasonal H3N2 subtype influenza were also identified, and both pandemic H1N1 virus and seasonal H3N2 subtype influenza virus were found in 2 of the 18 ILI clusters.

Subsequent investigation of the pandemic H1N1 cases or clusters

The first confirmed pandemic H1N1 case (flying from USA) and the first confirmed cluster of pandemic H1N1 in Qingdao were identified on June 13 and September 2, respectively.

Among the 38 pandemic H1N1 cases from air travellers, 10 were from Australia, 7 from the USA, 6 from South Korea, 6 from Japan, 2 from Indonesia, 2 from Hong Kong SAR, and 1 each from Canada, Chile, Philippines, UK and New Zealand, respectively. Twenty-eight (73.7%) of the 38 pandemic H1N1 cases were aged 11–30. Three of the 140 close contacts of the 38 pandemic H1N1 cases in air travellers were found to be pandemic H1N1 positive.

The infection source of the seven cases identified from patients with ILI in the sentinel hospitals could not determined during the subsequent investigation, and none of the 134 close contacts of the seven pandemic H1N1 cases were found to be pandemic H1N1 positive.

All the confirmed pandemic H1N1 cases within the seven pandemic H1N1 clusters and their close contacts were

isolated in the universities, or at home in the case of students in primary or middle schools, and no new cases were found at these sites 21 days after the isolation. The infection source of only one of the seven pandemic H1N1 clusters was found through subsequent investigation, and the infection source of the one cluster was a university student coming from another city to Qingdao.

Only 2 of all the 140 pandemic H1N1 cases identified from the four groups of high influenza risk had any ILI symptoms like fever, cough, dyspnea, rhinitis, headache, etc. Most of the identified pandemic H1N1 cases (≥ 93) took various traditional Chinese medicines, and some (≥ 26) took Oseltamivir. Only 2 of the 274 close contacts of the pandemic H1N1 cases identified from air travellers and patients with ILI of the sentinel hospitals had taken traditional Chinese medicine before the subsequent investigation

because they had developed some ILI symptoms, and both were subsequently confirmed as pandemic H1N1 cases. None of the 140 pandemic H1N1 cases including 92 from the 7 pandemic H1N1 clusters developed into severe infections.

Molecular characteristics of the influenza viruses detected in the surveillance

The whole-length HA sequences of 24 pandemic H1N1 viruses (15 from air travellers, 9 from 4 ILI clusters) and 21 seasonal H3N2 influenza viruses (2 from air travellers, 6 from patients with ILI of the sentinel hospitals, 13 from 6 ILI clusters) were obtained. Their GenBank accession numbers are CY050089–CY050140, CY050262–CY050276, GU144805, GU168018. Phylogenetic analysis of the whole-length HA sequences suggested that the pandemic H1N1

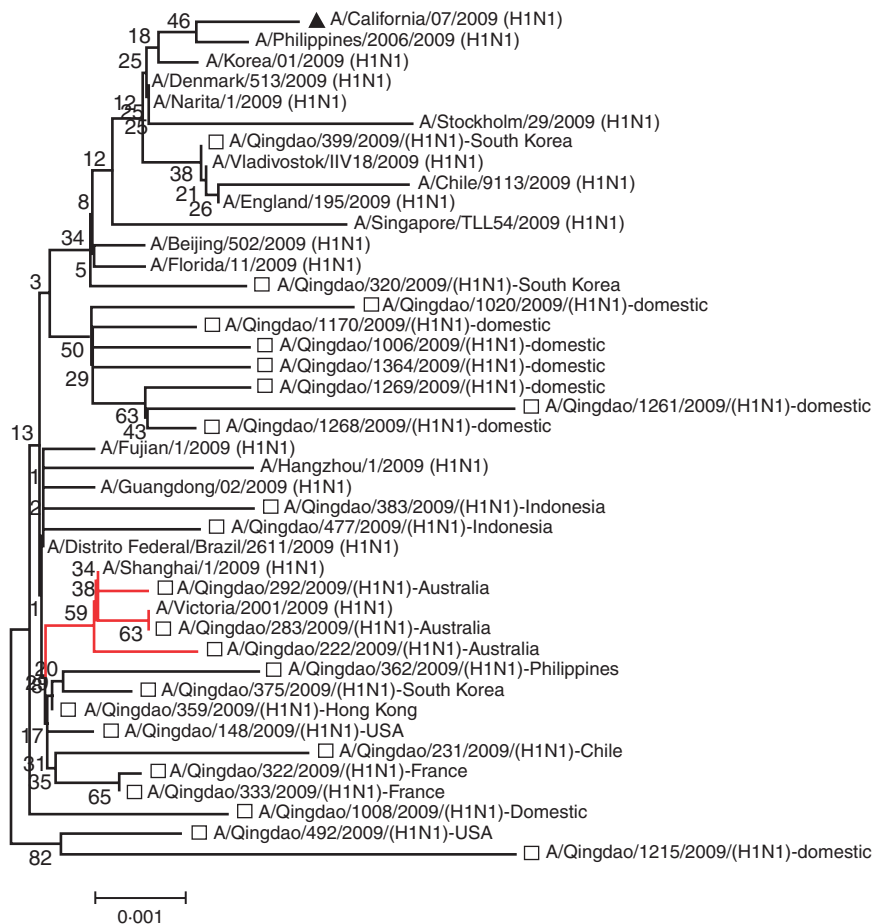


Figure 2. The phylogenetic tree of some pandemic H1N1 viruses isolated in 2009 based on their whole-length HA gene sequence. The viruses reported by this study are marked with squares, and the geographic background of the corresponding cases was given after the virus designations (for example, 'A/Qingdao/399/2009/(H1N1)-South Korea' means the case coming from South Korea). Some viruses in the figure were reported previously by others, including the one recommended by WHO for inclusion in vaccines for use in the 2009–2010 influenza seasons (marked with a triangle). Bootstrap values were given at the corresponding nodes. The tree suggested that all the viruses were similar to each other no matter where the cases came from, although the viruses of the clade marked with red lines were all from Australian cases or the cases imported from Australia [the virus A/Shanghai/1/2009(H1N1) was from a pandemic H1N1 case flying from Australia to Shanghai on May 23, 2009].

viruses detected in Qingdao were similar to their counterparts reported elsewhere in the world in 2009 (Figure 2). The seasonal H3N2 influenza viruses reported here were similar to most of those detected in other countries in 2009 (A/Perth/16/2009(H3N2)-like), but distinguishable from those dominating in the world in 2007–2008 (A/Brisbane/10/2007(H3N2)-like), in their HA gene sequences (Figure 3). Six amino acid mutations (E62K, N144K, K158N, K173Q, N189K and V213A) in the HA protein of the H3N2 influenza viruses were defined when compared with those isolated in 2007–2008. All these mutations were located in antigenic sites under positive selection.^{7,8}

Discussion

Representativity of the emergency surveillance

Other municipal cities in China also carried out emergency influenza surveillance similar to that reported here. Therefore, this report actually described the emergency influenza surveillance in China in 2009 at the municipal city level. This emergency surveillance targeting defined risk groups should be of significance for the city to establish its early detection system for acute respiratory diseases in the future.

One hundred and forty of pandemic H1N1 cases and seven pandemic H1N1 clusters were identified and investigated by the emergency surveillance. They were important for analysis of the risk of the pandemic in the city. However, because the surveillance only targeted some special groups, pandemic H1N1 cases outside the four groups could not be identified by the surveillance. Therefore, such surveillance would be inadequate if it were necessary to identify and isolate all cases to control a more dangerous respiratory disease, such as severe acute respiratory syndromes (SARS).

In addition, only a portion of pandemic H1N1 cases among the air travellers were identified by the emergency surveillance because some cases might not be febrile when they left the airport. Actually, only 2 of the first 12 confirmed pandemic H1N1 cases (all from air travellers) in China were detected with fever at the airport by temperature screening.⁹

Bias of the emergency surveillance

None of the real-time RT-PCR kits have been well evaluated, and their sensitivity as well as specificity was not known, although they were all accredited for emergency

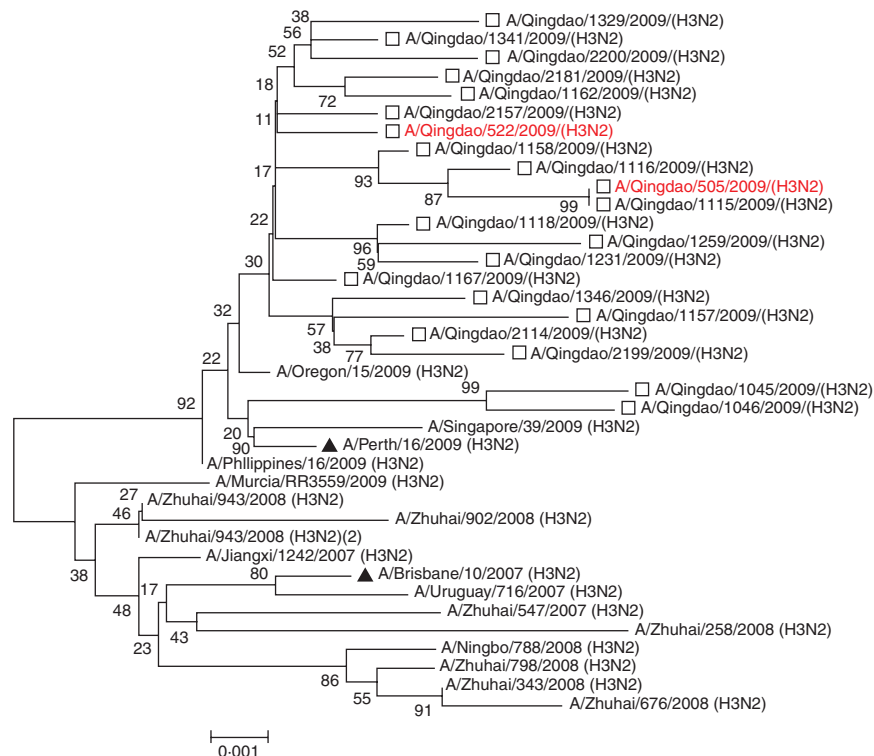


Figure 3. The phylogenetic tree of some seasonal H3N2 subtype viruses isolated in 2007–2009 based on their whole-length HA gene sequences. The viruses reported by this study are marked with squares, and the rest were reported previously by others including the two recommended by WHO for inclusion in vaccines for use in the 2008–2010 influenza seasons (marked with triangles). Bootstrap values are given at the corresponding nodes. The tree suggested that all the viruses reported herein were A/Perth/16/2009(H3N2)-like which became dominant around the world in 2009. The two viruses (with red designations) from air travellers were similar to the others identified in 2009 in the gene sequences.

use by the Chinese government on the basis of some preliminary comparison tests. This may create some biases of the surveillance.

Spread of the pandemic H1N1 virus in the city

All seven pandemic H1N1-positive samples from the randomly selected 271 patients with ILI of the sentinel hospitals were collected after August 26, 2009, which indicated that the disease had not spread widely in the city before then. This is also supported by the fact that no confirmed cluster of pandemic H1N1 in Qingdao were identified until September 2.

From the beginning of September, increasing numbers of pandemic H1N1 clusters were confirmed in the city, and virus circulation reached its peak in November, 2009 and declined in the beginning of 2010, as indicated by the subsequent routine surveillance (Table 1).

Among the 274 close contacts of the 45 confirmed pandemic H1N1 cases from air travellers or the patients with ILI of the sentinel hospitals, only three were found to be pandemic H1N1 positive during the subsequent investigation. This indicated that the pandemic virus spread slowly in the city in these months when compared with in other countries.^{10,11} Isolation of confirmed cases (which was stopped on September 30, 2009 in the city) and high

vigilance of the citizens should retard the spreading of the virus in the city in these months.

Virulence of the pandemic H1N1 virus

None of the 140 pandemic H1N1 cases including 92 from the 7 pandemic H1N1 clusters developed into severe infections. This indicated that the pandemic virus probably did not have the high virulence previously assumed for a pandemic influenza virus by some experts.¹²

Genetic characteristics of the pandemic H1N1 virus

No significant mutations were found by the surveillance in the HA gene sequences of the pandemic H1N1 viruses, and the seasonal H3N2 influenza viruses have become genetically distinguishable from those circulating in 2007–2008. This was consistent with the WHO report in September, 2009 (<http://www.who.int/csr/disease/influenza/recommendations2010south/en/index.html>, accessed on November 30, 2009).

The pandemic H1N1 viruses from some Australian cases and some Chinese cases imported from Australia formed a unique clade in Figure 3. This indicated that those viruses were possibly from the same close progenitor.

Air travel in the spread of the pandemic and seasonal influenza viruses

The surveillance identified not only 38 pandemic H1N1 cases but also two seasonal human H3N2 influenza cases from air travellers. This suggested that air travel facilitates spread of both pandemic and seasonal influenza even in the summer of the Northern Hemisphere, which is consistent with the rapid natural replacement of seasonal H3N2 influenza variants in the world in recent years, as shown by the example of the year 2009 mentioned earlier.^{12–14}

Co-circulation of pandemic H1N1 and seasonal influenza viruses

It is interesting to find the co-circulation of pandemic H1N1 and seasonal influenza viruses in the same clusters identified through emergency surveillance. The co-circulation also occurred in early September 2009 in Beijing,¹⁵ which indicated it is not a rare scenario. This phenomenon could facilitate re-assortment between the pandemic influenza viruses and seasonal influenza viruses. However, human influenza history has demonstrated that such re-assortment between different subtypes of human influenza viruses is of low frequency.¹⁶

The high activity of H3N2 in the summer of 2009 in Qingdao

Usually, it is believed that seasonal influenza activity in the summer remains high in southern China, but declines in

Table 1. Numbers of different type/subtypes of influenza cases confirmed by Qingdao CDC from May 1, 2009 to March 30, 2010, through the emergency and routine influenza surveillance*

Time	Pandemic H1N1	Seasonal H3N2	Seasonal H1N1	Type B	Total
05/2009	0	0	0	3	3
06/2009	6	0	0	2	8
07/2009	13	1	0	0	14
08/2009	8	8	1	0	17
09/2009	114	222	4	0	340
10/2009	105	33	5	0	143
11/2009	374	3	0	0	377
12/2009	206	3	0	6	215
01/2010	19	0	1	93	113
02/2010	8	0	0	86	94
03/2010	4	10	5	48	67

*The data did not indicate the real circulation intensity of each type or subtype of influenza in the city because the surveillance was not population based. However, the ratio among the numbers of each month suggested which type/subtype of influenza was dominant in the month, as the samples were collected at random from high-risk groups. The numbers indicated that pandemic H1N1 was dominant among the laboratory-confirmed cases in the last 3 months of 2009, but this dominance was replaced by influenza B in the first 3 months of 2010.

northern China.^{17,18} However, the results of detection of the ILI samples collected at the sentinel hospitals suggested that in the summer of 2009, seasonal H3N2 influenza spread actively in Qingdao which is located in northern China.

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Conflict of Interest

The authors have declared that no competing interests exist.

Author contributions

Conceived, designed and supervised the surveillance system: ZGW, ZYW, JMC. Conducted diagnostic assays: YY, TTY, XLL. Analyzed the data: ZGW, ZYW, FCJ, JMC. Wrote the paper: ZGW, JMC.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The number of influenza A(H1N1) cases and deaths in mainland China reported by the Ministry of Health of China from May 11 to October 30, 2009.

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