

Trends in seroprevalence of antibodies to pandemic influenza H1N1 (2009) virus among patients seeking care in China

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Received May 21, 2012; accepted October 29, 2012

Summary. – We determined seroprevalence of antibodies to pandemic influenza H1N1 (2009) virus in outpatients in China from December 2009 to March 2010. Serum antibody titers were determined by a hemagglutination-inhibition (HI) assay using the seroprevalence data for 2006–2008 (1.2%) as baseline. The overall seroprevalence was 7.6%, 18.6%, 20.5%, and 20.0% in December 2009, January 2010, February 2010, and March 2010, respectively. In comparison of monthly data, the seroprevalence values for the first three months exhibited statistically significant differences. As for the age-specific seroprevalence, the individuals aged <60 years exhibited significant increase in December 2009 and January 2010, while those aged ≥60 years showed a significant increase only in January and February 2010. The highest seroprevalence values were exhibited in the individuals of 6–24-year-old. This study showed that (i) the overall seroprevalence increased rapidly from December 2009 to February 2010, reaching a plateau, and (ii) the seroprevalence increased more quickly in individuals aged <60 years compared with those aged ≥60 years. It can be concluded that school-age children and young adults have had an important role in transmission of the pandemic virus.

Keywords: pandemic influenza H1N1 (2009); seroprevalence; China

Introduction

In April 2009, a novel swine-origin pandemic influenza H1N1 (2009) virus (2009 H1N1) emerged in the Americas and caused a pandemic (WHO, 2010). On 30 April 2009, nationwide surveillance of 2009 H1N1 as a notifiable infectious disease was established in China. From May to August 2009, 2009 H1N1 activity remained low, but increased significantly in September 2009. Clinical surveillance could not account for every case, since not every case will seek

medical care, and only a fraction of those were tested for 2009 H1N1. To understand trends of the seroprevalence of 2009 H1N1 virus antibodies in the population in China and to monitor age-specific differences, we conducted serological surveys in outpatients of five different age groups during the first wave of pandemic in December 2009 to March 2010.

Materials and Methods

Serum samples. The provincial CDCs of 31 provinces were enlisted to conduct the serial surveys using a uniform protocol issued by China CDC. In each province, 1 or 2 pediatric hospitals and 1 or 2 general hospitals were identified in the capital city. The first survey was conducted in November and December 2009 and 3 subsequent surveys were carried out monthly from January to March 2010. In each survey, serum samples were collected from

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Abbreviations: 2009 H1N1 = pandemic influenza H1N1 (2009); HI = hemagglutination-inhibition; CDC = Center for Disease Control and Prevention; IRR = incidence rate ratio; CNIC = Chinese National Influenza Center

outpatients and/or healthy people receiving routine physical examinations who had venous blood collected for clinical diagnosis of various diseases. Individuals who reported receiving 2009 H1N1 vaccine were not sampled and excluded from the study. Collected information includes age, gender, date of sample collection and vaccination history of 2009 H1N1 vaccine.

The study was approved by Chinese Ministry of Health as an emergency study for pandemic response. Informed consents were provided by all patients.

HI assay. Serum samples were tested for the presence of 2009 H1N1 virus antibodies by the HI assay (Kendal *et al.*, 1982; WHO 2009). Laboratory tests of all samples in the first survey were tested by CNIC of China CDC, and serum samples in subsequent surveys were tested by provincial CDCs. CNIC provided annual training for the serological HI assay for laboratories at provincial CDCs. All diagnostics were made according to the standard operational procedure of HI assay in Chinese National Influenza Surveillance Protocol (China CDC, 2011). The 2009 H1N1 antigen (A/California/07/2009 virus from USCDC) and the positive serum control (SPF Chicken anti-serum against A/California/07/2009) were provided by CNIC. Virus was inoculated into SPF embryonated chicken eggs and inactivated with 0.1% paraformaldehyde. Subjects with the HI titer of ≥ 40 were defined as seropositive (Miller *et al.*, 2010).

Surveillance of the pandemic. In April 2009, confirmed cases of 2009 H1N1 infection were reported to China CDC. All cases of

2009 H1N1 were identified through active surveillance by screening at borders, medical monitoring of close contacts of confirmed patients, and passive reporting by clinicians. A confirmed case was defined as a patient with acute respiratory illness and laboratory evidence of 2009 H1N1 infection diagnosed by RT-PCR testing of respiratory specimens (MoH China, 2009).

Statistical analysis. 1800 samples in each of five age groups (0–5 years, 6–15 years, 16–24 years, 25–59 years, and ≥ 60 years) in each survey were used to estimate age-specific seroprevalence. The age-adjusted seroprevalence or seroincidence were estimated by using the actual age-specific Chinese population data. Seroprevalence data from testing 2379 of stored serum samples collected from 5 provinces of mainland China between the years 2006 and 2008 was used as baseline for this study (Xu *et al.*, 2011). To estimate the cumulative seroincidence, the difference between the seroprevalence from each of the serological surveys and baseline survey was calculated. The cumulative incidence of confirmed cases of 2009 H1N1 was the ratio of cumulative number of confirmed cases reported via clinical surveillance to the actual Chinese population. The incidence rate ratio (IRR) was estimated as the ratio of the cumulative seroincidence to the cumulative incidence of confirmed cases. Differences in age-adjusted seroprevalence between each serological survey were tested with the chi-square test (significant level, $P < 0.05$). Multiple comparisons were made by the Bonferroni correction to examine the difference in seroprevalence between age groups within each serological survey and between the four sero-

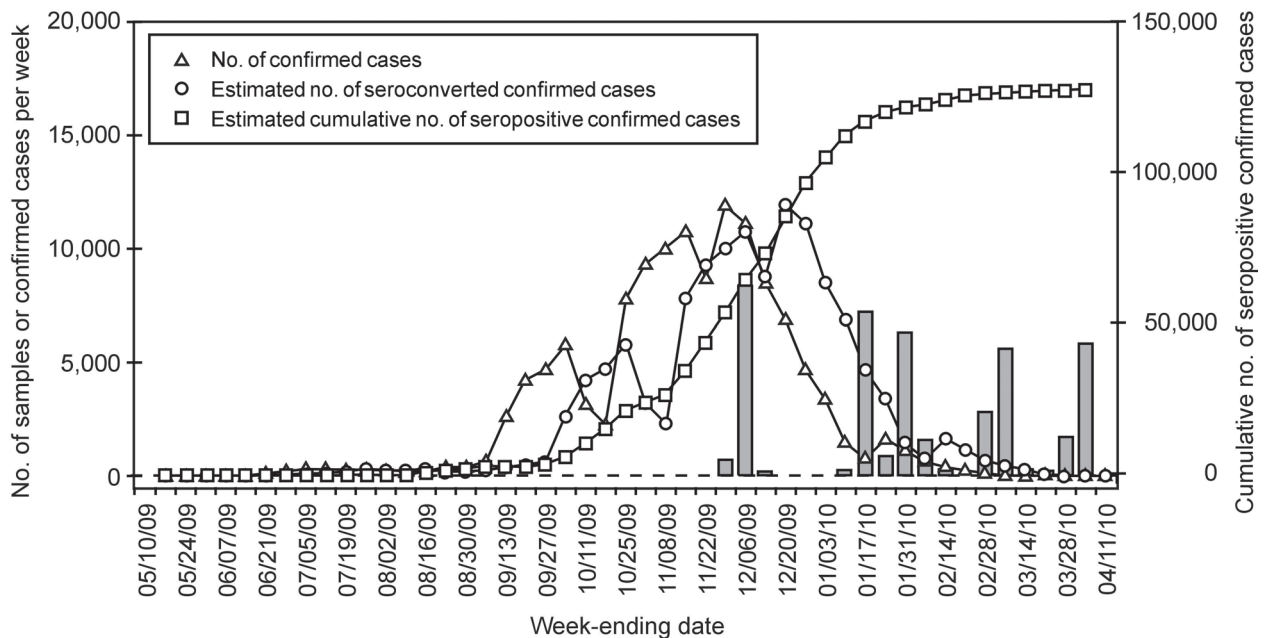


Fig. 1

Surveillance of the pandemic of influenza H1N1 (2009) in China in December 2009–March 2010

The left ordinate: No. of samples/confirmed cases; the right ordinate: Cumulative No. of seropositive confirmed cases; the abscissa: Time periods (m/d/y); the columns: No. of serum samples; the curve with triangles: No. of confirmed cases; the curve with circles: Estimated No. of seroconverted confirmed cases; the curve with squares: Estimated cumulative No. of seropositive confirmed cases.

logical surveys and the baseline survey for each age group and the total subjects. In this study the number of pairs for comparison was 11. An alpha <0.0045 was used to determine statistical significance for the multiple comparisons. All statistical analyses were done with SAS 9.1 statistical software (SAS Institute, NC, U.S.).

Results

A total of 43172 samples were collected from the four surveys between December 2009 and March 2010 (Fig. 1). 9738 samples were collected in December 2009, 16395 in January 2010, 8726 in February 2010, and 8313 in March 2010. There were statistically significant increases in the age-adjusted seroprevalence for the overall population between baseline (1.2%) and December 2009 (7.6%), and between December and January (18.6%) (Table 1, Fig. 2). Although the overall seroprevalence in February increased by only 1.9% in comparison with January, this difference was also statistically significant. There was no statistically significant difference in the overall seroprevalence between February and March. The national surveillance data showed that the number of confirmed 2009 H1N1 cases strikingly increased in early-September 2009, and culminated during the last week of November. The pandemic wave ended at the end of January 2010 (Fig.1). 89% of confirmed cases developed an HI titer of 40 or more after 3 weeks from onset of illness (Miller *et al.*, 2010). The trend of the estimated cumulative number of seropositive confirmed cases, which indicated a striking increase from October 2009 to February 2010 with a plateau thereafter, is similar to the trend of age-adjusted seroprevalence (Fig. 2).

For all four serological surveys, the seroprevalence of 2009 H1N1 was statistically significantly higher among individuals aged 6–15 and 16–24 years than that among the other age groups, and the lowest seroprevalence is among individuals aged ≥60 years (Table 1). The IRR of the total population increased more than 2–3 times for every age group in February or March 2010, in comparison to December 2009 (Table 2). For each of these four surveys, the IRR was the highest among those aged ≥60 years, and was the lowest among those aged 6–15 years and 16–24 years.

The seroprevalence of 2009 H1N1 within five age groups among the four surveys and baseline was compared by multiple comparisons. For all age groups younger than 60 years, there was a significant increase in seroprevalence comparing each of the four surveys with the baseline. A significant increase in the seroprevalence was observed in the surveys conducted in January, February and March compared with the survey conducted in December. However, the seroprevalence for these age groups were not significantly different among the surveys in January, February and March (Fig. 2). For individuals aged ≥60 years, there was no statistically

Table 1: Seroprevalence and seroincidence of antibodies to pandemic influenza H1N1 (2009) virus in December 2009–March 2010

Age (years)	Baseline			December, 2009			January, 2010			February, 2010			March, 2010		
	Pos/Total ^a	SP % (95% CI)	SI, % (95% CI)	Pos/Total ^a	SP, % (95% CI)	SI, % (95% CI)	Pos/Total ^a	SP, % (95% CI)	SI, % (95% CI)	Pos/Total ^a	SP, % (95% CI)	SI, % (95% CI)	Pos/Total ^a	SP, % (95% CI)	SI, % (95% CI)
0–5	0/436	0 (0–0.7)	7.5 (6.5–8.4)	214/2869	7.5 (6.5–8.4)	21.2 (19.9–22.4)	879/4150	21.2 (19.9–22.4)	21.2 (19.9–22.4)	479/2298	20.8 (19.2–22.6)	20.8 (19.2–22.5)	496/2028	24.5 (22.6–26.4)	24.5 (22.6–26.3)
6–15	6/556	1.1 (0.4–2.3)	12.5 (11.1–13.9)	271/2161	12.5 (11.1–13.9)	30.3 (28.4–32.2)	941/2997	31.4 (29.7–33.1)	30.3 (28.4–32.2)	553/1564	35.4 (33.0–37.8)	34.3 (31.8–36.8)	470/1477	31.8 (29.5–34.3)	34.3 (31.8–36.8)
16–24	12/360	3.3 (1.7–5.8)	17.5 (14.4–20.5)	106/607	17.5 (14.4–20.5)	24.7 (21.6–27.8)	343/1224	28.0 (25.5–30.5)	24.7 (21.6–27.8)	204/614	33.2 (29.5–37.1)	29.9 (25.7–34.1)	184/634	29.0 (25.5–32.7)	29.9 (25.7–34.1)
25–59	3/534	0.6 (0.1–1.6)	4.8 (3.9–5.8)	95/1973	4.8 (3.9–5.8)	14.2 (13–15.4)	733/4963	14.8 (13.8–15.8)	14.2 (13–15.4)	414/2676	15.5 (14.1–16.9)	14.9 (13.4–16.4)	395/2554	15.8 (14.0–16.9)	14.9 (13.4–16.4)
≥60	10/493	2.0 (1.0–3.7)	3.9 (3.1–4.7)	83/2128	3.9 (3.1–4.7)	6.4 (4.8–8)	259/3061	8.5 (7.5–9.5)	6.4 (4.8–8)	180/1574	11.4 (9.9–13.1)	9.4 (7.4–11.4)	176/1620	10.9 (9.4–12.4)	9.4 (7.4–11.4)
Total	31/2379	1.2 (0.7–1.6)	7.6 (7.1–8.1)	769/9738	7.6 (7.1–8.1)	17.4 (16.7–18.1)	3155/16395	18.6 (18.0–19.2)	17.4 (16.7–18.1)	1830/8726	20.5 (19.7–21.4)	19.4 (18.4–20.3)	1721/8313	20.0 (18.9–20.7)	19.4 (18.4–20.3)

^aNo. of positive samples/Total No. of samples.

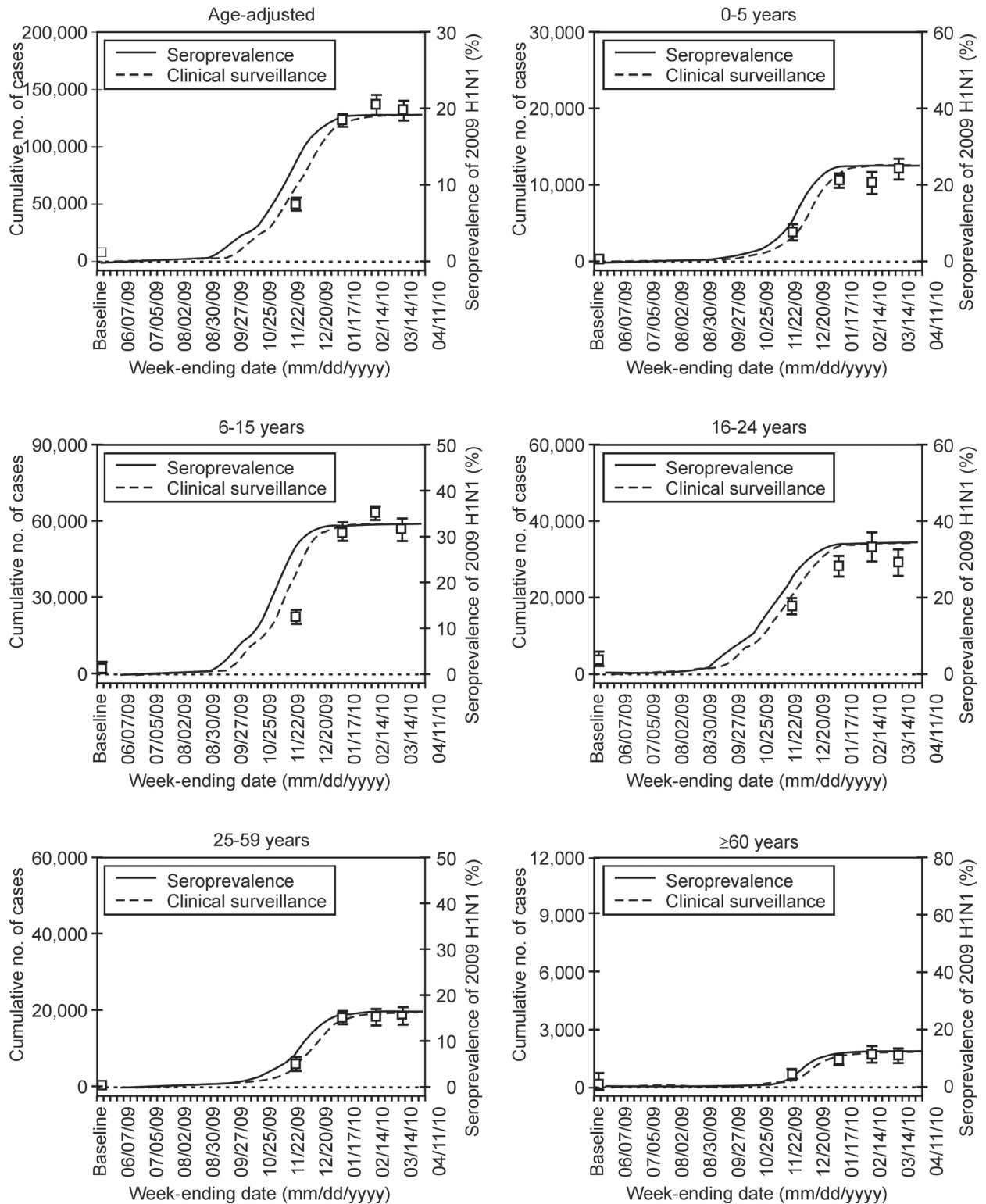


Fig. 2

Age-specific seroprevalence of antibodies to pandemic influenza H1N1 (2009) virus among outpatients in China

The left ordinate: Cumulative No. of confirmed cases; the right ordinate: Seroprevalence of antibodies (%); the abscissa: Time periods (m/d/y). Each data point of seroprevalence corresponds to the point estimates and vertical bars indicate 95% confidence intervals. In every figure panel, the age-specific seroprevalence match the age-specific clinical surveillance data.

significant difference in seroprevalence between baseline (2.0%) and December (3.9%), however there was a statistically significant increase in seroprevalence of 2009 H1N1 between December and January (8.5%) and between January and February (11.4%) (Fig. 2).

Discussion

The highlight of the series of serological surveys is the importance of estimating the changing seroprevalence of 2009 H1N1 among the Chinese population during different time points of 2009 H1N1 pandemic. We found that the overall seroprevalence of 2009 H1N1 among people without 2009 H1N1 vaccine was 7.6% in December 2009 when 2009 H1N1 pandemic culminated. Even higher seroprevalence rates were observed in the months following the pandemic peak. Seroprevalence for all age groups younger than 60 years increased significantly from December to January and from December to February for age group of 60 years or older.

In this study, we found that the trend of the overall seroprevalence of 2009 H1N1 antibodies in the unvaccinated population was consistent with the overall trend of cumulative seropositive confirmed cases estimated by clinical surveillance data. The overall seroprevalence significantly increased before February 2010, and culminated thereafter. These findings were similar to findings from a similar serological study conducted in Hong Kong, which showed a striking increase in seroprevalence during the pandemic wave and then followed by a plateau in the next month (Wu *et al.*, 2010). These findings indicate that clinical surveillance system is able to identify trends in influenza activity for the total population, although only a fraction of all cases was detected.

The IRR between seroincidence and cumulative incidence of confirmed patients was 785 in the first survey, while in the following three surveys it was 2000. These findings indicated that the clinical surveillance underestimated actual number of infections in the widespread transmission of the 2009 H1N1. In addition, our study showed that the clinical surveillance data detected a lower number of infections among individuals aged 60 years or older compared with the number of infections for those aged 6-24 years of age. This could be due to the difference in health seeking behavior by age group and could also be due to the severity of illness varying in age group.

In our study, testing results were obtained within 3 weeks after specimen collection in the last 3 surveys, and for the first survey, it was about 7 weeks after specimen collection. These serological data were helpful in terms of providing valuable information for the Chinese government to develop and adjust control strategy. We found that the overall seroprevalence of 2009 H1N1 antibodies among unvaccinated

Table 2: Comparison of serological and clinical incidences of pandemic influenza H1N1 (2009) in December 2009–March 2010

Age (years)	December, 2009			January, 2010			February, 2010			March, 2010						
	Cumulative No. of cases	Incidence ^a	Serological incidence (%)	Cumulative No. of cases	Incidence ^a	Serological incidence (%)	Cumulative No. of cases	Incidence ^a	Serological incidence (%)	Cumulative No. of cases	Incidence ^a	Serological incidence (%)	IRR ^b			
0-5	8634	101.7	7.5	733	12399	146	21.2	1450	12566	148	20.8	1405	12665	149.2	24.5	1642
6-15	55570	281.7	11.5	471	51284	259.9	30.3	1166	51337	260.2	34.3	1318	51394	260.5	34.3	1317
16-24	29276	177.9	14.1	632	41754	253.7	24.7	973	41993	255.1	29.9	1172	42108	255.8	29.9	1169
25-59	13222	19.2	4.3	2219	19230	27.9	14.2	5096	19563	28.4	14.9	5253	19692	28.6	14.9	5219
≥60	1141	6.7	1.9	2786	1772	10.4	6.4	6164	1809	10.7	9.4	8823	1822	10.7	9.4	8760
Total	107843	82.6	6.5	785	126439	96.8	17.4	1797	127268	97.4	19.4	1991	127681	97.7	19.4	1985

^aIncidence per million population. ^bIRR is the ratio of the seroincidence to the incidence rate of pandemic influenza H1N1 (2009) from clinical data.

individuals in January 2010 was 18.6% (95% CI, 18.0–19.2%), which was similar to the seroprevalence of unvaccinated individuals (17.1%; 95% CI, 16.1–18.0%) from another random-sampling serological survey conducted in China (Xu *et al.*, 2011). Therefore we believe that the serological survey conducted with convenient outpatients samples collected from clinics can provide relatively accurate estimation of 2009 H1N1 seroprevalence.

We also observed age-specific differences in the timing of the significant increase in 2009 H1N1 seroprevalence rates. The seroprevalence increased more rapidly in all age groups younger than 60 years, compared with the ≥ 60 year old age group. This might be explained by more frequent social contact of children and adults younger than 60 years, which may have as well contributed to transmission. Results from the four surveys showed that school-aged children and young adults had the highest seroprevalence of 2009 H1N1 antibodies, which was consistent with serological studies conducted in other countries, as well as the other studies conducted in China after the emergence of 2009 H1N1 (Miller *et al.*, 2010; Xu *et al.*, 2011; Wu *et al.*, 2010; Allwinn *et al.*, 2010; Ross *et al.*, 2010; Deng *et al.*, 2010). The seroincidence of 2009 H1N1 was 6.5% for 7 months from May to December 2009, and then increased to 17.4% in a period of less than 2 month period from December 2009 to January 2010. This seroincidence in December 2009 was lower than that reported during the same time in other countries or regions (Miller *et al.*, 2010; Wu *et al.*, 2010; Allwinn *et al.*, 2010; Ross *et al.*, 2010; Deng *et al.*, 2010). Our finding may be explained by relatively low and inconsistent transmission in the early phase of the 2009 H1N1 pandemic, which was similar to the clinical and laboratory surveillance data (CNIC, 2011).

However, this study has several limitations. One of the limitations is a problem regarding self-reported vaccination history or misclassification of vaccine (e.g. some individuals may report receiving 2009 H1N1, but may have actually received a different vaccine). Second, the baseline serum samples were only collected from 5 provinces or autonomous regions which may not have reflected the entire population of China. However, the baseline seroprevalence of cross-reactive antibodies to 2009 H1N1 by age were consistent with the two other Chinese studies. Additionally, our analyses were conducted among outpatients and/or healthy people receiving physical examinations in capital cities, which may not be representative of the whole Chinese population.

These serological surveys indicated that the overall seroprevalence sharply increased before February 2010, and culminated thereafter. This trend was similar to surveillance data collected during the same time period. Serial serosurveys can be used to monitor the trend of 2009 H1N1 activity and to compare with clinical surveillance data for consistency. They may also allow for the calculation of a less biased estimate of the number of infections in various age

groups. One important outcome from this study was that individuals under the age of 60 years contributed more to transmission of 2009 H1N1 compared with individuals older than 60 years of age. Our findings of the distribution and trend in the seroprevalence by age groups at different time points during the pandemic enhanced our understanding of the pandemic influenza H1N1 (2009) virus and provided valuable information for the Chinese government to develop and adjust the control strategy.

Acknowledgements. This work was (in part) supported by the NIH/NIAID Grant No.5-U54-AI-057157-08 (Epidemiology and Molecular Mechanism of Highly Pathogenic Avian Influenza H5N1 and Pandemic Influenza H1N1). The authors thank the participated hospitals, local health departments, China CDCs for sample collection and data coordinating, and the China Ministry of Health for supporting this study. The authors thank Dr. Danielle Iuliano, epidemiologist of Centers for Disease Control and Prevention, USA and Dr. Marc-Alain Widdowson, epidemiologist of Centers for Disease Control and Prevention, USA for their critical revision of the manuscript.

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