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Serological survey of antibodies to influenza A viruses on a group of people without a history of influenza vaccination

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Abstract

A serological survey for antibodies to influenza viruses was performed in China on a group of people without a history of influenza vaccination. Using the hemagglutination inhibition (HI) assay, we found seropositivity rates for seasonal H3N2 to be significantly higher than for seasonal H1N1. Samples positive for antibodies to pandemic (H1N1) 2009 virus increased from 0.6% pre-outbreak to 4.5% ($p<0.01$) at one year post-outbreak. Interestingly, HI and neutralization tests showed that 1.4% of people in the group have antibodies recognizing H9N2 avian influenza viruses, suggesting that infection with this subtype may be more common than previously thought.

Keywords

influenza; prevalence; antibody; pandemic H1N1; H9N2

Serological data may provide information for understanding general immunity, and retrospectively determining the infection rate of previously-circulating strains of influenza virus. The outbreak of the swine-origin pandemic (H1N1) 2009 virus led to numerous studies revealed some variations in pre-existing or cross immunity to the pandemic (H1N1) 2009 virus strain between people from different regions[1-6]. These are most likely due to geopolitical differences in access to annual influenza vaccination programs, as well as past exposure to genetically-related influenza virus strains. In addition to the seasonal influenza viruses, including the 2009 H1N1 virus, human infections caused by avian H5N1, H7N7 and H9N2 viruses have been observed in recent years [7]. While infection with highly pathogenic H5N1 and H7N7 strains causes severe disease, infection with H9N2 virus results in mild symptoms, similar to seasonal influenza. Avian H9N2 virus is highly prevalent in poultry, particularly chickens, and has plenty of opportunities for cross species transmission. There is concern that repeated avian-human transmissions may result in gradual host adaptation of H9N2, leading to another pandemic. Estimation of unrecognized infection in the general population is therefore extremely important.

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Transparency Declaration All authors declare no conflicts of interest.

In this study, a serological survey of people from rural areas of China was conducted in order to understand the rate of natural infection with strains of influenza viruses which are circulating in humans and also strains which are circulating in poultry, but have the potential to cross the species barrier and infect humans. A total of 1571 serum samples collected in March 2009, prior to the outbreak of pandemic (H1N1) 2009 influenza, and a further 9936 serum samples collected in May 2010, all from blood donors who were born between 1942 and 1991 and resident in Jiangsu Province in eastern China, were analyzed. Micro-well cell neutralization assays performed in MDCK cell cultures and hemagglutination inhibition (HI) assays were used to detect antibodies against influenza virus, as previously described [8, 9].

We first examined 1039 sera collected from residents of villages in Jiangsu Province in May 2010 for the presence of antibodies to eight selected influenza viruses, using the HI assay. As shown in Table 1, the positive rate (HI ≥40) for the presence of antibodies to seasonal H1N1 virus is only 1.4% and the higher positive rate, 16.8%, for H3N2-specific antibodies is likely attributed to the predominant circulation of A/Perth/16/09-like H3N2 strain from 2009 season. The pandemic (H1N1) 2009 virus started to circulate in China in June 2009. To find the rate of natural infection with pandemic (H1N1) virus among this group of people, we examined sera for the presence of antibodies to A/California/7/09. Antibodies were detected in 3.1% of people from this group. To better understand the rate of infection with pandemic (H1N1) 2009 virus, 1571 sera samples collected in March 2009, before the outbreak of the pandemic, and all 9936 sera samples collected in May 2010 were compared. While only 0.6% of people sampled in March 2009 exhibited positive antibodies to A/California/7/09 virus, the positive rate was 4.5% among sera samples collected in 2010. We were able to further confirm this observation by examining 1295 sets of paired sera, each pair consisting of samples from the same donors, taken in March 2009 and May 2010. As shown in Table 2, the positive rate is 0.6% and 4.7% among the 2009 and 2010 sera samples, respectively. These results suggest that the natural infection rate with pandemic (H1N1) 2009 in rural areas of China (3.1-4.7%) is well below the levels reported in other places [3-5, 10]. While reports from other studies, conducted mainly in urban areas, found a drastic increase in seropositivity in humans following the peak period of pandemic (H1N1) 2009 virus circulation [3-5, 10], only about 4.5% of people sampled in this study exhibited detectable levels of antibodies to this virus, suggesting that the majority of people in rural areas of China are still naïve, in terms of exposure to pandemic (H1N1) 2009 virus.

The 1039 sera initially described above were further examined for antibodies to avian H5N1, H9N2 and H6N1 subtype viruses. Antigenically distinct strains of avian H5N1 virus are known to circulate in different places, causing sporadic human infections [8]. We used four H5N1 virus strains, representing different antigenic groups, for the HI assays conducted in this study. No positive samples (HI ≥40) were found among the 1039 sera examined. Likewise, none of the sera tested positive for antibodies to H6 subtype avian influenza virus. Genetic and antigenic analysis has revealed that there are two lineages of H9N2 viruses, G1-like and Ck/Bei-like, co-circulating in domestic poultry and viruses from both lineages have been reported to cause human infections [11]. We therefore used viruses representing both genetic lineages to conduct HI tests. In contrast to the results for H5 and H6 subtype viruses, 1.4% of people tested positive for antibodies to one of the H9N2 viruses. To confirm that the positive antisera were specific for H9N2 virus, micro-neutralization assays were conducted, and demonstrated that the antibodies detected by HI assay do indeed represent antibodies which can specifically neutralize H9N2 virus but not H3N2, H1N1 or H6N1 influenza viruses used as controls. Our result is not unexpected, given that the H5N1 virus is highly pathogenic and asymptomatic infection is unlikely. However, infection with avian H9N2 virus causes only mild symptoms. It is possible that G1-like H9N2 virus may be prevalence in the poultry in the area from where those sera were collected. While only 11 human cases have been identified up to date, the avian H9N2 virus was not considered to infect human

regularly. Because we found a similar rate of positivity for antibodies against seasonal H1N1 virus (Table 1), the detection of antibodies specific to H9N2 virus suggests that natural infection with this virus in the general population may occur at higher levels than previously thought. While we have just experienced the swine-origin H1N1 pandemic and continue to closely watch the activity of avian H5N1 virus, the potential for H9N2 virus or one of its derivatives to cause a pandemic should be further assessed.

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TABLE 1

Prevalence of antibodies against different influenza A viruses in sera from unvaccinated people in Jiangsu, China

Subtype	Virus strain	Positive number at different HI (rate, 95% CI) (n=1039)			
		HI≥10	HI≥20	HI≥40	HI≥80
H1N1	Brisbane/59/2007	126 (12.1%,10.2-14.3)	51 (4.9%,3.7-6.4)	14 (1.4%,0.7-2.2)	4 (0.4%,0.1-1.0%)
panH1N1	California/07/2009	88 (8.5%,6.9-10.3)	57 (5.5%,4.2-7.0)	32 (3.1%,2.1-4.3)	11 (1.1%,0.5-1.9)
H3N2	Perth/16/2009-like	435 (41.9%,38.8-44.9)	335 (32.2%,29.4-35.2)	174 (16.8%,14.5-19.2)	69 (6.6%,5.2-8.3)
H5N1	Shenzhen/406H/2006(Clade 2.3.4)	27 (2.6%,1.7-3.8)	3 (0.3%,0.1-0.8)	0 (0.0%,0.0-0.4)	0 (0.0%,0.0-0.4)
	BGs/Qinghai/15C/2005(Clade 2.2)	3 (0.3%,0.1-0.8)	2 (0.2%,0.0-0.7)	0 (0.0%,0.0-0.4)	0 (0.0%,0.0-0.4)
H9N2	A/HK/2103/03	9 (0.9%,0.4-1.6)	1 (0.1%,0.1-0.5)	0(0.0%,0.0-0.4)	0 (0.0%,0.0-0.4)
	A//HK//464419/09	149 (14.3%,12.3-16.6)	62 (6.0%,4.6-7.6)	15(1.4%,0.8-2.4)	3 (0.3%,0.1-0.8)
H6N1	A/ duck/ JX227/03	0 (0.0%,0.0-0.4)	0 (0.0%,0.0-0.4)	0 (0.0%,0.0-0.4)	0 (0.0%,0.0-0.4)

1039 sera samples with approximately 200 samples in each of five birth year groups five birth year groups (1942-1951, 1952-1961, 1962-1971, 1972-1981, 1982-1991) were collected from residents of two villages in Jiangsu Province, China in May 2010. Antibodies to reference strains of influenza A virus were examined by hemagglutination inhibition assay (9). For H5N1 virus, HI assays were also conducted using two strains belonging to clade 7: no positive reactions were detected. For H9N2, two strains representing the G1 and CK/Bei lineages were used and all positive sera with HI ≥40 were tested against other major influenza virus subtypes to exclude cross-reactivity. Neutralization assays were also performed to confirm the presence of specific antibodies to H9 that did not cross-react with other subtypes of influenza virus. Specimens were tested in duplicate. Estimation of the 95% CI was performed with exact binomial methods. BGs, bar-headed goose; A/HK/2103/03 (CK/Bei-like); A/HK/464419/09 (G1-like).

TABLE 2

Serum antibodies to pandemic (H1N1) 2009 virus A/California/07/2009

Collection place	Collection date	No. of subjects	Positive number at different HI (rate, 95%CI)			
			HI≥10	HI≥20	HI≥40	HI≥80
Jiangsu[a]	March 2009	1571	52 (3.3%,2.5-4.3)	25 (1.6%,1.0-2.3)	9 (0.6%,0.3-1.1)	2 (0.1%,0.0-0.5)
	May 2010	9936	1385 (13.9%,13.3-14.6)*	874 (8.8%,8.2-9.4)*	448(4.5%,4.1-4.9)*	182(1.8%,1.6-2.1)*
Jiangsu serial[b]	March 2009	1295	43 (3.3%,2.4-4.4)	20 (1.5%,1.0-2.4)	8 (0.6%,0.3-1.2)	2 (0.2%,0.0-0.6)
	May 2010	1295	178 (13.8%,11.9-15.7)*	119 (9.2%,7.7-10.9)*	61 (4.7%,3.6-6.0)*	24 (1.8%,1.2-2.7)*

Specimens were tested in duplicate.

*p<0.01, the unadjusted Chi-square or Fisher's exact test was used for categorical independent variables. Estimation of the 95% CI was performed with exact binomial methods. Calculations were conducted with SPSS statistical software, version 17.0 (SPSS, Chicago, IL, USA).

[a]1517 and 9936 sera samples were collected from residents of two villages in Jiangsu province, eastern China, in March 2009 and May 2010, respectively. None of the people had a history of influenza vaccination.

[b]Among the 1517 and 9936 sera samples, there are 1295 serial sample pairs taken from the same individuals in 2009 and 2010, respectively.

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