Seroprevalence of influenza viruses in Shandong, Northern China during the COVID-19 pandemic

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Abstract Nonpharmaceutical interventions (NPIs) have been commonly deployed to prevent and control the spread of the coronavirus disease 2019 (COVID-19), resulting in a worldwide decline in influenza prevalence. However, the influenza risk in China warrants cautious assessment. We conducted a cross-sectional, sero-epidemiological study in Shandong Province, Northern China in mid-2021. Hemagglutination inhibition was performed to test antibodies against four influenza vaccine strains. A combination of descriptive and meta-analyses was adopted to compare the seroprevalence of influenza antibodies before and during the COVID-19 pandemic. The overall seroprevalence values against A/H1N1pdm09, A/H3N2, B/Victoria, and B/Yamagata were 17.8% (95% CI 16.2%–19.5%), 23.5% (95% CI 21.7%–25.4%), 7.6% (95% CI 6.6%–8.7%), and 15.0% (95% CI 13.5%–16.5%), respectively, in the study period. The overall vaccination rate was extremely low (2.6%). Our results revealed that antibody titers in vaccinated participants were significantly higher than those in unvaccinated individuals ($P < 0.001$). Notably, the meta-analysis showed that antibodies against A/H1N1pdm09 and A/H3N2 were significantly low in adults after the COVID-19 pandemic ($P < 0.01$). Increasing vaccination rates and maintaining NPIs are recommended to prevent an elevated influenza risk in China.

Keywords influenza virus; seroprevalence; antibody; COVID-19; cross-sectional study

Introduction

Influenza, particularly seasonal influenza, imposes a major burden on global public health. Every year, 10% to 20% of the world’s population contracts influenza, resulting in a severe burden of morbidity, mortality, and economic losses [1]. The seroprevalence of seasonal influenza among the general public varies sharply between seasons and across regions in China. For example, the A/H1N1 subtype seroprevalence values are 9.9% in Changzhi during the 2018–2019 influenza season [2] and 56.8% in Hong Kong during the summer of 2015 [3]. The A/H3N2 seroprevalence values are 7.9% in Shenzhen and 7.1% in Changzhou in 2018–2019 [4], and 73.1% in Hong Kong in 2017 [5]. In addition, the reported incidence and mortality of influenza increase with a steep upward trend in China in 2018–2019, and the reported cases of influenza reach 1.77 million in China in the first five months of 2019, which exceed the...
cumulative total number of the reported influenza cases from 2015 to 2018 [6].

Since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many countries formulated control strategies to prevent the spread of COVID-19 particularly nonpharmaceutical interventions (NPIs) [7,8]. These measures can provide effective protection against respiratory infections in the community [9] and have concomitantly resulted in a remarkable reduction in influenza transmission [10]. According to the Global Influenza Surveillance and Response System, only 0.3% of the respiratory samples are diagnosed as influenza viral infections between April 2020 and the end of December 2020, indicating a substantial decrease in incidence from that of the previous five years [11]. The incidence of influenza is extremely low in China and globally after the onset and during the COVID-19 pandemic, and mass vaccination has been deployed against COVID-19 in many countries. However, the global pandemic risk of seasonal influenza strains, particularly A/H1N1pdm09 and A/H3N2, should not be ignored. Experimental and clinical data showed that the coinfection of COVID-19 and seasonal influenza enhances the severity of pneumonia [12–14]. Therefore, investigating the antibody titers among the general public is crucial for assessing the risk of seasonal influenza transmission and outbreaks during the COVID-19 pandemic era.

In the present study, we have conducted a cross-sectional, sero-epidemiological study to investigate the antibody titers against A/H1N1pdm09, A/H3N2, B/Victoria, and B/Yamagata in Shandong Province, China. We have characterized low seroprevalence profiles for each of the four currently circulating seasonal influenza viruses among different age groups, which is indicative of an elevated pandemic risk of seasonal influenza at present.

**Characteristics of participants**

Between May and June 2021, 2666 individuals from Taian and Zibo cities in Shandong Province agreed to participate in this study. Of the participants, 4 did not complete the questionnaire, and 12 did not provide blood samples (Supplementary materials and methods). Finally, 2650 participants were included in the study (Fig. 1A). Among the 2650 participants, 820 (30.9%) were under the age of 15 years, 999 (37.7%) were aged between 15 and 59 years, and 831 (31.4%) were aged ≥60 years. Most of the participants were female (60.6%), and the proportion of female urban residents (62.7%) was significantly higher than that of female rural residents (58.3%; \( \chi^2 = 5.468, P = 0.019; \) Table 1). After the outbreak of COVID-19, people gradually developed the

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**Fig. 1** Inclusion criteria of participants and studies in the meta-analysis. (A) Participant selection. Light gold color represents urban communities, and light green color represents rural communities. (B) Study selection in the meta-analysis. *(1)–(5) represent the inclusion criteria for the literature screening in the meta-analysis, which have been described in the supplementary materials and methods.
practice of wearing face masks, and 2079 (78.5%) individuals often or always wore face masks when they went out. However, the proportion wearing face masks in rural participants (70.6%) was significantly lower than that in urban participants (85.5%; $\chi^2 = 88.603, P < 0.001$; Table 1). Notably, only 2.6% (69/2650) of the participants received influenza vaccines in the past two years, and the influenza vaccination rate of urban participants was higher than that of rural participants (3.3% vs. 1.8%; $\chi^2 = 7.508, P = 0.023$; Table 1), which was slightly higher than those between 2004 and 2014 in China (1.5%–2.2%) but was far lower than those of high-income countries [3,15]. The prevalence of self-reported influenza-like symptoms was 23.2% (614/2650) since October 2020 (Table 1), of which 19.4% (119/614) of the cases with influenza-like symptoms occurred within the last two weeks before the study (data not shown).

### Table 1 Demographic characteristics of the study participants

|                | Total, $n$ (%) | Urban, $n$ (%) | Rural, $n$ (%) | $P$ value |
|----------------|----------------|----------------|---------------|-----------|
| **Age**        |                |                |               |           |
| <15 years      | 820 (30.9)     | 443 (31.7)     | 377 (30.1)    | 0.354     |
| 15–59 years    | 999 (37.7)     | 524 (37.5)     | 475 (37.9)    |           |
| ≥ 60 years     | 831 (31.4)     | 430 (30.8)     | 401 (32.0)    |           |
| **Sex**        |                |                |               | 0.019     |
| Female         | 1606 (60.6)    | 876 (62.7)     | 730 (58.3)    |           |
| Male           | 1044 (39.4)    | 521 (37.3)     | 523 (41.7)    |           |
| **Wearing face masks** |            |                |               | <0.001    |
| Occasionally   | 541 (20.4)     | 188 (13.5)     | 353 (28.2)    |           |
| Regularly      | 2079 (78.5)    | 1194 (85.4)    | 885 (70.6)    |           |
| Missing        | 30 (1.1)       | 15 (1.1)       | 15 (1.2)      |           |
| **Influenza vaccination** |          |                |               | 0.023     |
| Yes            | 69 (2.6)       | 46 (3.3)       | 23 (1.8)      |           |
| No             | 2418 (91.2)    | 1285 (92.0)    | 1133 (90.4)   |           |
| Unclear        | 163 (6.2)      | 66 (4.7)       | 97 (7.7)      |           |
| **Influenza-like symptoms** |          |                |               | 0.332     |
| Yes            | 614 (23.2)     | 317 (22.7)     | 297 (23.7)    |           |
| No             | 1951 (73.6)    | 1041 (74.5)    | 910 (72.6)    |           |
| Unclear        | 85 (3.2)       | 39 (2.8)       | 46 (3.7)      |           |

Data are presented as $n$ (%). $P$ values were calculated by $\chi^2$ test or Mann–Whitney test, as appropriate.

Seroprevalence of antibodies against influenza viruses

For the 2650 participants, the overall seroprevalence rates of antibodies against A/H1N1pdm09, A/H3N2, B/Victoria, and B/Yamagata were 17.8% (95% CI 16.2%–19.5%), 23.5% (95% CI 21.7%–25.4%), 7.6% (95% CI 6.6%–8.7%), and 15.0% (95% CI 13.5%–16.5%, Table 2). The seroprevalence of antibodies in the young group was significantly higher than those in the middle-aged and elderly groups (A/H1N1pdm09: 36.3% vs. 9.6% and 9.3%, respectively ($\chi^2 = 280.158, P < 0.001$); A/H3N2: 42.0% vs. 15.1% and 15.3%, respectively ($\chi^2 = 225.755, P < 0.001$); and B/Yamagata: 20.2% vs. 15.8% and 8.8%, respectively ($\chi^2 = 43.430, P < 0.001$). Previous studies reported that school-aged children have the highest seroprevalence of seasonal and pandemic influenza strains likely because they have high population densities, which may increase potential for the transmission of influenza viruses [2,16]. The seroprevalence of antibodies for A/H1N1pdm09, B/Victoria, and B/Yamagata did not differ significantly between males and females (all $P > 0.05$). However, the seroprevalence of A/H3N2 in the female group (22.0%) was lower than that in the male group (25.7%; $\chi^2 = 4.673, P = 0.031$).

With respect to wearing face masks, the seroprevalence of antibodies against A/H3N2 in the group occasionally wearing masks was significantly higher than those in groups regularly wearing masks (30.3% vs. 21.7%; $\chi^2 = 17.762, P < 0.001$). However, the seroprevalence values of antibodies against A/H1N1pdm09, B/Victoria, and B/Yamagata were not statistically significant between the groups occasionally and regularly wearing masks. The seroprevalence of antibodies for the four influenza strains were significantly higher in the vaccinated group than those in the unvaccinated group: 42.0% vs. 16.2% for A/H1N1pdm09 ($\chi^2 = 62.568, P < 0.001$), 46.4% vs. 21.9% for A/H3N2 ($\chi^2 = 25.212, P < 0.001$), 21.7% vs.
These results suggested that the vaccination of influenza could provide consistent and effective antibody levels. Compared with participants without influenza-like symptoms in the past two years, those with influenza-like symptoms had higher seroprevalence of antibodies against A/H3N2, but no significant difference was found for other strains (Table 2).

### Meta-analysis for comparing the seroprevalence before and during the COVID-19 pandemic

To compare the seroprevalence of antibodies against influenza strains before and during the COVID-19 pandemic, we performed a meta-analysis to retrieve the seroprevalence of antibodies against the major circulating seasonal influenza strains in the scientific literature published since 2015. A total of 475 potentially relevant articles were retrieved after systematic search, of which 12 met all the inclusion criteria (Fig. 1B) [2–5,17–24]. A total of 9656 individuals were included in our meta-analysis (Table S1). Among them, 11, 10, 7, and 5 studies reported serum A/H1N1pdm09 [2–4,17–24], A/H3N2 [2–5,17–20,22,23], B/Victoria [2–4,18–20,22], and B/Yamagata antibodies [2–4,18,20], respectively (Table S1). Other information in the articles, such as study site, study participants, collection date of the serum sample, study design, method of serologic assays and cutoff, were also extracted (Table S2).

Only two studies reported the serum influenza antibodies in children or adolescents, and we only calculated the pooled estimates of seroprevalence in adults in the meta-analysis. Seroprevalence against A/H1N1pdm09 in the literature ranged from 7.8% to 7.3% for B/Victoria ($\chi^2 = 19.778$, $P < 0.001$), and 29.0% vs. 14.3% for B/Yamagata ($\chi^2 = 18.564$, $P < 0.001$). These results suggested that the vaccination of influenza could provide consistent and effective antibody levels. Compared with participants without influenza-like symptoms in the past two years, those with influenza-like symptoms had higher seroprevalence of antibodies against A/H3N2, but no significant difference was found for other strains (Table 2).
58.2% in adults, with the pooled estimate of 25.2% (95% CI 14.9%–39.2%) by using the random model (Fig. 2A and Table S3). Seroprevalence against A/H3N2 in the literature ranged from 7.3% to 96.1%, with the pooled estimate of 48.9% (95% CI 27.5%–70.8%) by using the random model (Fig. 2B and Table S3). Seroprevalence

![Fig. 2](https://example.com/fig2)

Subgroup analysis of the seroprevalence of influenza antibodies between the relevant literature and our study: (A) A/H1N1pdm09, (B) A/H3N2, (C) B/Victoria, and (D) B/Yamagata.
against B/Victoria in the included literature ranged from 2.4% to 59.6%, with the pooled estimate of 16.8% (95% CI 6.5%–36.8%) by using the random model (Fig. 2C and Table S3). Seroprevalence against B/Yamagata in the literature ranged from 2.9% to 33.7%, with the pooled estimate of 8.5% (95% CI 3.5%–19.1%) by using the random model (Fig. 2D and Table S3). By comparing the seroprevalence of influenza before and during the COVID-19 pandemic, we found a significantly low seropositive rate of influenza in adults following the onset of the COVID-19 pandemic particularly for A/H1N1pdm09 and A/H3N2. As expected, results of seroprevalence of influenza were consistent with the low reporting of laboratory-confirmed influenza cases [25]. In the sensitivity analysis, no individual study substantially influenced the pooled seroprevalence rates (Fig. S1). In addition, no publication bias was detected by funnel plots and Egger tests (A/H1N1pdm09: \( P = 0.743 \), A/H3N2: \( P = 0.855 \), B/Victoria: \( P = 0.517 \), and B/Yamagata: \( P = 0.098 \); Table S3).

Subgroup analysis was used to assess the differences in the seroprevalence of antibodies against influenza strains between the meta-analysis and our study. Accordingly, significant differences were observed in the seroprevalence rates of A/H1N1pdm09 (meta-analysis: 25.2%, 95% CI 14.9%–39.2%; our study: 9.4%, 95% CI 8.1%–10.9%; \( P < 0.01 \)) and A/H3N2 (meta-analysis: 48.9%, 95% CI 27.5%–70.8%; our study: 15.2%, 95% CI 13.6%–16.9%; \( P < 0.01 \))(Fig. 2A and 2B). No significant difference was found for the seroprevalence of antibodies against the B/Victoria and B/Yamagata strains between the data from the meta-analysis and our study (Fig. 2C and 2D). These findings indicated that antibodies for A/H1N1pdm09 and A/H3N2 might have lowered after the onset of the COVID-19 pandemic.

By the end of December 2021, an estimated 57.6% of the global population has received at least one dose of a COVID-19 vaccine [26]. Therefore, the relaxation of NPIs and other pandemic prevention measures, such as the abolition of the “lockdowns” and the easing of restrictions on social and physical distancing, has been implemented in some countries. However, China reported 110,691 influenza cases in November 2021, compared with 22,783 cases in November 2020 [6]. The global circulation of influenza viruses has also been on the rise since autumn 2021 [27]. Therefore, relaxing the NPIs will likely increase the pandemic risk of influenza.

**Summary**

We reported low seroprevalence to four influenza vaccine strains among the general public in Shandong Province, China during the COVID-19 pandemic, which highlighted the increased pandemic risk of seasonal influenza in northern China and potentially elsewhere. We strongly recommend that the vaccination of seasonal influenza and SARS-CoV-2 should be substantially increased to contain the COVID-19 pandemic and lower the pandemic risk of seasonal influenza. Active influenza surveillance should also be performed longitudinally to assess influenza antibody titers among the general public and monitor the genetic variation of influenza viruses for the mitigation of future influenza pandemics.

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**Compliance with ethics guidelines**

Chuansong Quan, Zhenjie Zhang, Guoyong Ding, Fengwei Sun, Hengxia Zhao, Qinghua Liu, Chuannin Ma, Jing Wang, Liang Wang, Wenbo Zhao, Jinjie He, Yu Wang, Qian He, Michael J. Carr, Dayan Wang, Qiang Xiao, and Weifeng Shi declare that they have no actual or potential competing financial interests. This study was approved by the Ethics Committee of Shandong First Medical University & Shandong Academy of Medical Sciences (approved No. R202105170154). The study conformed to the principles of the Declaration of Helsinki, and the standards of Good Clinical Practice defined by the International Conference on Harmonization. Written informed consent was obtained from all adult participants, and from legal guardians for participants ≤ 15 years old.

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