

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

## Supplementary Appendix

(This appendix has been provided by the authors to give readers additional information about their work)

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## Appendix: Questionnaire of influenza serum antibody used in our study

### DEMOGRAPHIC INFORMATION

Questionnaire code: _____		Serum code: _____	
Community:	<input type="checkbox"/> Zhouidian (Taian urban community) <input type="checkbox"/> Fangcun (Taian rural community) <input type="checkbox"/> Daxinzhuang (Zibo urban community) <input type="checkbox"/> Badou (Zibo rural community)		
Participant's name: (family name), (given name(s)) _____			
Age:	<input type="checkbox"/> <15 years <input type="checkbox"/> 15~59 years <input type="checkbox"/> ≥ 60 years		
Sex:	<input type="checkbox"/> Male <input type="checkbox"/> Female		

### VACCINATION INFORMATION

Frequency of wearing a mask when you go out every day:	<input type="checkbox"/> Occasionally <input type="checkbox"/> Regularly
Receiving influenza vaccine in the past 2 years:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear
Influenza vaccine type:	<input type="checkbox"/> Trivalent lysis inactivated influenza vaccine (Date: _____) <input type="checkbox"/> Tetravalent lytic inactivated influenza vaccine (Date: _____) <input type="checkbox"/> Unclear (Date: _____)

### HISTORY OF INFLUENZA-LIKE SYMPTOMS

Exposure to influenza-like symptoms since October 2020:	<input type="checkbox"/> Yes (Number of influenza-like symptoms: _____) <input type="checkbox"/> No (If answer this term, skip to end) <input type="checkbox"/> Unclear (If answer this term, skip to end)
Exposure to influenza-like symptoms in the past two weeks:	<input type="checkbox"/> Yes <input type="checkbox"/> No (If answer this term, skip to end)
Treatment for influenza-like symptoms:	<input type="checkbox"/> Yes <input type="checkbox"/> No (If answer this term, skip to end) <input type="checkbox"/> Unclear (If answer this term, skip to end)
The way of treatment for influenza-like symptoms:	<input type="checkbox"/> Purchase drugs in pharmacies <input type="checkbox"/> Treatment in outpatient or emergency <input type="checkbox"/> Treatment in hospitalization <input type="checkbox"/> Resting at home without medication <input type="checkbox"/> Unclear

Investigator: \_\_\_\_\_ Date: \_\_\_\_\_

**Table S1** Characteristics of the included studies on seroprevalence of antibodies against the influenza strains

First author, year	Virus strain (A/H1N1)	Virus strain (A/H3N2)	Virus strain (B/Victoria)	Virus strain (B/Yamagata)	Positive no./N (A/H1N1)	Positive no./N (A/N3N2)	Positive no./N (B/Victoria)	Positive no./N (B/Yamagata)	
Gao, 2016	A/California/7/2009	A/Texas/50/2012	B/Massachusetts/02/2012	NR	136/292	191/292	174/292	NR	
Zhu, 2018	NR	A/Hong Kong/4801/2014	NR	NR	NR	318/435	NR	NR	
Tam, 2018	A/California/07/2009	A/Switzerland/9715293/2013	B/ Brisbane/60/2008	B/Phuket/3073/2013	499/878	550/878	286/878	82/878	
Xu, 2021	A/Michigan/45/2015	A/Singapore/0019/2016	INFIMH-16- huan-Gaoxin/531/2018	B/Colorado/06/2017;B/Sic huan-Gaoxin/531/2018	B/Phuket/3073/2013	Total: 61/616 Children: 20/203 Adults: 41/413	Total: 74/616 Children: 35/203 Adults:39/413	Total: 17/616 Children: 7/203 Adults:10/413	Total: 20/616 Children: 8/203 Adults:12/413
Tiffany, 2020	A/California/7/2009	A/Switzerland/9715293/2013	B/Brisbane/60/2008	B/Phuket/3073/2013	466/800	587/800	248/800	78/800	
Quan, 2019	A/California/04/2009	A/Beijing/CAS0001/2007	NR	NR	348/2124	772/2124	NR	NR	
Shu, 2020	A/Michigan/45/2015	A/Singapore/INFIMH16-0019/2016	B/Colorado/06/2017	B/Phuket/3073/2013	8/68	5/68	4/68	2/68	
Wang, 2017	A/California/7/2009	A/Switzerland/9715293/2013	B/Brisbane/60/2008	B/Phuket/3073/2013	Total: 929/3536 Children and adolescents: 662/1780 Adults: 267/1756	Total: 2061/3536 Children and adolescents:1254/1780 Adults: 807/1756	Total:160/3536 Children and adolescents:72/1780 Adults: 88/1756	Total: 1144/3536 Children and adolescents: 553/1780 Adults: 591/1756	
Wang, 2015	A/California/7/2009	A/Perth/16/2009	B/Brisbane/60/2008	NR	12/26	25/26	9/26	NR	
Wu, 2015	A/California/07/2009	NR	NR	NR	45/502	NR	NR	NR	
Ma, 2015	A/California/07/2009	A/Victoria/210/2009	NR	NR	9/115	36/115	NR	NR	
Chen, 2015	A/Guangdong/1057/2010	A/Brisbane/10/2007(H3N2)	NR	NR	134/264	NR	NR	NR	

No. number; NR, not reported.

**Table S2** Other characteristics of the included studies on seroprevalence of antibodies against the influenza strains

First author, year	Study site	Study participants	Age (years)	Collection date of the serum	Study design	Test	Cutoff
Gao, 2016	Beijing	Healthy servicemen	18-34	2014.9	RCT	HAI	1:40
Zhu, 2018	Hong Kong	Hospital in- and out-patients not symptomatic for influenza or any acute respiratory illness	≥18	2017.07-2017.09	Cross-sectional study	HAI	1:40
Tam, 2018	Hong Kong	Older adults	≥75	Summer of 2015	Follow-up study	HAI	1:40
Xu, 2021	Changzhi	Local residents	5-59	2018.11-2019.05	Follow-up study	HAI	1:40
Tiffany, 2020	Hong Kong	Older adults	≥75	2015.10-2015.12	Observational study	HAI	1:40
Quan, 2019	Seven areas in China	Poultry workers	≥18	2014.12-2016.04	Longitudinal seroepidemiologic study	HAI and MN	1:40 and 1:80
Shu, 2020	Shenzhen & Changzhou	Volunteers	≥18	2018.11-2019.12	RCT	HAI	1:40
Wang, 2017	Lianyungang	Healthy children, adolescents and adults	≥3	2016.01-2016.08	RCT	HAI	1:40
Wang, 2015	Guangdong	Healthcare workers	24-52	2011.03	Paired experimental design	HAI	1:40
Wu, 2015	Guangdong	Swine workers and blood donors	Swine workers: 39.7±12.4 Blood donors: 32.3±16.8	2013.04-2014.05	Observational study	HAI	1:40
Ma, 2015	Guangdong	Swine workers and urban residents as control	≥18	2014.07-2014.09	Cross-sectional study	HAI	1:40
Chen, 2015	Guangdong	Animal-exposed participants and volunteers with no occupational animal exposure	mean: 38.4	2013.12-2014.01	Cross-sectional study	HAI	1:40

RCT, randomized controlled trial; HAI, hemagglutination inhibition assays; MN, microneutralization assay.

**Table S3** Meta-analysis of the seroprevalence of antibodies against the influenza strains in adults

Influenza subtype/lineage	Seropositive (%)	95% CI (%)	<i>I</i> <sup>2</sup> (%)	<i>P</i> <sub>heterogeneity</sub>	Statistical model	Egger's test
A/H1N1pdm09	25.2	14.9-39.2	99.1	<0.001	Random	0.743
A/H3N2	48.9	27.5-70.8	98.8	<0.001	Random	0.855
B/Victoria	16.8	6.5-36.8	98.9	<0.001	Random	0.517
B/Yamagata	8.5	3.5-19.1	98.8	<0.001	Random	0.098

CI: confidence interval.

## **Materials and methods**

### **Study design and participants**

We conducted a cross-sectional study to assess the seropositivity of the four major influenza subtypes/lineages. To assess the risk of influenza pandemic in next season, participants were recruited from four communities in Taian (n=2) and Zibo (n=2) cities between May and June 2021. The two communities of each city included an urban and rural area, respectively. The minimum sample size was estimated based on a 2%-allowable error and a type I error rate (two sided) of 0.05. It was performed using PASS (version 15, NCSS, LLC, USA) by the predictor variable, i.e., overall seroprevalence of 24.5% of seasonal influenza viruses, which was the overall seroprevalence of seasonal influenza infection in Changzhi in northern China during the 2018-2019 influenza season. If we assume a similar seroprevalence, 2190 participants are sufficient to detect this rate allowing for a loss of power of 20%. In this study, each community included three age groups: < 15 years (young group), 15-59 years (middle-aged group) and  $\geq 60$  years (elderly age group), with each age group including > 190 participants.

A questionnaire was designed to gather information from the participants via face-to-face interviews by trained graduate students. The information included demographic data (community, age, and sex), vaccination information (seasonal influenza vaccines), and history of influenza-like symptoms since October 2020 (Supplementary questionnaire).

## Serologic Assays

A single venous whole blood sample from each participant was collected by phlebotomy nurses between May and June 2021 using a Vacutainer blood collection tube (Becton Dickinson, <https://www.bd.com>). Sera was divided into two aliquots and frozen at -80°C until use.

The collected sera were tested by hemagglutination inhibition (HAI) assays using the four vaccine strains recommended by the World Health Organization (WHO) for the 2020-2021 northern hemisphere influenza season: A/Guangdong-Maonan/SWL1536/2019 (A/H1N1pdm09), A/Hong Kong/2671/2019 (A/H3N2), B/Washington/02/2019 (B/Victoria), and B/Phuket/3073/2013 (B/Yamagata). These influenza strains were provided by the Chinese National Influenza Center, Chinese Center for Disease Control and Prevention (China CDC), a WHO collaborating center for reference and research on influenza.

The procedure of the HAI assay was performed following standard WHO guidelines. The standardized human type O red blood cells (RBCs) were added to the sera to remove non-specific agglutinins (10% RBCs) and the adsorbed sera were transferred to remove the packed RBCs. Serum samples were tested with a starting dilution of 1:20, followed by 2-fold serial dilutions to 1:640. The HAI titers were calculated as the reciprocal of the serum dilution at which erythrocyte agglutination was complete. In this study, HAI titers  $\geq 1:40$  were considered seropositive.



## Statistical analysis

A combination of descriptive and meta-analyses was adopted to estimate the seroprevalence of antibody titers against influenza strains in Shandong Province, China. The descriptive analysis was performed to evaluate the seroprevalence and geometric mean titers (GMT) of antibodies against influenza strains. Qualitative data were presented as a rate or percentage. Seroprevalence was expressed as a percentage and 95% confidence interval (CI). GMT and 95% CI for HAI assays were also calculated for antibody titers. Chi-square tests were used to compare qualitative data between groups. Since antibody titers against A/H1N1pdm09, A/H3N2, B/Victoria, and B/Yamagata are dependent variables and do not conform to normal distributions, we applied a logarithmic transformation of the antibody titers for statistical tests. We used a *t* test or a one-way analysis of variance (ANOVA) to compare the differences of antibody titers between groups. Statistical significance was defined as *P*-value < 0.05 and all the statistical analyses mentioned above were performed using SPSS (version 25, IBM, New York, USA).

In addition, we performed a meta-analysis of the scientific literature published since 2015 to investigate the seroprevalence of antibodies against influenza viruses in China. We systematically searched PubMed, Embase and Web of Science to identify the relevant literature. The search terms included subject term and free words for H1N1, H3N2, Victoria, Yamagata and serum, and the full search strategy of the three databases is listed in Table S4. The subjects of these studies were defined as humans, the

publication date of studies was set as after 2015, the study area was China, and the language of articles was limited to English and Chinese. References from all searched literature were manually reviewed to identify additional studies. To be included in the meta-analysis, a study met five criteria: (1) serum samples were collected after the 2009 H1N1 pandemic; (2) the antigens of influenza viruses were from humans; (3) sera were studied with HAI assay; (4) HAI titers of  $\geq 1:40$  were considered seropositive; (5) seroprevalence or data to calculate it was reported.

We extracted the following items from each study: name of the first author, year of publication, study site, study participants, age of participants, collection date of the serum, study design, influenza virus strains, and data to calculate seroprevalence. For the randomized controlled trial (RCT) studies included, the seroprevalence of the baseline or control was extracted. For studies involving animal-exposed participants, we calculated the seroprevalence from the control. Article screening and data extraction were performed by two independent investigators to minimize potential errors.

The meta-analysis was completed using the meta package in the R statistical software (version 3.6.2, R Foundation for Statistical Computing). The Logit transformation of seroprevalence of antibodies was used to allow for studies with seroprevalence less than 20%. The  $Q$  test and the  $I^2$  statistic were used to assess the heterogeneity among studies. In instances of substantial heterogeneity ( $I^2 > 50\%$ ), we estimated the seroprevalence using the random-effect meta-analysis model. Otherwise, the fixed effects model was applied as the pooling method. We also used the subgroup analysis to assess the

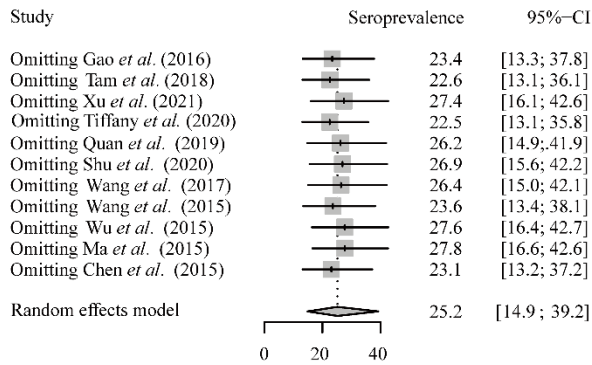
discrepancies in the seroprevalence between existing literature and our study. A sensitivity analysis was performed to validate the stability of the pooled seroprevalence of the included literature by removal of every individual study. A funnel plot and Egger linear regression test were estimated to assess the publication bias. All tests were 2-sided and a *P*-value of less than 0.05 was considered statistically significant.

**Table S4** Search strategy for three databases

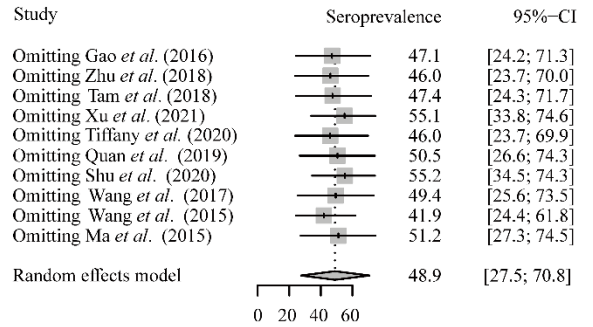
Database	Step	Searching strategy	Number of articles
PubMed	#1	"influenza a virus, h1n1 subtype"[MeSH]	16281
	#2	"influenza a virus, h3n2 subtype"[MeSH]	4501
	#3	"Influenza B virus"[MeSH]	4436
	#4	"sero"[All Fields]	7271
	#5	"serum"[MeSH] OR "serum"[All Fields] OR "serums"[All Fields] OR "serum s"[All Fields] OR "serumal"[All Fields]	1181156
		#1 OR #2 OR #3	21770
	#7	#4 OR #5	1187063
	#8	#6 AND #7	1378
	#9	Publication Date: 2015/01/01-2021/12/31	414
	#10	Language: English and Chinese	410
	#11	Species: Humans	307
Embase	#1	'influenza a virus (h1n1)/exp	15026
	#2	'influenza a virus (h3n2)/exp	5403
	#3	'influenza virus b/exp	6574
	#4	'serum'/exp	207575
	#5	#1 OR #2 OR #3	22374
	#6	#4 AND #5	85
	#7	#6 AND (2015:py OR 2016:py OR 2017:py OR 2018:py OR 2019:py OR 2020:py OR 2021:py) AND 'human'/de	24
Web of Science	#1	TS=H1N1	18902
	#2	TS=H3N2	4780
	#3	TS=Victoria	16082
	#4	TS=Yamagata	869
	#5	TS=sero	3257
	#6	TS=serum	587176
	#7	((#1) OR #2) OR #3) OR #4	36842
	#8	(#5) OR #6	589419
	#9	(#7) AND #8	1750
	#10	Publication Years: 2015 or 2016 or 2017 or 2018 or 2019 or 2020 or 2021	823
	#11	Document Types: Articles	788
	#12	Countries/Regions: PEOPLES R CHINA	144

**Fig. S1.** Sensitivity analysis of the pooled seroprevalences for A/H1N1pdm09 (A), A/H3N2 (B), B/Victoria(C) and B/Yamagata (D).

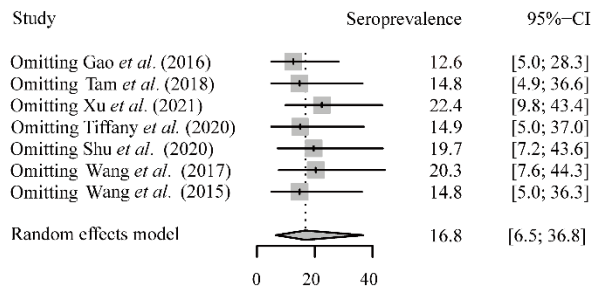
**A**



**B**



**C**



**D**

