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Mild and asymptomatic influenza B virus infection among unvaccinated pregnant persons: Implication for effectiveness of non-pharmaceutical intervention and vaccination to prevent influenza

Liling Chen[a], **Min Z. Levine**[b], **Suizan Zhou**[b], **Tian Bai**[c], **Yuanyuan Pang**[a], **Lin Bao**[a], **Yayun Tan**[a], **Pengwei Cui**[a], **Ran Zhang**[b], **Alexander J. Millman**[b], **Carolyn M. Greene**[b], **Zhongwei Zhang**[d], **Yan Wang**[e], **Jun Zhang**[a,*]

[a]Suzhou Center for Disease Control and Prevention, Suzhou, Jiangsu Province, PR China

[b]U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA

[c]Chinese National Influenza Center, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, PR China

[d]Suzhou Municipal Hospital, Suzhou, Jiangsu Province, PR China

[e]Wuzhong Maternal and Child Health Care Institute, Suzhou, Jiangsu Province, PR China

Abstract

Background: We estimated symptomatic and asymptomatic influenza infection frequency in community-dwelling unvaccinated pregnant persons to inform risk communication.

Methods: We collected residue sera from multiple antenatal-care blood draws during October 2016–April 2017. We determined influenza infection as seroconversion with ≥ 4-fold rise in antibody titers between any two serum samples by improved hemagglutinin-inhibition assay including ether-treated B antigens. The serology data were linked to the results of nuclei acid testing (rRT-PCR) based on acute respiratory illness (ARI) surveillance.

Results: Among all participants, 43 %(602/1384) demonstrated serology and/or rRT-PCR evidenced infection, and 44 %(265/602) of all infections were asymptomatic. ARI-associated rRT-PCR testing identified only 10 %(61/602) of total infections. Only 1 %(5/420) of the B Victoria cases reported ARI and had a rRT-PCR positive result, compared with 33 %(54/165) of the H3N2 cases. Among influenza ARI cases with multiple serum samples, 19 %(11/58) had seroconversion to a different subtype prior to the illness.

*Corresponding author. sz_zhangj@163.com (J. Zhang).

Disclaimer.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conclusions: The incidence of influenza B infection in unvaccinated pregnant persons is underestimated substantially. Non-pharmaceutical intervention may have suboptimal effectiveness in preventing influenza B transmission due to the less clinical manifestation compared to influenza A. The findings support maternal influenza vaccination to protect pregnant persons and reduce consequent household transmission.

Keywords

Pregnancy; Influenza virus infection; Seroconversion

1. Introduction

Pregnant persons infected with influenza virus are at increased risk for illness complications and influenza-related morbidity and mortality [1,2]. An active surveillance study of pregnant persons in China found a 0.7–2.1 % risk of seasonal influenza-associated acute respiratory illness (ARI) per month during epidemic periods in 2015–2018 [3]. Another multisite study among pregnant persons reported a comparable 0.7–0.9 % risk of influenza ARI illness in 2017 and 2018 seasonal epidemics [4]. However, these data likely underestimate the risk of influenza virus infections in this population because they do not include infections with non-ARI symptoms or asymptomatic infections.

Misconceptions about influenza virus infection risks during pregnancy can create challenges for effectively promoting prevention measures such as influenza vaccination during pregnancy [5]. We estimated symptomatic and asymptomatic influenza virus infection frequency in a cohort of persons during pregnancy from October 2016 to April 2017 to better understand the incidence of influenza virus infection during pregnancy with the goal of developing targeted risk communication messages for healthcare providers and the public in China.

2. Methods

2.1. Study population and design

We conducted a serology sub-study within prospective active respiratory illness surveillance among a cohort of persons during pregnancy enrolled from two antenatal care facilities in Suzhou, China. A description of the complete cohort profile and methods was published previously [6]. For this sub-study, we included persons in different trimesters of pregnancy who had regular antenatal healthcare visits in any of the study facilities in October 2016 and were living in and planning to deliver in Suzhou.

We collected residue serum samples drawn during routine antenatal care visits in the study period. Some participants had multiple samples available at the end of the study period as they may have had blood drawn repeatedly throughout the study period from routine care around gestational weeks 12, 16, 22, 26, 30, 37 or delivery. Persons with only one serum sample available throughout the follow-up period were excluded from this analysis. We also confirmed no influenza vaccination 12 months prior to enrollment and during follow-up in the enrollees. Laboratories at the antenatal care facilities stored the sera after routine tests

at 4 °C for no more than one week. Participants' sera were transported at 4 °C to Suzhou Center for Disease Control and Prevention (Suzhou CDC) and stored at −80 °C until testing.

2.2. Serology methods

Serum samples were tested with the hemagglutinin-inhibition assay (HI) in the Suzhou CDC laboratory to determine antibody titers against local circulating influenza strains during the study period. The HI assay used 0.5 % turkey red blood cells (RBCs) for influenza A(H1N1)pdm09 and influenza B viruses, and 0.75 % guinea pig RBCs for influenza A(H3N2) as it reacted poorly to turkey RBCs. As local circulating influenza strains showed antigenic similarity to four vaccine strains in the 2016–2017 northern hemisphere season (data not shown), the vaccine strains or vaccine-like viruses were used: A/California/7/2009 (pdmH1N1), A/HK/7127/2014 (H3N2, A/Hong Kong/4801/2014-like virus), B/Brisbane/60/2008 (BV), and B/Phuket/3073/2013 (BY). Viruses were provided from the Chinese National Influenza Center and propagated in Madin-Darby Canine Kidney cells. Influenza B viruses were treated with ether. To inhibit interference of the neuraminidase protein of influenza A(H3N2) virus, oseltamivir (40 nM) was added in the HI assay when measuring antibodies against influenza A(H3N2). Serum samples were treated with receptor destroying enzyme (RDE, DENKA SEIKEN II) at 37 °C for 16–18 h followed by incubation at 56 °C for 30 min. For sera that showed nonspecific agglutination with RBCs, adsorption with packed RBCs was performed before measurement. Serum samples were duplicate titrated in 2-fold dilutions in phosphate-buffered saline.

2.3. Data linkage

Serology data were linked to participants' demographic characteristics and illness surveillance data, and illness triggered real-time reverse transcription-polymerase chain reaction (rRT-PCR) testing of respiratory samples (Fig. 1). In the primary cohort surveillance, study nurses conducted twice weekly phone and text message follow-ups to identify new episodes of ARI, defined as ≥ 1 respiratory symptom (cough, sore throat, stuffy nose, chest pain, difficulty breathing) and ≥ 1 systemic symptom (feverish, temperature ≥ 38 °C, chills, headache), or ≥ 2 respiratory symptoms [6]. Combined nasal and throat swabs were collected within 10 days of ARI onset, and tested by rRT-PCR for typing and subtyping/lineage classification of influenza viruses in the Suzhou CDC laboratory within 72 h of sample collection [6].

2.4. Result definition

Seroconversion between any two time points of sera collection was used as an indication of serological evidence of influenza virus infection. Seroconversion criteria were: 1) ≥ 4-fold rise of HI antibodies between any pair of sera, and 2) a convalescent titer ≥ 40. Antibody waning following seroconversion was defined as at least a 4-fold decrease between the convalescent titer and a post-infection titer. Among rRT-PCR confirmed positive participants with multiple timepoint sera collections, repeat infection was defined as seroconversion to a different influenza strain prior to the rRT-PCR confirmed illness. Seroconversion to two influenza strains during the same period or to a different influenza strain after the rRT-PCR confirmed illness was not defined as repeat infection as this scenario may have indicated co-infection and delayed immune-response.

2.5. Statistical analysis

To estimate the proportion of influenza virus infections during pregnancy, we calculated percentages and 95 % confidence intervals (CI) assuming a binomial distribution. We compared the characteristics and the percentage of each symptom between influenza virus-infected and non-infected persons using a χ^2 or Fisher's exact test where appropriate. All tests were two sided, and a $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using R version 3.6.1 (R Foundation for Statistical Computing).

2.6. Ethics

This study was approved by the Institutional Review Board (IRB) of Jiangsu Provincial Center for Disease Control and Prevention, and the United States (U.S.) Centers for Disease Control and Prevention relied upon the Jiangsu Provincial IRB.

3. Results

3.1. Participants

1384 persons during pregnancy were enrolled in October 2016 with median age of 28 years (range: 19–47). 27 (2 %) reported underlying disease, 981 (71 %) were in the second trimester (13–27 gestational weeks) and all were unvaccinated for seasonal influenza 12 months prior to enrollment and throughout follow-up (Table 1).

3.2. Influenza virus infection

From October 2016–April 2017, 602 (43 %) of all enrollees had laboratory evidence of influenza virus infection. Of the 602 cases, 61 (10 %) reported ARI and had respiratory samples that were rRT-PCR positive for influenza, while 541 (90 %, 95 % CI: 87 to 92) only had serologic evidence of influenza virus infection. No case with respiratory sample positive for influenza by rRT-PCR was negative by serology (Table 1).

Among the 602 cases, 337 (56 %) reported at least one symptom during the study period, and 272 (45 %) met the ARI case definition. The remaining 265 (44 %) cases were asymptomatic. Among 272 influenza-positive ARI cases, 78 % (211/272, 95 % CI: 72 to 82) were detected by serology only despite the collection of respiratory samples and testing with rRT-PCR. Among all 337 influenza cases with any symptoms, 82 % (276/337, 95 % CI: 77 to 86) were detected by serology only (Table 1).

Influenza B virus infection was associated with a higher proportion of asymptomatic and non-ARI symptomatic infection compared with influenza A. The proportion of participants with any seroconversion to B Victoria was 30 % (420/1384). However, only 1 % (5/420) of the B Victoria infection cases reported ARI and had a rRT-PCR positive result for influenza; the remaining 99 % (415/420, 95 % CI: 97 to 99) of the B Victoria infections were identified by serology only. In comparison, of the 165 influenza A (H3N2) cases, 33 % (54/165) reported ARI and had a positive rRT-PCR result, and 67 % (111/165, 95 % CI: 60 to 74) of H3N2 cases was detected by serology only (Table 1).

Among all participants, those more likely to have influenza virus infection by either rRT-PCR or serology were of younger age, earlier trimester, and reported ARI or any symptom such as cough, temperature ≥ 38 °C, fever/feverish/chills, sore throat, runny nose, chest pain, headache, muscle ache and fatigue (Table 1).

3.3. Antibody waning, repeat infection and cross-reactivity

Among 20 participants who had post-infection sera available for at least 3 months' observation after the rRT-PCR confirmed positive illness, 5 (25 %) had an observed ≥ 4-fold antibody titer decrease during the study period (Fig. 2).

Among 58 rRT-PCR confirmed positive participants with multiple timepoint sera collection available throughout the season, 11 (19 %) had an earlier infection with a different subtype/lineage detected by seroconversion before their illness onset date. Among these, 9 influenza A(H3N2) cases by rRT-PCR had an earlier sero-conversion corresponding to influenza B infection (7 influenza B Victoria and 2 influenza B Yamagata); 1 influenza A(H1N1) pdm09 case by rRT-PCR had an earlier seroconversion for an influenza B Victoria infection. In addition, 1 influenza B Victoria case confirmed by rRT-PCR had an earlier infection for an influenza B Yamagata infection (Table 2) (Fig. 3).

3 of 4 influenza B Victoria cases by rRT-PCR had a rise in antibody titer against influenza B Yamagata at the same time-period as the rise in antibody titer against influenza B Victoria.

4. Discussion

During October 2016–April 2017, 43 % of community-dwelling persons during pregnancy enrolled in our study had laboratory evidence of influenza virus infection, and 44 % of those infections were asymptomatic. ARI-triggered rRT-PCR testing identified only 10 % of the total infections in our study population. We found that those with laboratory evidence of influenza B virus infection frequently had atypical symptoms or were asymptomatic compared to those infected with influenza A.

This is the first study to assess influenza virus infection risk during pregnancy by both rRT-PCR and seroconversion, and we reported a high proportion of infection, and more specifically asymptomatic infection in this population. Although there are no comparable community population data from pregnant persons, the Flu Watch cohort study in England reported rates of seroconversion against any circulating influenza virus among unvaccinated community population aged 16 to 44 years old as 22 % in the 2006/07 influenza season, 17 % in 2007/08 and 32 % in 2008/09 [7]. Several factors may account for the higher seroconversion rate of young adults seen in our study. First, we used ether-treated B antigens which can increase the sensitivity of the HI testing, and therefore enhance the detection of infections caused by influenza B. With non-ether treated B antigens, a community household serology study in Hong Kong showed half of PCR-confirmed influenza B virus infection cases had no detectable rise in HI titers against the infecting strain [8]. In a previous validation study, non-ether treated B antigens were five times less likely to be detected compared with ether-treated B antigens [9]. Second, in our study multiple blood draws

during the winter season may have increased the chances of detecting seroconversion in the setting of possible antibody waning or a delay in immune response.

Our findings suggest that influenza B virus infection in our study population was less likely to have clinical manifestations compared with influenza A virus infection and therefore might be under-estimated in existing surveillance systems that rely upon clinical illness for sampling and testing. In our study, persons with influenza A virus infection, especially A(H3N2) infection, presented with symptoms more likely to meet the ARI case definition than patients with influenza B virus infection. Therefore, when influenza A and influenza B viruses are co-circulating, influenza A virus infection may be more likely to be detected in clinical care-based surveillance systems that sample and test patients with typical ARI. During COVID pandemic, global wide non-pharmaceutical interventions (NPI), including social distancing, masking, hand hygiene, and travel restriction significantly reduced influenza transmission [10]. During post-COVID pandemic period, when NPI gradually lifted, it's population-level effectiveness in preventing influenza transmission reduced. And the global influenza activity increased consequently. In area continuing strict NPI including international travel restriction and immigrant quarantine, such as in China, influenza B activity resumed much faster than influenza A [11]. It may be because of less compliance to hand hygiene, masking, or social distancing in patients with mild clinical manifestation of influenza B virus infection. Furthermore, NPI such as temperature check lack sensitivity to identify these patients, thus they could maintain active social activities. It could be anticipated that, in future influenza pandemic, NPI would be less effective in preventing community transmission after mass vaccination attenuating disease severity. As a public health measure, maternal influenza vaccination could more sustainably protect pregnant persons and reduce consequent household transmission to populations at higher risk for influenza complications such as children by either symptomatic or asymptomatic infection [12 13].

In this study, we demonstrated repeat infection with a different influenza virus among unvaccinated persons during pregnancy over a typical winter epidemic season. Repeat infection is likely due to co-circulation of multiple strains during one season and lack of cross-protection from antibodies against different strains [14]. The national influenza surveillance reports from southern China, indicated that both influenza A and influenza B viruses were co-circulating during the study period, with influenza A(H3N2) pre-dominating in the early winter of 2016, followed by increased circulation of influenza A(H1N1)pdm09, B Victoria, and B Yamagata in the spring of 2017 [15]. Even though influenza A(H3N2) virus was the predominant subtype early in the season in the national surveillance system, our study detected repeat infection with influenza B virus prior to infection with A(H3N2), likely because most influenza B virus infections detected in our study population did not manifest with ARI and therefore would not have been detected by national surveillance.

Increases in HI titers may not always lead to sero-protection. As observed in a previous vaccination trial study, while vaccination boosted antibody response, subsequent infections still occurred in some vaccinees [16]. Additional studies focused on sero-protection failure are warranted. Of note, the increases in HI titers we observed might be cross-reactive, with cross-lineage influenza B and heterologous influenza A antibody response [17,18]. A

community household study in Hong Kong found that certain influenza B lineage infections lead to cross-lineage reaction with > 4-fold rise in titer of the opposing lineage, with higher antibody titer increases after infection observed in children compared with adults [8]. In addition, due to “antigenic seniority”, the HI titers for influenza B lineage may be highest for the strain that is most similar to the one encountered earliest in life [19]. However, the increase in HI titers suggests new infection did occur, leading to non-specific or specific antibody response.

ARI-triggered rRT-PCR testing identified only 10 % of all influenza virus infections in our study population over one influenza season. This finding may not be generalizable to other populations that may have higher risk of presenting with more severe respiratory illness, such as populations with underlying conditions. Our finding is also not comparable to studies with more frequent sampling strategies for rRT-PCR testing regardless of symptoms, or to studies within healthcare settings where the population may not represent the community [20]. Our study population represented community-dwelling pregnant persons who are more likely to have mild or asymptomatic infections compared to hospitalized patients. In the community household study (PHIRST) in South Africa, influenza infection average rate was 43.6 per 100 person-seasons. Similarly, only 56 % of infections were symptomatic, and 17 % of individuals who had one influenza infection had a repeat influenza infection during the same season [13]. The community household study in Hong Kong identified 13 % paucisymptomatic (1 symptom only) and 11 % asymptomatic cases among healthy individuals who were household contacts of index influenza cases [21]. A *meta*-analysis of the unvaccinated arms of influenza vaccine randomized controlled trials also found half of influenza infections were asymptomatic. The prevalence of asymptomatic cases ranged from 5.2 % to 35.5 % and subclinical cases with illness that did not meet the criteria for acute respiratory or influenza-like illness ranged from 25.4 % to 61.8 % [22]. Thus, symptom-triggered rRT-PCR testing alone would have decreased detection of infection cases in this population compared to a case finding strategy that employed molecular and serologic techniques.

Our study enrolled unvaccinated persons in a country where only 2 % of the population was vaccinated [23]. Sero-conversion in this study can be attributed to infection rather than vaccination. China CDC has recommended maternal influenza vaccination since 2014. However, pregnancy was listed as a contraindication for seasonal influenza vaccination in the Chinese Pharmacopeia and the vaccine package insert. Although pregnancy was removed as a contraindication from the China Pharmacopeia in 2020, it continues to be listed on the package insert contraindications. Our findings of substantial influenza virus infection among pregnant persons in Suzhou, China, support China CDC’s maternal influenza vaccination recommendation and can be used for risk communication to both pregnant persons and healthcare providers.

Our study has the following limitations. First, we were unable to perform microneutralization assays to confirm HI positive result. We undertook the following steps to increase the confidence in the quality of our results. Recognizing that fluctuations in ambient room temperatures or small reagent differences may potentially affect virus-antibody interactions and impact a sample’s titer reading [24], we tested all samples from

each enrollee in one day to reduce this bias as much as possible. Furthermore, we performed duplicates of each assay. If the 2 titers from each set of duplicates differed by > 4-fold, we repeated the testing. With titers from multiple replicates, we used a geometric mean titer as the final titer. In addition, we used ≥ 4-fold antibody titer increases between paired sera to determine influenza virus infection rather than relying upon the seroprevalence from a single sera titer, thus reducing the number of false positives for infection. Second, although our study observed several cases of antibody waning after influenza virus infection, the sample size of participants with multiple post-infection sera available was not large enough to explore the risk factors of this sub-group further. Third, the baseline influenza B HI titer observed in this study could represent the residue of previous-season infections. The sero-conversion we observed may miss those infections without significant titer increase especially if the previous-season infection residue titer was still high [16,25].

5. Conclusion

The incidence of influenza virus infection in community-dwelling persons during pregnancy is substantial. Nearly-two in five unvaccinated pregnant persons in our study had laboratory evidence of influenza virus infection during the 2016–2017 winter season. Most influenza B virus infections were associated with non-ARI or were asymptomatic. Non-pharmaceutical intervention may have suboptimal effectiveness in preventing influenza B transmission due to the less clinical manifestation compared to influenza A. Although further research on the impact of influenza virus infection on pregnancy and infant health outcomes is needed, our findings can support risk communication to both pregnant persons and healthcare providers and provide data to support China CDC's recommendation for maternal influenza vaccination to reduce influenza virus infection among pregnant persons in China.

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Data availability

Data will be made available on request.

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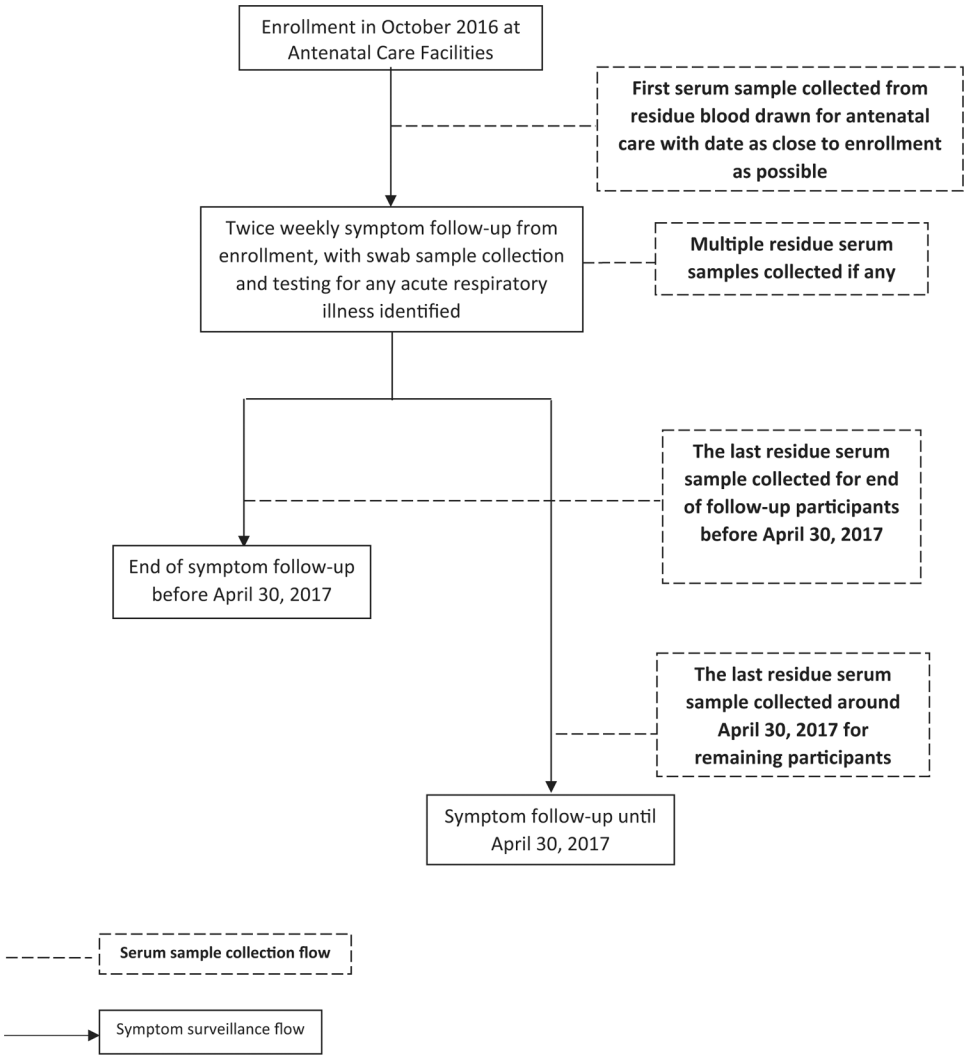


Fig. 1.

Study flowchart. End of symptom follow-up included loss of follow-up (deliver out of Suzhou, lost contact despite multiple attempts of call, or voluntarily withdraw), abortion, and delivery before April 30, 2017.

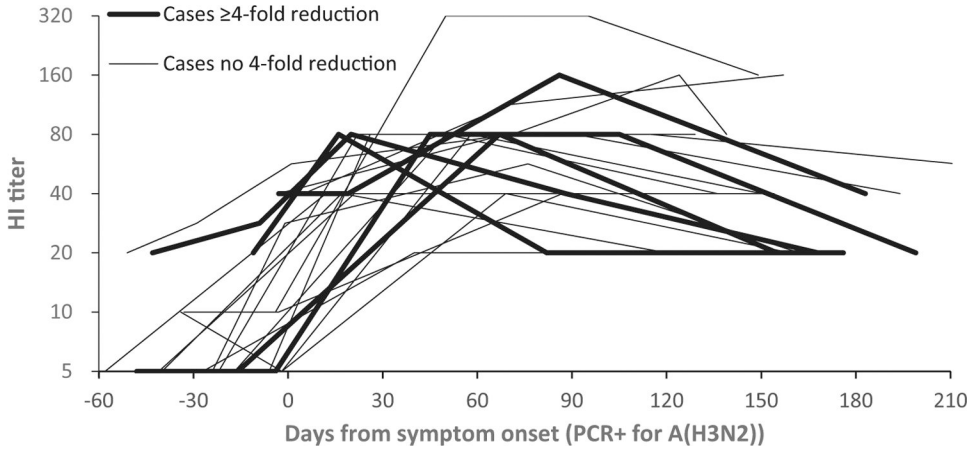
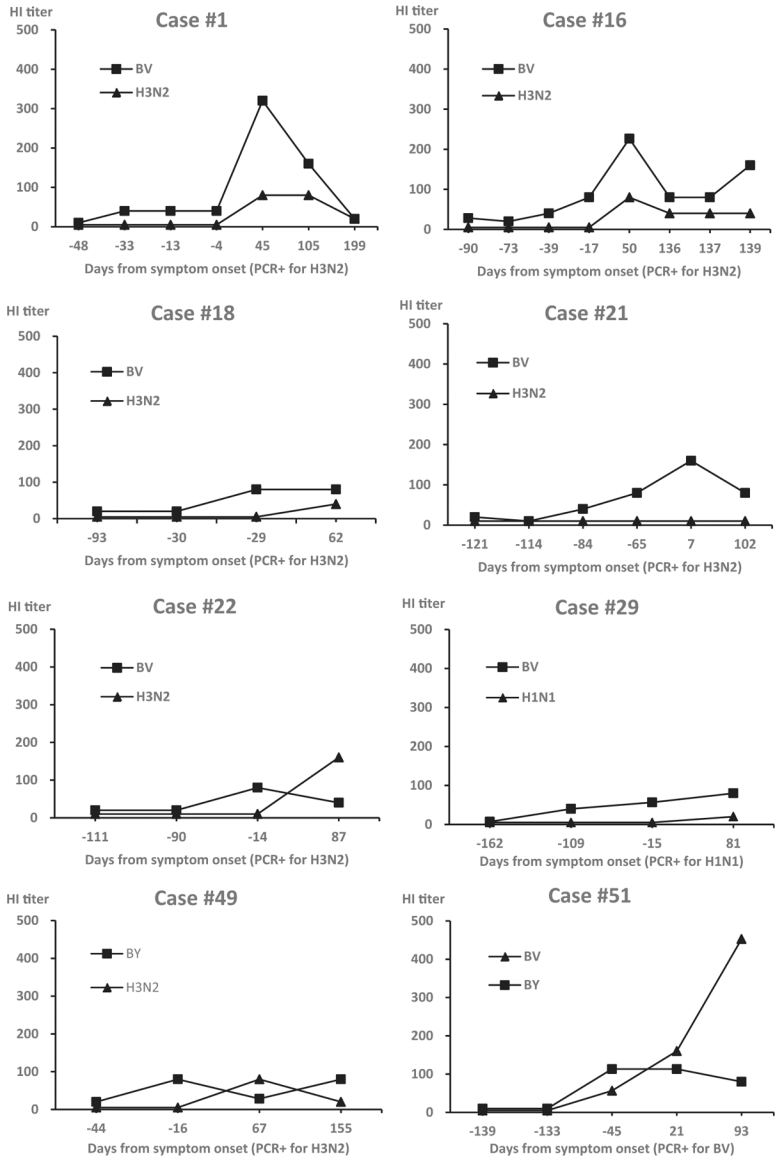


Fig. 2.

Influenza antibody waning in five pregnant persons with post-infection sera available for at least 3 months' observation after the real-time reverse transcription-polymerase chain reaction detected influenza positive (PCR +), Suzhou China, 2016–2017.



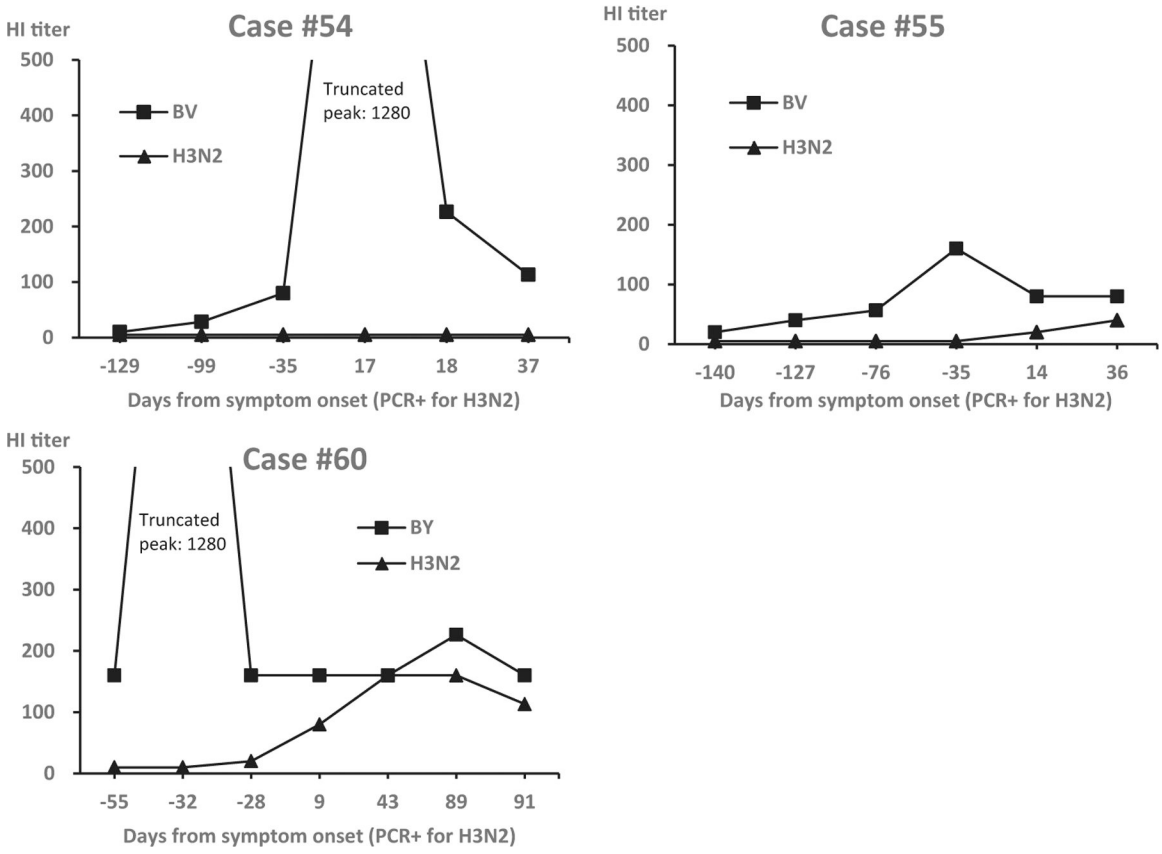


Fig. 3.

Influenza seroconversion to a different virus strain among 11 pregnant persons who tested positive for influenza by real-time reverse transcription-polymerase chain reaction (PCR +) testing, Suzhou China, 2016–2017.

Table 1

Characteristics of influenza virus infections among pregnant persons in Suzhou, China, October 2016 to April 2017.

	Influenza negative by both rRT-PCR and serology[a]	Influenza positive by rRT-PCR testing for ARI[b]	Influenza positive by serology only[c]	Influenza positive by either rRT-PCR or serology		Proportion of confirmed cases identified by serology but not rRT-PCR	
	n (row %)	n (row %)	n (row %)	n (row %)	p value[d]	% (95 % CI)[e]	p value[e]
All (n = 1384)	782 (57)	61 (4)	541 (39)	602 (43)	NA	90 (87 to 92)	NA
Age (years) (median: 28; range: 19–47)					**0.019**		0.530
<30 (n = 999)	545 (55)	44 (4)	410 (41)	454 (45)		90 (87 to 93)	
≥30 (n = 385)	237 (62)	17 (4)	131 (34)	148 (38)		89 (82 to 93)	
Han ethnic (n = 1373)	774 (56)	60 (4)	539 (39)	599 (44)	0.438	90 (87 to 92)	1.000
Underlying disease[g] (n = 27)	15 (56)	2 (7)	10 (37)	12 (44)	0.920	83 (55 to 95)	1.000
Trimester upon enrollment					**<0.001**		0.988
First (n = 302)	121 (40)	19 (6)	162 (54)	181 (60)		90 (84 to 93)	
Second (n = 981)	590 (60)	38 (4)	353 (36)	391 (40)		90 (87 to 93)	
Third (n = 101)	71 (70)	4 (4)	26 (26)	30 (30)		87 (70 to 95)	
ARI syndrome[h]					**0.002**		NA
No ARI syndrome (n = 823)	493 (60)	NA	330 (40)	330 (40)		100 (99 to 100)	
Met ARI definition (n = 561)	289 (52)	61 (11)	211 (38)	272 (48)		78 (72 to 82)	
Reported symptom					**0.005**		NA
No symptom (n = 669)	404 (60)	NA	265 (40)	265 (40)		100 (99 to 100)	
At least one symptom (n = 715)	378 (53)	61 (9)	276 (39)	337 (47)		82 (77 to 86)	
Cough (n = 405)	194 (48)	49 (12)	162 (40)	211 (52)	**<0.001**	77 (71 to 82)	0.076
Temperature ≥ 38 °C (n = 18)	4 (22)	8 (44)	6 (33)	14 (78)	**0.003**	43 (21 to 67)	0.333
Fever, feverish or chills (n = 188)	85 (45)	31 (16)	72 (38)	103 (55)	**0.001**	70 (60 to 78)	0.089
Sore throat (n = 502)	251 (50)	57 (11)	194 (39)	251 (50)	**<0.001**	77 (72 to 82)	0.050
Runny nose (n = 599)	309 (52)	58 (10)	232 (39)	290 (48)	**0.001**	80 (75 to 84)	0.083
Chest ache (n = 33)	10 (30)	5 (15)	18 (55)	23 (70)	**0.003**	78 (58 to 90)	0.773
Headache (n = 155)	62 (40)	23 (15)	70 (45)	93 (60)	**<0.001**	75 (66 to 83)	0.261
Muscle ache (n = 62)	26 (42)	14 (23)	22 (35)	36 (58)	**0.025**	61 (45 to 75)	0.182
Dyspnea (n = 133)	70 (53)	17 (13)	46 (35)	63 (47)	0.392	73 (61 to 82)	0.306
Fatigue (n = 147)	67 (46)	24 (16)	56 (38)	80 (54)	**0.006**	70 (59 to 79)	0.150

	Influenza negative by both rRT-PCR and serology[a]	Influenza positive by rRT-PCR testing for ARI[b]	Influenza positive by serology only[c]	Influenza positive by either rRT-PCR or serology		Proportion of confirmed cases identified by serology but not rRT-PCR	
	n (row %)	n (row %)	n (row %)	n (row %)	p value[d]	% (95 % CI)[e]	p value[e]
Vomiting (n = 33)	16 (48)	4 (12)	13 (39)	17 (52)	0.446	76 (53 to 90)	0.800
Diarrhea (n = 34)	15 (44)	4 (12)	15 (44)	19 (56)	0.194	79 (57 to 91)	0.836
Influenza virus subtype/lineage							
A(H1N1)pdm09	NA	1	48	49		98 (89 to 100)	
A(H3N2)	NA	54	111	165		67 (60 to 74)	
B Victoria	NA	5	415	420		99 (97 to 99)	
B Yamagata	NA	1	207	208		100 (97 to 100)	

rRT-PCR: real-time reverse transcription-polymerase chain reaction; ARI: acute respiratory illness; NA: not applicable.

[f] $\chi 2$ or Fisher's exact test of association between each variable and influenza-positive diagnosed only by serology but not by rRT-PCR.

[a] Seroconversion was defined as ≥ 4-fold rise between any pair of residue blood draw, with the post-infection titer ≥ 40.

[b] Influenza positive by rRT-PCR testing for ARI: all influenza ARI confirmed by rRT-PCR had a seroconversion between certain time points during follow-up.

[c] Influenza positive by serology only: including participants who reported ARI but rRT-PCR tested negative, and participants who reported other symptom(s) or no symptom therefore not meeting rRT-PCR testing criteria.

[d] $\chi 2$, Fisher's exact test, or one-way ANOVA test for differences between each variable and influenza positivity by either rRT-PCR or serology.

[e] CIs were calculated using binomial method.

[g] Underlying disease referred to any medical problem diagnosed before pregnancy by a doctor or other health care provider before pregnancy that lasted for at least six months such as diabetes, asthma, heart disease, or cancer.

[h] ARI was defined as ≥ 1 respiratory symptom (cough, sore throat, stuffy nose, chest pain, difficulty breathing) and ≥ 1 systemic symptom (feverish, temperature ≥ 38 °C, chills, headache); or ≥ 2 respiratory symptoms.

Table 2

Repeat infection by different influenza viruses among pregnant persons in Suzhou, China, October 2016 to April 2017.

	Repeat infection, n (%)[a]
Index influenza illness with respiratory swab positive (n = 58)	11 (19)
A(H3N2) index illness (n = 52)	9 (17)[b]
A(H1N1)pdm09 index illness (n = 1)	1 (100)[c]
B Victoria index illness (n = 4)	1 (25)[d]
B Yamagata index illness (n = 1)	0 (0)

[a]Repeat infection referred to a seroconversion to a different influenza strain prior to the real-time reverse transcription-polymerase chain reaction testing confirmed illness since October 2016.

[b]9 repeat infections included 7 with influenza B Victoria and 2 with B Yamagata.

[c]1 repeat infection was with influenza B Victoria.

[d]1 repeat infection was with influenza B Yamagata.