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The added value of serologic testing: A comparison of influenza incidence among pregnant persons based on molecular-based surveillance versus serologic testing

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Author contributions

FD conceived the study. All authors planned or advised on the study and contributed to the design of data collection instruments as well as coordinated and supervised data collection. SM performed initial data analysis. WK completed data analysis and drafted the paper with critical inputs from FD. All contributed to the interpretation of the results, provided feedback and helped shape the research, analysis, and this paper.

Declarations 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.

Ethical approval

The study protocol was reviewed and approved by ethical committees in each hospital, and by the Institutional Review Board (IRB) of U.S. Naval Medical Research Unit SOUTH IRB (protocol NAMRU6.2016.0015) in compliance with all applicable federal regulations governing the protection of human subjects, and by the IRBs of Abt Associates and the Walter Reed Army Institute of Research (protocol 2463). The IRB of the U.S. CDC relied on the determination of the Abt Associates' IRB. Written informed consent was obtained from all study participants.

Supplementary materials

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Abstract

Background: We examined the added value of serologic testing for estimating influenza virus infection incidence based on illness surveillance with molecular testing versus periodic serologic testing.

Methods: Pregnant persons unvaccinated against influenza at <28 weeks gestation were enrolled before the 2017 and 2018 influenza seasons in Peru and Thailand. Blood specimens were collected at enrollment and ≤14 days postpartum for testing by hemagglutination inhibition assay for antibodies against influenza reference viruses. Seroconversion was defined as a ≥4-fold rise in antibody titers from enrollment to postpartum with the second specimen's titer of ≥40. Throughout pregnancy, participants responded to twice weekly surveillance contacts asking about influenza vaccination and influenza-like symptoms (ILS). A mid-turbinate swab was collected with each ILS episode for influenza real-time reverse transcription polymerase chain reaction (rRT-PCR).

Results: Of 1,466 participants without evidence of influenza vaccination during pregnancy, 296 (20.2%) had evidence of influenza virus infections. Fifteen (5.1%) were detected by rRT-PCR only, 250 (84.4%) by serologic testing only, and 31 (10.5%) by both methods.

Conclusions: Influenza virus infections during pregnancy occurred in 20% of cohort participants; >80% were not detected by a broad illness case definition coupled with rRT-PCR.

Keywords

Influenza; Peru; Pregnancy; Serology; Thailand

Background

The World Health Organization (WHO) recommends prioritizing pregnant persons for influenza vaccination and influenza vaccines have been shown to effectively reduce the risk of influenza illness among both pregnant persons and their infants during the first few months of life [1]. However, use of influenza vaccines among pregnant persons remains low globally, in part because of insufficient information about the local burden of influenza. A WHO systematic review from 2017 highlighted the continued paucity of data about influenza disease burden among pregnant persons in low- and middle-income countries [2, 3].

Influenza disease burden is typically estimated either by relying on passive surveillance of medical record data or through prospective molecular surveillance for influenza illness. For studies examining disease burden in groups at increased risk for severe illness, including pregnant persons, medical record data are limited by inconsistent and often infrequent testing for influenza viruses during medical encounters for febrile or respiratory illness episodes [4]. Influenza surveillance studies address these limitations by implementing systematic testing with standard illness case definitions that trigger testing, but such studies are expensive, limited by participant adherence to surveillance, and miss influenza virus

infections that do not meet the surveillance case definition or are reported outside of the virus shedding window [5]. The addition of periodic serologic testing for antibodies against circulating influenza viruses may optimize infection detection in surveillance studies by identifying infections that do not meet influenza case definitions (e.g., pauci or asymptomatic) or are not reported by participants. However, serologic testing requires blood collection which may not be acceptable to all participants and requires additional resources. Data are needed to examine the added value of serologic testing in influenza surveillance studies. A previous prospective single-season surveillance study among pregnant persons in China compared influenza virus infection detection by an illness case definition coupled with molecular testing versus by serologic testing alone and found that infections missed by molecular surveillance may account for a large proportion (up to 39%) of influenza virus infections in pregnant persons [6].

Using data from a prospective two-season multinational cohort study of pregnant persons that included both systematic molecular surveillance using a broad illness case definition and serologic surveillance [7, 8], we examined the added value of serologic testing for estimating influenza virus infection incidence, estimated the proportion of influenza virus infections that were missed by molecular surveillance alone, and examined characteristics of participants with concordant and discordant infection statuses based on illness surveillance with molecular testing versus periodic serologic testing. Findings from this analysis may help estimate the fraction of influenza virus infections missed in influenza disease burden assessments based solely on surveillance using an illness case definition and molecular testing to inform future evaluations of the value of influenza vaccination programs for pregnant persons.

Methods

Setting and participant enrollment and follow-up

A detailed description of the Pregnancy and Influenza Multinational Epidemiologic (PRIME) study was previously published [7, 8]. The PRIME study was conducted during the 2017 and 2018 influenza seasons at study sites in India, Peru, and Thailand. Pregnant persons ≥18 years of age with an estimated delivery date that permitted an eight-week period of follow-up during influenza season were considered eligible for enrollment in the PRIME study. Additionally, potential participants must have planned to stay in the study area and deliver in the study hospitals, and be willing to be contacted two times per week for respiratory symptom surveillance during their pregnancy periods [7]. Participants were ineligible for enrollment if they received the current season's influenza vaccine prior to enrollment. Enrollment started up to 10 weeks before the anticipated start of the influenza season through the first four weeks of the respective influenza season. Participants were contacted twice weekly and asked whether they had symptoms that met the study case definition from the start of the influenza season or enrollment (whichever occurred later) through the end of their pregnancies. The study case definition was defined as new onset or sudden worsening of one or more of the following symptoms within the past seven days: myalgia, cough, runny nose or nasal congestion, sore throat, or difficulty breathing. Participants reporting symptom(s) that met the study case definition within seven days

of illness onset were asked to submit mid-turbinate nasal swab specimens (staff-collected swabs in Peru and participants self-collected swabs in Thailand). Specimens were tested for influenza viruses by real-time reverse transcription polymerase chain reaction (rRT-PCR) using U.S. Centers for Disease Control and Prevention (U.S. CDC)-provided primers and probes and following the WHO-U.S. CDC's protocols [9]. Detection of influenza viruses from staff- versus participant-collected swabs was similar, as previously reported [10].

Blood collection

At the PRIME Peru and Thailand study sites, participants who were not vaccinated with influenza vaccines were invited to this serology substudy that included blood specimen collection until up to 700 participants were enrolled per site per season. Serology substudy participants had blood collected at enrollment and within 14 days of the end of pregnancy to estimate rates of seroconversion to circulating influenza viruses during the study follow-up period. Participants who received seasonal influenza vaccines in between two blood collections (self-reported and/or medical record verified) were excluded from the analysis (Figure 1). Blood specimens were transported to local laboratories in each country and processed for serum collection within 18 hours of collection. Sera were stored at −20°Celsius or colder and shipped at the end of each influenza season to a single central laboratory for testing.

Serologic testing

Sera were tested by hemagglutination inhibition (HAI) assays at a U.S. CDC designated laboratory that completed proficiency testing prior to testing. The HAI assays were performed following the WHO-U.S. CDC's protocols [9]. Egg-grown viruses representative of the influenza vaccine strains for each season [9] were used because vaccine strains were antigenically well matched to circulating viruses during both influenza seasons (Supplementary Table 1) and the cell grown influenza A/H3N2 viruses from these two seasons did not work in the HAI assays. HAI assays were performed with 0.5% turkey erythrocytes for influenza A(H1N1), B/Yamagata, and B/Victoria antigens as previously described [9]. Influenza A(H3N2) antigens were tested with 0.75% guinea pig erythrocytes in the presence of 20 nM oseltamivir to prevent interference of the viral neuraminidase protein [9].

Analytic definitions

In this study, symptomatic rRT-PCR-confirmed influenza virus infection was defined by a respiratory specimen collected from a participant who reported an ILS during study follow-up that tested positive for influenza viruses. Serologically confirmed influenza virus infection was defined as seroconversion based on a ≥4-fold rise in antibody titers between specimens taken at enrollment and at the end of pregnancy (with a minimum titer of 40 in the end of pregnancy specimen), regardless of whether an ILS was reported during the study follow-up. Discordant influenza virus infection status was defined as influenza virus infection detection by either rRT-PCR-based surveillance or serology without detection by the other method.

Data analysis

Descriptive analysis was conducted to summarize participants' clinical and demographic characteristics; the cumulative incidence of influenza virus infections by rRT-PCR-based surveillance (e.g., symptomatic infections), serologic testing (e.g., asymptomatic infections, infections not meeting the study case definition, or infections with rRT-PCR false negative), or either method combined; and the frequency of seroconversion to 1 or >1 influenza reference virus. The frequency of concordant versus discordant influenza virus infection status by rRT-PCR-based surveillance and serologic testing was examined by study site, season, and influenza subtype (for influenza A) and lineage (for influenza B). Summary statistics were calculated and reported among those who were serologically testing positive for at least one influenza reference virus. To identify factors associated with having an influenza virus infection identified by serologic testing alone, baseline and surveillance characteristics of participants with infections identified by serologic testing alone were compared to those with concordant infection status by rRT-PCR-based surveillance and serologic testing using Chi Square or Fisher's Exact test; participants with infections identified by rRT-PCR-based surveillance only ($n = 15$) were excluded. Similarly, factors associated with having an influenza virus infection identified by rRT-PCR testing alone were examined in the same manner; in this analysis, participants with concordant infection status by rRT-PCR-based surveillance and serologic testing served as a reference group while those with infections identified by serologic testing only ($n = 250$) were excluded. Data analyses were conducted using Stata version 16 (Stata Corp, College Station, Texas, USA). Two-tailed *P*-values of < 0.05 were considered statistically significant.

Results

Characteristics of study participants

Of 1,466 participants with paired blood specimens who had no evidence of influenza vaccination during pregnancy, 363 (24.8%) were from Peru (173 enrolled in 2017 season and 190 in 2018 season) and 1,103 (75.2%) were from Thailand (545 enrolled in the 2017 influenza season and 558 in 2018 season; Table 1). Most participants (1,371, 93.5%) were ≥20 years of age, while 95 (6.5%) were aged <20 years old. At enrollment, 350 (23.8%), 924 (63.0%), and 191 (13.0%) were in the first, second, and third trimesters of pregnancy, respectively. Overall, 221 (15.1%) self-reported being diagnosed with a chronic medical condition by healthcare personnel during the previous 24 months. Three participants were living with HIV or had other immunosuppressive conditions. The average time participants were observed in the respective influenza season was 15.5 weeks (standard deviation [SD] 7.1 weeks). Enrollment and end of pregnancy blood specimens were collected at a mean of 18.7 weeks apart (SD 7.1 weeks). During the study follow-up, 662 ILS episodes were identified, of which 579 (87.4%) had mid-turbinate nasal swab specimens collected. The average time from illness onset to nasal swab specimen collection was 2 days (SD 1.8 days).

Influenza virus infections

During the 2017 and 2018 influenza seasons, 296 (20.2%) of the enrolled participants had molecular and/or serologic evidence of influenza virus infections, while 1,170 (79.8%) did not (Figure 1). Among the 296 participants with laboratory evidence of influenza virus

infections, 15 (5.1%) were detected by rRT-PCR only, 250 (84.4%) were detected by serologic testing only, and 31 (10.5%) were detected by both methods. The Cohen's Kappa which indicates agreement between the two methods ranged from 0.00 (95% confidence interval [CI] 0.0 0-0.0 0) for influenza B/Victoria to 0.27 (95% CI 0.23-0.31) for influenza A/H1N1 (Supplementary Table 2). Among participants with discordant statuses, 15/15 (100%) and 72/250 (22.8%) reported at least one ILS episode during pregnancy.

Overall, the cumulative incidence proportions based on combined rRT-PCR-based surveillance and/or serologic testing results were higher in Peru than in Thailand in 2017 (24.3% [42/173] versus 19.1% [104/545]) and 2018 (21.1% [40/190] versus 19.7% [112/566]; Figure 2). Of all 1,466 participants, 214 (14.6%) seroconverted to one influenza reference virus (range 13.8-16.8% across study site and season), while 67 (4.6%) seroconverted to more than one influenza reference virus (range 3.5-6.9% across study site and season; Figure 3). Among the 67 participants who seroconverted to more than one influenza reference virus, the most common dual seroconversion was influenza B/Yamagata and influenza B/Victoria (38; 56.7%), followed by influenza B/Yamagata and influenza A/H1N1 (21; 31.3%), influenza B/Victoria and influenza A/H1N1 (14; 20.9%), influenza A/H1N1 and influenza A/H3N2 (7; 10.4%), and influenza A/H3N2 and influenza B/Victoria (5; 7.5%).

Added value of burden estimates by serologic testing

Among 1,466 participants, 46 (3.1%) individuals with infections were identified during the study period by rRT-PCR-based surveillance only. These included 26 (1.8%) cases of influenza A/H1N1, 11 (~1%) influenza A/H3N2, and 9 (~1%) influenza B/Yamagata (Supplementary Table 2). When serologic testing was added to the rRT-PCR-based surveillance and assumed that all seropositives were true positive, an additional 204 (13.9%) individuals with infections were identified. These included 82 (5.4%) cases of influenza A/H1N1, 69 (4.7%) cases of influenza A/H3N2, 112 (7.6%) cases of influenza B/Yamagata, and 61 (4.2%) cases of influenza B/Victoria.

Antibody titers by influenza type and rRT-PCR status are shown in Supplementary Table 3. Sizable proportions of participants had antibody titers indicating seroprotection (i.e., titers ≥ 1:40) at baseline: 277 out of 1,466 (18.9%) for influenza A/Michigan, 626 out of 718 (87.2%) for influenza A/Hong Kong, 652 out of 748 (87.2%) for influenza A/Singapore, 365 of 1,466 (24.9%) for influenza B/Phuket, and 185 out of 1,466 (12.6%) for influenza B/Brisbane. Among participants with rRT-PCR negative, the seroconversion proportions at end of pregnancy ranged from 4-8% depending on influenza subtype/lineage. These proportions were higher among those with rRT-PCR positive, ranging from 0-46%. In an analysis which included only participants who had evidence of seroprotection at baseline, the seroconversion proportions at end of pregnancy dropped to 1-5% among those with rRT-PCR negative and 0-15% among those with rRT-PCR positive (Supplementary Table 4). The rise in antibody titer between enrollment and end of pregnancy blood specimens among participants with concordant influenza virus infection status by both rRT-PCR-based surveillance and serologic testing versus infection detected by serology alone is shown in Supplementary Figure 1.

Characteristics associated with identification of infections by one laboratory method but not the other

The study year, age, trimester at enrollment, monthly household income level, presence of immunosuppressive conditions, weeks between enrollment and end of pregnancy blood collection, and weeks pregnant during influenza season were similar between participants with concordant infection status based on rRT-PCR-based surveillance and serologic testing compared to those with infections detected only by serologic testing. The odds of infection detection by serologic testing only, however, were higher among those with lower response rates to twice weekly illness surveillance compared to those with ≥90% adherence (odds ratio [OR] 1.40, 95% CI 1.01-1.96; Table 2). Even among participants with very high response rate (≥90% adherence), 16.3% had influenza virus infections detected by serologic testing only. The detection proportion was 21.5% among those with 0-89% adherence.

The odds of infection detection by rRT-PCR testing only were higher among those with body temperatures >100.4°Fahrenheit compared to those with lower body temperatures (OR 13.04, 95% CI 2.69-63.28; data not shown). Among participants whose body temperatures >100.4°Fahrenheit, 12.5% had influenza virus infections detected by rRT-PCR testing only. The detection proportion was 1.1% among those with body temperatures ≤100.4°Fahrenheit.

Discussions

Among cohorts of ~1,500 influenza unvaccinated pregnant persons, approximately one in five had influenza virus infections detected by rRT-PCR-based surveillance and/or serologic testing during the 2017 and 2018 influenza seasons in Peru and Thailand. More than 80% of influenza virus infections were detected by serologic testing only, highlighting the added value of serologic testing in influenza surveillance studies that aim to fully characterize the burden of influenza virus infections besides those meeting an illness-based case definition. Nearly a quarter of participants with influenza virus infections had serologic evidence of infection with more than one virus subtype/lineage, although the overall frequency of dual influenza virus infections detected by serology was low (5%). Most dual infections included evidence of infection with at least one influenza B virus, highlighting the contribution of influenza B viruses to overall influenza disease burden.

Seasonal influenza vaccination during pregnancy prevents influenza illnesses and associated hospitalizations in pregnant persons [1, 11, 12] as well as in their infants during their first few months of life when they are too young to receive influenza vaccines [12–14]. The limited data about influenza disease burden among pregnant persons have been cited as a potential contributor to persistently low vaccine uptake among pregnant persons in many settings [15]. In a prospective surveillance study among pregnant persons in China during October 2016-April 2017, Chen et al. found that 43% of persons had evidence of influenza virus infection by rRT-PCR-based surveillance or serologic testing [6]. Our findings corroborate those reported by Chen et al. and document the substantive influenza virus infection burden in pregnant persons over two seasons in two middle-income countries. While almost all influenza virus infections detected in our community cohort were mild or asymptomatic, studies have documented that viral shedding is detectable during paucisymptomatic and asymptomatic influenza virus infections, and the possibility

of influenza transmission during these infections is plausible [16–18]. Furthermore, other studies have documented that pregnancy confers an increased risk for hospitalization with influenza, and hospitalization with acute respiratory illness during pregnancy may be associated with adverse perinatal outcomes such as preterm birth and low birthweight [19]. Additionally, a previous analysis from our study cohort found that symptomatic rRT-PCR-confirmed influenza virus infection during pregnancy was associated with stillbirth and decreased infant birthweight [8]. The high incidence of influenza virus infections among pregnant persons coupled with the increased risk for influenza-associated hospitalization and other adverse outcomes in this population underscore the importance of improving influenza vaccine uptake among pregnant persons. Additional studies are needed to elucidate the clinical significance of asymptomatic influenza infections among pregnant persons with respect to effects on pregnancy and perinatal outcomes and infection transmission. Data may inform not only the clinical management and infection prevention and control strategies but also economic analysis of vaccination programs targeting pregnant persons, providing a better understand if these infections are associated with adverse birth outcomes in ways that might improve the cost-benefit of influenza vaccination.

Our study documents the added value of serologic testing in studies assessing influenza virus infection incidence [6, 20]. For example, similar to Chen et al. [6], we found that the majority of influenza virus infections were identified by serologic testing only (90% of infections in the study by Chen et al. compared to 84% of infections in our study). We found a strong inverse association between adherence to twice weekly illness surveillance and detection of infections by serologic testing only, suggesting that a fraction of these infections may have been symptomatic but unreported. However, even among participants who responded to at least 90% of surveillance contacts, 16% had influenza virus infections detected by serologic testing only suggesting that many infections were asymptomatic or had mild symptoms that did not meet the broad illness case definition. These findings highlight the necessity of frequent follow-up of participants in epidemiological cohorts and the implication on interpreting results when follow-up of study participants is limited. It should be noted that while serologic testing can help us understand the true incidence of infection, it comes with added costs for specimen collection and laboratory testing while the clinical significance and health and economic consequences of this portion of the burden pyramid are still less understood.

Strengths of our study include enrollment and retention of a large cohort over two influenza seasons in two middle-income countries, prospective twice weekly surveillance using a broad illness case definition, high rates of adherence to surveillance, and paired serum collection and testing at a single centralized laboratory that completed proficiency testing. Nonetheless, several limitations should be considered when interpreting study findings. Antibodies induced by influenza virus infection are known to wane over time [21, 22]. Because of the long interval between the two blood draws pre- and post-influenza season, it is possible that antibodies might have waned before the second blood collection and the proportions of those who seroconverted reported in this study are likely the minimum estimates. Additionally, while we used two laboratory methods for influenza virus infection detection that are commonly used in epidemiological studies, both have different fundamental purposes (one detects viral genomes while the other detects antibodies). They

also have different performance in terms of sensitivity and specificity [6, 20] and might yield false positive or negative results. Furthermore, we used egg-grown antigens rather than the cell grown ones in the HAI testing that may also impact the sensitivity of the serological detection of infection. We were unable to determine which fractions of discordant pairs were associated with the imperfection of the detection methods. Additionally, while our case definition was fairly sensitive and time from illness onset to nasal swab specimen collection in this study was rather short, we did not investigate whether adjusting the case definition to be even more sensitive, narrowing the specimen collection window after illness onset, or collecting/testing among individuals who did not report symptoms may have increased the sensitivity of rRT-PCR to detect more cases [23–26]. Moreover, although the sample size target was initially met at enrollment, more than half of the participants in Peru were excluded from the analysis because of the high influenza vaccine uptake during pregnancy. It is also possible that there were some participants with influenza vaccination (i.e., unreported, undocumented) that were not excluded from our analysis. Last, antibody titers we observed might be cross-reactive between influenza viruses, particularly among participants with dual seroconversion [27, 28].

Influenza virus infections during pregnancy are common and a large number of infections may not be detected even using a broad case definition for respiratory illness and rRT-PCR testing. Findings from this study expand upon those from previous studies that a fraction of these infections (i.e., those not meeting the study case definition) may only be ascertained by serologic testing. The findings also support the WHO's recommendation for influenza vaccination during pregnancy to reduce disease burden and may better inform influenza vaccine cost-benefit and other economic analyses for sustainable influenza vaccination programs among pregnant persons. They may also help inform the selection of laboratory assays for more accurate assessment of number of infected individuals in epidemiological studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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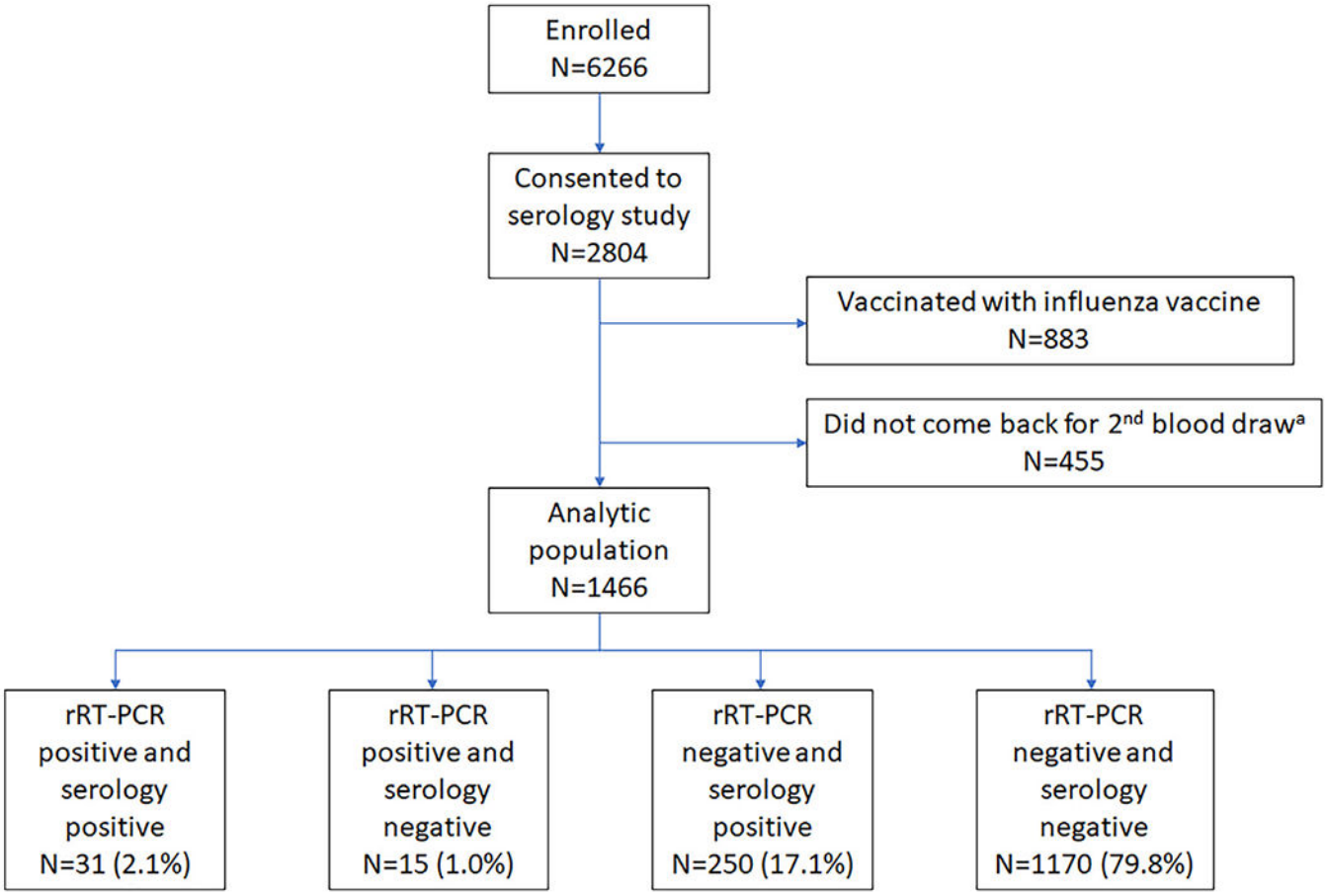

Figure 1.

Study flow diagram, Pregnancy and Influenza Multinational Epidemiologic Study, Peru and Thailand. rRT-PCR: Real-time reverse transcription polymerase chain reaction.

[a] After excluding those who were vaccinated.

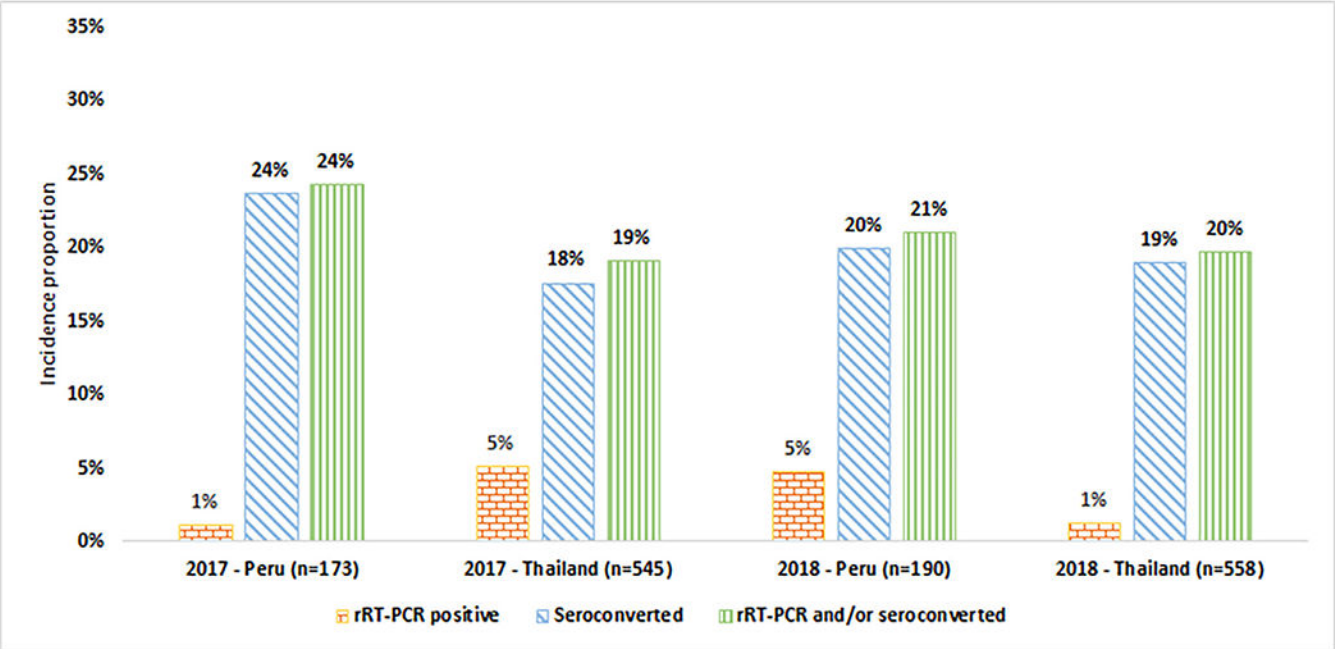

Figure 2.

Influenza virus infection positive proportions by detection method, site, and season, Pregnancy and Influenza Multinational Epidemiologic Study, Peru and Thailand, $N = 1466$. rRT-PCR: Real-time reverse transcription polymerase chain reaction. Percentages reflect the proportions of infections detected by each detection method among total participants included in the analysis for each country and season.

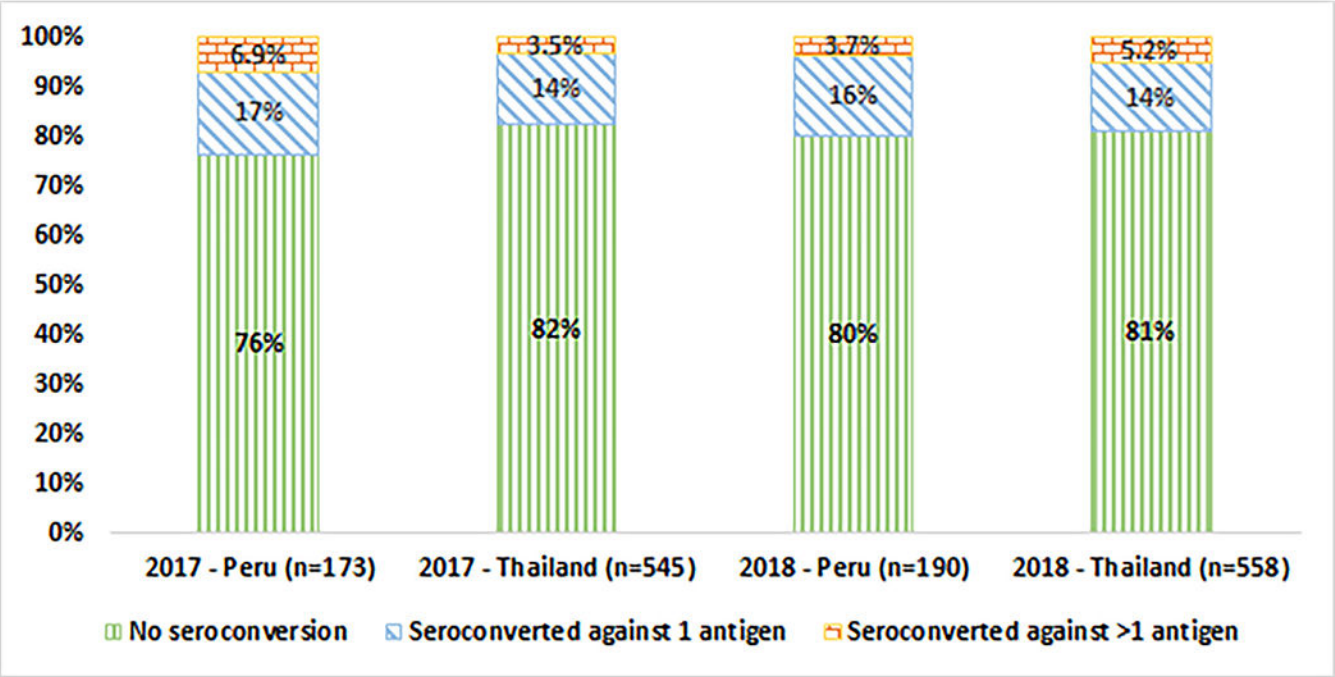

Figure 3.

Seroconversion to circulating influenza viruses by number of seroconversions, site, and season, Pregnancy and Influenza Multinational Epidemiologic Study, Peru and Thailand, *N* = 1466. rRT-PCR: Real-time reverse transcription polymerase chain reaction. Percentages reflect the proportions of individuals in each category among total participants included in the analysis for each country and season.

Table 1

Baseline, illness, and surveillance characteristics of participants enrolled in the serology study, Pregnancy and Influenza Multinational Epidemiologic Study, Peru and Thailand, $N = 1466$.

	Total (N = 1466)	
	Number	**(%)**
Study year		
Year 1	718	49
Year 2	748	51
Age (years)		
<20	95	6
20-29	747	51
≥30	624	43
Trimester at enrollment		
First	350	24
Second	924	63
Third	191	13
Unknown	1	<1
Monthly household income level [a]		
>75th percentile	207	14
≤75th percentile	1259	86
Chronic medical conditions [b]		
Yes	221	15
HIV	1	<1
Any immunosuppressive conditions not including HIV	2	<1
No	1245	85
Gestational diabetes	141	10
Gestational hypertension	84	6
Weeks between two blood collections (pre- and post-influenza season)		
0-10	134	9
11-20	689	47
21-30	539	37

	Total (*N* = 1466)	
	Number	**(%)**
>30	104	7
Weeks pregnant during influenza season		
0-10	274	19
11-20	840	53
21-30	295	20
>30	57	4
Number of rRT-PCR confirmed influenza events		
0	1420	97
1	46	3
rRT-PCR positive		
Influenza A/H1N1	26	2
Influenza A/H3N2	11	1
Influenza B/Yamagata	9	1
Influenza B/Victoria	0	0
Seroconversion		
Influenza A/H1N1	103	7
Influenza A/H3N2	73	5
Influenza B/Yamagata	120	8
Influenza B/Victoria	64	4

rRT-PCR: Real-time reverse transcription polymerase chain reaction.

[a] 75th percentile was calculated using data of participants from each respective country. Those with missing data were considered having income below the 75th percentile.

[b] Participants replied yes to one of the following questions: 1) D3. Please tell me if you have had any of these types of chronic medical conditions over the past 24 months (two years). Only include conditions diagnosed by a healthcare provider: (choice = Problems with the immune system, excluding HIV); 2) E5. I will now read you a list of types of chronic medical conditions. Please tell me which of these types of chronic medical conditions you developed during this pregnancy. (choice = Problems with the immune system, excluding HIV); 3) A11. Collect information below for underlying medical conditions that the participant had during this pregnancy. (Check all that apply): (choice = Immunosuppressive condition not including cancer or transplant [in the last six months]) date was before start of influenza season.

Table 2

Demographic, health, and surveillance characteristics of participants with concordant influenza virus infection status by both rRT-PCR-based surveillance and serologic testing versus infection detected by serology alone, Pregnancy and Influenza Multinational Epidemiologic Study, Peru and Thailand, *N* = 1451.

	Concordance[a]	Row %[b]	Infection detected by serologic testing alone	Row %[b]	Total	Odds ratio (95% confidence interval)
Total	1201	83	250	17	1451	
Country						
Peru	289	80	71	20	360	1.25 (0.92-1.70)
Thailand	912	84	179	16	1091	Reference
Study year						
Year 1	593	87	116	16	709	Reference
Year 2	608	82	134	18	742	1.13 (0.86-1.48)
Age (years)						
< 20	78	83	16	17	94	Reference
20-29	611	82	131	18	742	1.05 (0.59-1.85)
≥30	512	83	103	17	615	0.98 (0.55-1.75)
Trimester at enrollment [c]						
First	289	84	56	16	345	Reference
Second	747	82	169	18	916	1.17 (0.84-1.63)
Third	164	87	25	13	189	0.79 (0.47-1.31)
Monthly household income level [d]						
> 75th percentile	184	89	22	11	206	0.53 (0.34-0.85)
≤75th percentile	1017	82	228	18	1245	Reference
Influenza						
Detected by rRT-PCR	31	100	0	0	31	n/a
Not detected by rRT-PCR	1170	82	250	18	1420	
Fever > 100.4°Fahrenheit	14	82	3	18	17	1.03 (0.29-3.61)
Immunosuppressive condition [e]						
Yes	2	67	1	33	3	2.41 (0.22-26.66)
No	1199	83	249	17	1448	Reference

	Concordance[a]	Row %[b]	Infection detected by serologic testing alone	Row %[b]	Total	Odds ratio (95% confidence interval)
Gestational diabetes						
Yes	118	85	21	15	139	0.84 (0.52-1.37)
No	1083	83	229	17	1312	Reference
Gestational hypertension						
Yes	71	85	13	15	84	0.87 (0.48-1.60)
No	1130	83	237	17	1367	Reference
Weeks between two blood collections (pre- and post-influenza season)						
0-10	117	88	16	12	133	Reference
11-20	566	83	119	17	685	1.54 (0.88-2.69)
21-30	428	81	102	19	530	1.74 (0.99-3.67)
> 30	90	87	13	13	103	1.06 (0.48-2.31)
Weeks pregnant during influenza season						
0-10	229	84	43	16	272	Reference
11-20	688	82	146	18	834	1.13 (0.78-1.64)
21-30	236	82	52	18	288	1.17 (0.75-1.83)
> 30	48	84	9	16	57	1.00 (0.46-2.18)
% Adherence to surveillance [f]						
0-89	205	79	56	21	261	1.40 (1.01-1.96)
≥90	996	84	194	16	1190	Reference

rRT-PCR: Real-time reverse transcription polymerase chain reaction.

[a] Either infection detected by both real-time reverse-transcription polymerase chain reaction (rRT-PCR) and serologic testing or absence of infection based on rRT-PCR and serologic testing.

[b] The denominator for the row percentage was the total number of women in the category.

[c] One individual with missing information was excluded.

[d] 75^{th} percentile was calculated using data of participants from each respective country. Those with missing data were considered having income below the 75^{th} percentile.

[e] HIV and other immunosuppressive conditions.

[f] The response rate was calculated as number of successful contacts made to either participant or proxy divided by the number of expected contacts during the study.

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