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Influenza virus seroincidence in a cohort of healthy and high-risk children enrolled in infancy, Bangkok, Thailand

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ABSTRACT

Background: We measured seroconversion to influenza viruses and incidence of symptomatic influenza virus infection in a cohort of children in Bangkok, Thailand.
Methods: Children aged ≤6 months were followed for two years for acute respiratory illness (ARI) and had serum specimens taken at 6-month intervals and tested by hemagglutination inhibition (HI) assay. Seroconversion was defined as a ≥4-fold rise in the HI titers between time points with a titer of ≥40 in the second specimen. Respiratory swabs were tested by rRT-PCR for influenza. Data were analyzed using generalized linear models.
Results: Of 350 children, 266 (76%, 147 were healthy and 119 were high-risk) had ≥2 serum specimens collected before influenza vaccination. During the 2-year follow-up, 266 children contributed 370 person-years of observation, excluding post-vaccination periods. We identified 32 ARI cases with rRT-PCR-confirmed influenza virus infection (7 infections/100 person-years, 95% confidence interval [CI], 4–11). There were 126 episodes of influenza virus infection, resulting in a seroconversion rate of 35 infections/100 person-years (95% CI, 30–42). Rates in healthy and high-risk children did not differ.
Conclusions: Influenza virus infection is common during the first two years of life among Thai children. A large proportion of infections may not be detected using the ARI case definition.

Background

Annual influenza epidemics cause between 3–5 million cases of severe illness and approximately 290,000 to 600,000 deaths globally (World Health Organization, 2018). Young children experience a high burden of influenza-associated illness, including hospital admissions for acute lower respiratory infection (ALRI) and pneumonia (Thompson et al., 2004; Neuzil et al., 2002; Poehling et al., 2006; Nair et al., 2011). Influenza virus infections also account for a substantial proportion of all pneumonia deaths of viral etiology in young children globally (Rudan et al., 2008), with up to 111,500 global deaths attributed to influenza-associated ALRI in children aged <5 years in 2008 (Nair et al., 2011) and an average of 9,000–106,000 deaths annually among children aged <5 years in 92 countries with high respiratory infection mortality rates (Iuliano et al., 2018).

In Thailand, children aged <5 years are more likely to be hospitalized with influenza-associated pneumonia compared to the general Thai population (Katz et al., 2007). Estimated annual incidence of laboratory confirmed-influenza virus infection ranged from <100 cases per 100,000 children in 2003-2004 to >400 cases per 100,000 children in the pandemic year (2009) (Katz et al., 2007; Olsen et al., 2010; Baggett et al., 2012). Published studies of influenza disease burden in Thailand have focused on medically attended illness, and have used case definitions that may not capture milder or atypical infections, particularly among young children. It may be helpful to quantify asymptomatic infection although its role in transmission is unclear. Studies of persons

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exposed to influenza who may or may not get ill using influenza serologic testing to identify infection based on changes in antibody titers over time have demonstrated that influenza serology may identify more infections than influenza reverse transcription polymerase chain reaction (RT-PCR) testing alone (Leung et al., 2015; Monto and Sullivan, 1993). However, studies with longitudinal serology testing for influenza virus infection among young children are scarce (Leung et al., 2015). In this study, we measured seroconversion to influenza at six-month intervals over two years in a cohort of children, enrolled during the first six months of life, in Bangkok, Thailand, to calculate subtype-specific cumulative incidence of infection by age and compare incidence of influenza virus infection by health status. This study was part of a larger cohort study of respiratory infection in the pediatric population in Bangkok, Thailand (Kittikraisak et al., 2018).

Methods

Ethical approvals

The study was approved by the ethics committees of the Queen Sirikit National Institute of Child Health (QSNICH; Bangkok, Thailand) and the Walter Reed Army Institute of Research (Maryland, USA), with the U.S. Centers for Disease Control and Prevention's Institutional Review Board (Georgia, USA) relied on the QSNICH's determination. Written parental informed consent was sought for all children.

Study population

The study population consisted of children receiving care at the QSNICH, the largest children's tertiary care public hospital in Thailand serving exclusively pediatric population from birth to 18 years of age. All study participants were residents of the metropolitan area of Bangkok and its vicinity.

Enrollment method and study procedures

Enrollment method and study procedures have been previously described (Kittikraisak et al., 2018; Kittikraisak et al., 2017). Briefly, a prospective cohort study (Pediatric Respiratory Infection Cohort Evaluation, the PRICE study) to determine influenza burden among children living in the Bangkok Metropolitan areas who sought care at QSNICH (for any cause except acute respiratory illness) was established in August 2011. Healthy children aged 0–36 months were matched by age (±1 month for children aged <1 year and ±1 year for those aged ≥1 year) and calendar time with children at high-risk for severe influenza complication (hereafter referred to as high-risk children), defined as any child with one or more of the underlying medical conditions as described in Supplementary Table S1. None of the children were immunocompromised. At enrollment, study nurses administered structured questionnaires to collect demographic and health history data. Within two months of enrollment, study nurses visited children's homes to record family and household characteristics, and childhood immunizations from children's vaccine books (including influenza vaccination if ≥6 months old). Children's influenza vaccination statuses were determined approximately every six months thereafter.

For two years during the study follow-up, caregivers were contacted weekly by telephone to identify any acute respiratory illness (ARI) in the enrolled children, defined as presence of ≥2 of the following symptoms: fever/feverish, cough, sore throat, or runny nose with onset during the preceding seven days. Caregivers of children reporting ARI were encouraged to take the child to the study hospital, on that day or the next day, for symptom verification and treatment; all had nasal and throat specimens collected during hospital visit. The specimens were tested by real-time reverse transcription PCR (rRT-PCR) for influenza virus.

All children had 2 mL of venous blood taken every six months during the course of the study to measure influenza antibody titers. Specimens from children who were ≤6 months of age at enrollment were tested as part of this study.

Laboratory testing

All laboratory testing was performed at the Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand. The rRT-PCR assay was conducted on respiratory specimens according to the U.S. Centers for Disease Control and Prevention's standard protocol (specimens with a cycle threshold ≤38 were classified as positive) (WHO Collaborating Centre for Influenza, 2009). The hemagglutination inhibition (HI) assay to measure influenza antibody titers in blood specimens was conducted using guinea pig erythrocytes according to the World Health Organization's protocol (WHO Collaborating Center for surveillance, epidemiology, and control of influenza, 2019; WHO Global Influenza Surveillance Network, 2011). Specimens were tested against a panel of representative influenza viruses circulating in Thailand at the time of the specimen collection: A/California/07/2009, A/Texas/50/2012, A/Switzerland/9715293/2013, A/Victoria/361/2011, B/Brisbane/60/2008, B/Wisconsin/1/2010, B/Massachusetts/2/2012, and B/Phuket/3073/2013. A set of specimens from each child was tested on the same 96-well plate. All blood specimens were tested in three batches to minimize inter-batch variation as much as possible.

Data analysis

Children enrolled at ≤6 months of age were included in the analytical dataset if they were born to a mother who self-reported not having received influenza vaccination during pregnancy. Enrolled children provided at least two blood specimens six months apart to permit measurement of seroconversion. All intervals and specimens collected after a child was vaccinated with influenza vaccine were excluded from the analysis. Titers from the HI assay recorded as <10 were converted to 5 for analytical purposes. Seroconversion was defined as a ≥4-fold rise in the HI titers for each influenza virus between two consecutive time points with a minimum titer on the second specimen of 40. A child could experience more than one seroconversion to the same influenza virus. Children's age for which the seroconversion occurred was determined using age at the start of the interval during which the seroconversion was measured.

At each blood collection visit, we determined whether the child had any ARI symptoms, ascertained weekly through telephone contact, during the preceding six months up to two weeks before blood collection since it usually takes 1–2 weeks for the antibodies to develop (Rothbarth et al., 1999; Kumar and Henrickson, 2012). A laboratory-confirmed episode of influenza virus infection with ARI symptoms was defined as an illness episode with symptoms that met the ARI definition and had laboratory-confirmed influenza virus infection (by rRT-PCR or serology). A laboratory-confirmed episode of influenza virus infection without ARI symptoms was defined as laboratory-confirmed influenza virus infection without any ARI symptoms reported in the preceding six months up to two weeks before blood collection (by serology) or in the proceeding ten days of respiratory specimen collection (by rRT-PCR).

The Chi-Square test was used to compare baseline demographic and household characteristics between healthy and high-risk children. We used a generalized linear model with a log link and a Poisson distribution and the natural log of person-time in years as

an offset to calculate incidence rates with 95% confidence intervals by age and health status (Dilorio, 1991). Because influenza viruses circulate perennially in Thailand, there was no need to account for seasonality in the analysis. All data analyses were conducted using SAS software version 9.0 (SAS Institute, Cary, North Carolina, USA).

Results

Characteristics of study participants

Between August 2011 and September 2013, 1,149 children were enrolled into the cohort; 350 (30%) children were enrolled at ≤6 months of age. At least two blood specimens were obtained from 306 (87%) eligible children, but 40 were excluded because they received an influenza vaccination before the collection of their second blood specimens, resulting in an analysis of 266 participants. Among these, 147 (55%) were healthy and 119 (45%) were considered high-risk at the time of enrollment.

Among those included in the analysis, the median age at enrollment was 3 months (interquartile range, 2–4). Healthy and high-risk children were similar with respect to sex, age at enrollment, daycare attendance, distance from home to hospital, mother's education, monthly household income of parents, and number of siblings aged <10 years old (Table 1). However, healthy children were more likely than high-risk children to have been breastfed at enrollment (63% vs. 50%; *P* value = 0.04). None of the mothers of children included in this analysis reported receipt of influenza vaccination during pregnancies.

Influenza rRT-PCR positivity and seroconversion rates

During the 2-year follow-up, 266 children contributed 370 person-years of observation after excluding post-vaccination periods; healthy children contributed 200 person-years (54%) and high-risk children contributed 170 person-years (46%) (Table 2). Thirty-two cases of rRT-PCR-confirmed influenza virus infection were identified when children came for routine blood draw (total of 706 visits). Eleven (35%) of the influenza viruses identified were influenza A (H1N1)pdm09, 12 (38%) were influenza A (H3N2), and 9 (28%) were influenza B. The overall rate of rRT-PCR-confirmed influenza virus infection in this cohort was 7 infections per 100 person-years (95% confidence interval [CI], 4–11), with no statistical difference between healthy (8 infections per 100 person-years, 95% CI, 5–15) and high-risk children (6 infections per 100 person-years, 95% CI, 3–12; *P* value = 0.42; Table 3A). Among 32 rRT-PCR positive cases, 6 (19%) were in children who experienced ARI symptoms during the ten days preceding the respiratory specimen collection. There were 3 (9%) rRT-PCR positive cases who had ARI symptoms without fever, while 3 (9%) reported having fever and cough.

As measured through seroconversions, there were 126 episodes of influenza virus infection over 370 person-years of observation, resulting in a rate of 35 infections per 100 person-years (95% CI, 30–42; Table 3B). Serology data show a significantly higher rate of influenza virus infection than the incidence of influenza virus infection measured through rRT-PCR (35 vs. 7 infections per 100 person-years; *P* value < 0.01). Among 126 seroconverted cases, 56 (44%) experienced ARI symptoms during the preceding six months up to two weeks before blood collection (35 infections per 100 person-years; 95% CI, 28–44) while the rest denied ARI symptoms on re-interview (67; 53%) or had only a fever (3 [2%]; 33 infections per 100 person-years; 95% CI, 25–43; *P* value = 0.78; Supplementary Table S2). There were 6 (5%) seroconverted cases who had ARI symptoms but absence of fever, while 44 (35%) reported having fever and cough.

The seroincidence rate of influenza virus infection was lowest for children between 0 and 6 months of age (23 infections

Table 1
Baseline characteristics of healthy and high-risk children enrolled in the seroincidence study, Bangkok, Thailand.

Characteristics	All children N = 266 n (%)	Healthy N = 147 n (%)	High-risk[a] N = 119 n (%)	*P* value
Male	138 (52)	74 (50)	64 (54)	0.58
Age at enrollment				0.32
<2 months	40 (15)	22 (15)	18 (15)	
2–3 months	106 (40)	53 (36)	53 (45)	
4–6 months	120 (45)	72 (49)	48 (40)	
Reported breast feeding at enrollment	153 (58)	93 (63)	60 (50)	0.04
Attended daycare at enrollment	15 (6)	8 (5)	7 (6)	0.88
Distance from home to hospital				0.18
<5 km	89 (33)	56 (38)	33 (28)	
5–9 km	47 (18)	28 (19)	19 (16)	
10–14 km	32 (12)	16 (11)	16 (13)	
≥15 km	98 (37)	47 (32)	51 (43)	
Mother completed primary school	205 (77)	120 (82)	85 (71)	0.05
Monthly household income ≤20,000 Thai Baht (570 USD)	125 (47)	61 (42)	64 (54)	0.05
Had ≥1 sibling aged <10 years	95 (36)	52 (35)	43 (36)	0.90

[a] At high risk for complications of influenza if infected (refer to Supplementary Table S1 for detail).

Table 2
Number of children at the start of each interval and person-time of observation.

Age interval (month)[b]	Number of children at the start of the interval			Person-years		
	All children	Healthy	High-risk[a]	All	Healthy	High-risk
0–6	266	147	119	140	79	64
7–12	178	94	84	94	52	43
13–18	145	76	69	75	39	36
19–24	117	63	54	58	31	27
All	266	147	119	370	200	170

[a] At high risk for complications of influenza if infected (refer to Supplementary Table S1 for detail).
[b] Represents children by age at the start of the interval during which seroconversion was measured.

Table 3
Incidence of influenza virus infection per 100 person-years in healthy and high-risk children enrolled into the seroincidence study, Bangkok, Thailand.

A. rRT-PCR-confirmed influenza virus infection

Age interval (month)[b]	All children		Healthy		High-risk[a]	
	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)
0–6	10	4 (2–12)	9	11 (6–21)	1	2 (0–11)
7–12	10	10 (5–19)	7	14 (7–30)	3	7 (2–22)
13–18	9	12 (6–23)	4	11 (4–28)	5	14 (6–33)
19–24	3	5 (1–16)	1	3 (0–23)	2	7 (2–30)
All	32	7 (4–11)	21	8 (5–15)	11	6 (3–12)

P value for comparison of healthy vs high-risk: 0.42, *P* value for age at the start of the interval: 0.22, *P* value for interaction term between arm and health status at start of the interval: 0.13

B. Seroincidence of any influenza virus

Age interval (months)[b]	All children		Healthy		High-risk[a]	
	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)
0–6	33	23 (16–33)	19	23 (14–36)	15	23 (14–39)
7–12	39	41 (30–57)	18	37 (23–58)	20	47 (30–72)
13–18	31	41 (29–58)	18	46 (29–74)	13	36 (21–62)
19–24	23	40 (26–60)	13	42 (24–73)	10	37 (20–69)
All	126	35 (30–42)	68	34 (27–43)	58	35 (27–45)

P value for comparison by arm: 0.88, *P* value for age at the start of the interval: 0.04, *P* value for interaction term between arm and age at start of the interval: 0.78.

C. Seroincidence of influenza virus A (H1N1) pdm09

Age interval (month)[b]	All children		Healthy		High-risk[a]	
	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)
0–6	8	6 (3–11)	4	5 (2–14)	4	6 (2–17)
7–12	13	13 (8–23)	5	10 (4–23)	8	19 (10–37)
13–18	11	14 (8–26)	7	18 (9–38)	4	11 (4–30)
19–24	7	12 (6–26)	4	13 (5–35)	3	11 (4–35)
All	39	11 (8–15)	20	10 (7–16)	19	11 (7–18)

P value for comparison by arm: 0.86, *P* value for age at the start of the interval: 0.14, *P* value for interaction term between arm and age at start of the interval: 0.57.

D. Seroincidence of influenza virus A (H3N2)

Age interval (month)[b]	All children		Healthy		High-risk[a]	
	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)	Number	Rate[c] (95% CI)
0–6	22	15 (10–24)	11	14 (8–25)	11	17 (10–31)
7–12	19	20 (13–32)	9	17 (9–33)	10	34 (13–43)
13–18	23	30 (20–46)	14	36 (21–61)	9	25 (13–48)
19–24	16	28 (17–45)	9	36 (21–61)	7	26 (12–55)
All	80	23 (18–28)	43	22 (17–30)	37	22 (16–31)

P value for comparison by arm: 0.98, *P* value for age at the start of the interval: 0.12, *P* value for interaction term between arm and age at start of the interval: 0.70.

E. Seroincidence of influenza virus B

Age interval (month)[b]	All children		Healthy		High-risk[a]	
	Number	Rate[c] (95% CI)	Number	Rate[c](95% CI)	Number	Rate[c] (95% CI)
0–6	6	3 (1–9)	5	6 (3–15)	1	2 (0–11)
7–12	7	7 (3–15)	5	10 (4–23)	2	5 (1–19)
13–18	4	5 (2–14)	2	5 (1–21)	2	6 (1–22)
19–24	8	12 (5–27)	6	19 (9–43)	2	7 (2–30)
All	25	6 (4–10)	18	9 (5–15)	7	4 (2–9)

P value for comparison by arm: 0.10, *P* value for age at the start of the interval: 0.24, *P* value for interaction term between arm and age at start of the interval: 0.77.

95% CI, 95% confidence interval.
[a] At high risk for complications of influenza if infected (refer to Supplementary Table S1 for detail).
[b] Represents children by age at the start of the interval during which seroconversion was measured.
[c] Incidence rates and 95% confidence intervals by age and health status were calculated using a generalized linear model with a log link and a Poisson distribution and the natural log of person-time in years as an offset.

per 100 person-years, 95% CI, 16–33; Table 3B). The overall rates did not differ between healthy (34 infections per 100 person-years, 95% CI, 27–43) and high-risk children (35 infections per 100 person-years, 95% CI, 27–45; *P* value = 0.88). Seroincidence rates were higher for influenza A (H3N2) (23 infections per 100 person-years; 95% CI, 18–28) than for influenza A (H1N1)pdm09 (11 infections per 100 person-years; 95% CI, 8-15) or influenza B (6 infections per 100 person-years; 95% CI, 4–10) viruses (Table 3C–E).

Discussion

Of 266 children followed for 370 person-years, one in three seroconverted to influenza virus infection in this study. We found no difference in seroincidence rates between healthy children and children at high risk of influenza complications. Influenza virus infection without ARI symptoms occurred in more than half of children in this study and did not differ between healthy and high-risk children.

Data on seroconversion in response to influenza virus infection in Thailand are scarce; no study has reported seroconversion data for seasonal influenza and most studies of seroconversion to influenza A (H1N1)pdm09 virus during the 2009 influenza pandemic focused on seroconversion in special populations (Lerdsamran et al., 2011; Garg et al., 2014; Khuntirat et al., 2014; Simmerman et al., 2011). For instance, using specimens collected in 2009, Lerdsamran et al. reported a substantial influenza A (H1N1) pdm09 virus-specific infection rate among healthy children aged <15 years old compared to healthy adults (Lerdsamran et al., 2011). A Thai study by Garg et al. reported influenza A (H1N1)pdm09 seroincidence in the population of men who have sex with men between 2009–2011 (Garg et al., 2014). In our study, the overall incidence of influenza virus infection was high (35 infections per 100 person-years). Although it can be difficult to compare results across studies and settings, rates of seroconversion to influenza virus infection in our study were of the same magnitude as rates reported from prior studies of seasonal influenza in older children, which ranged from 15–42 per 100 person-years (Horby et al., 2012; Hayward et al., 2014). Further, the simulated incidence among children by stochastic mechanistic models was estimated to range from 7–29% annually based on the USA's influenza serology data (Tokars et al., 2018; Sullivan et al., 1993; Ranjeva et al., 2019). In this study, we observed substantial proportions of individuals who seroconverted but did not report ARI symptoms. An earlier study suggested that infected individuals with few or no signs of illness may shed infectious virus (Foy et al., 1987), highlighting the role of infections without symptoms meeting traditional respiratory illness case definitions in possible influenza transmission. Quantifying the proportion of serologically confirmed infections without ARI symptoms can inform our understanding of influenza transmission dynamics.

In the PRICE study, which was a parental study of this work, we observed a higher incidence of influenza virus infection as determined by rRT-PCR in healthy children compared to high-risk children in the entire cohort (Kittikraisak et al., 2018). However, we did not observe a similar difference in a subset of very young children, possibly because of the small sample size. Another possible explanation is that younger children had fewer social contacts outside the home (i.e., being nursed at home instead of a daycare/school) and thus fever opportunities for infection. In the current report, only 6% of children attended daycare at enrollment, and follow-up ended when children were around two years of age. In contrast, 13% of healthy children and 11% of high-risk children in the PRICE study, more than half enrolled after one year of age, reported attending daycare/pre-school at enrollment.

Our study has several strengths. First, we collected sera every six months, making it less likely that our results underestimate seroconversion because of antibody decline, which is a concern when sera are collected at longer intervals (Hsu et al., 2014). Second, we were able to evaluate seroconversion as a marker of natural infection among a cohort of children born to women who had not received influenza vaccine during pregnancy, thereby avoiding maternal vaccination effects that would complicate interpretation of sera results during early infancy. Third, high-risk children were oversampled in our study, allowing the estimation of seroincidence in this sub-population.

Several limitations should be considered when interpreting our findings. First, seroconversions without rRT-PCR-confirmed ARI may have been associated with atypical presentations of influenza (Polkinghorne et al., 2011; Silvennoinen et al., 2011; Blumental et al., 2011; Launay et al., 2011; Lochindarat and Bunnag, 2011; Wright et al., 1980) that may be more common in young infants and were not captured by our case definition. Second, blood and respiratory specimens were not collected at the same time although we determined whether the child had any ARI symptoms during the preceding six months up to two weeks before blood collection. Third, although we excluded children who were born to vaccinated mothers, influenza virus infection among the mothers during pregnancy regardless of vaccination status was not assessed in our cohort.

Our study shows that influenza virus infection is common during the first two years of life among children in Thailand and that a large proportion of infections may not be detected using the ARI case definition.

Contributions

WK, SJO, PS, TC, FSD, KAL designed the research study. KR, WK, DD, SJO, PS, TC, CK, IY, SF, LM, KAL performed the research. KR prepared the dataset. WK and KAL analyzed the data. KR prepared the first draft of the manuscript. WK, SJO, FSD, KAL critically reviewed and revised the manuscript. All authors reviewed and approved the manuscript.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention, the Department of the Army, the Department of Defense, or the U.S. Government.

Conflict of interest statement

The authors have no conflicts of interest or funding to disclose.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.08.022.

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