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Influenza vaccine effectiveness in young children:
Thailand Influenza Network for Evaluation (TINE)

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Abstract Cover Page

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Thailand Influenza Network for Evaluation (TINE)

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An abstract of

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Abstract

The Thailand Influenza Network for Evaluation (TINE) was established to assess influenza vaccine effectiveness (VE) in the Thai population. During the 2017 Thailand influenza season (from June 1, 2017, through May 31, 2018), we applied a test-negative case-control design to evaluate the effectiveness of influenza vaccine against medically-attended, laboratory-confirmed influenza among children aged 6-36 months who presented at the seven participating hospitals in the TINE. To determine the evaluation period, all seven participating hospitals performed a rapid influenza test in children who presented with influenza-like-illness, at a rate of ten tests per week. The VE evaluation was conducted during periods when the proportion of positive rapid influenza tests from all hospitals combined was above 4%. For VE evaluation, we prospectively enrolled children 6-36 months seeking ambulatory care for acute respiratory illness (ARI) with onset ≤ 10 days. Consenting participants provided nasal and throat swabs for influenza real-time reverse transcription polymerase chain reaction (rRT-PCR) testing. Vaccination status was ascertained by vaccination booklets or hospital records. We applied a logistic regression model to determine the odds of PCR-confirmed influenza infection in vaccinated versus unvaccinated children. The VE was calculated as (1 – adjusted odds ratio) x 100.

We enrolled 2,823 children from July 23 through December 23, 2017, and 836 children from February 12 through April 12, 2018. Of 3,646 enrolled children with available influenza testing results for the VE analysis, 446 (12.2%) tested positive for influenza; of those, 310 (69.5%) were influenza A and 136 (30.5%) were influenza B. Influenza A(H3N2) and A(H1N1)pdm09 virus comprised 62.3% and 37.7% of influenza A positive tests respectively while influenza B-Yamagata lineage comprised 92.6% of influenza B positive tests. Influenza vaccination coverage was 6.8% and 4.7% for full vaccination and partial vaccination respectively. The VE against PCR-

confirmed medically-attended influenza illness was 54.6% (95% confidence interval (CI): 23.0, 73.2) for full vaccination. The VE for influenza A(H1N1)pdm09, A(H3N2), and influenza B were 84.1% (95% CI: 34.6, 96.1), 50.4 (95% CI: -8.4, 77.4), and 15.4 (95% CI: -87.1, 61.8) respectively. According to the Thai National Influenza Center surveillance data, the reduced VE against influenza A(H3N2) is due to the antigenic drift of circulating influenza A(H3N2) virus to subclade 3C.2a1. And the VE against influenza B is likely to be impacted due to the fact that the predominant circulating influenza B was a Yamagata lineage virus while the influenza B composition of the trivalent influenza vaccine 2017 used in Thailand is a strain of influenza B-Victoria lineage virus. Overall, influenza vaccine provided moderate protection for Thai children during 2017 season. Additional efforts to increase vaccination coverage and continue effectiveness monitoring are warranted.

INFLUENZA VACCINE EFFECTIVENESS IN YOUNG CHILDREN:

THAILAND INFLUENZA NETWORK FOR EVALUATION (TINE)

Piyarat Suntarattiwong

Thesis submitted to Hubert Department of Global Health

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In partial fulfillment of the requirements for the degree of

MASTER OF PUBLIC HEALTH

In

Infectious Diseases

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Chapter 1

Introduction

Influenza is a serious global public health problem that accounts for 3–5 million severe illnesses and an average of 290,000–650,000 deaths worldwide each year[1]. Certain conditions or comorbidities confer a higher risk of severe influenza including pregnancy, children under 59 months, the elderly (65+ years) and individuals with chronic medical conditions or immunosuppressive conditions. Additionally, healthcare workers are at high risk of acquiring influenza virus infection due to increased exposure to patients and risk further spreading influenza, particularly to vulnerable individuals[1]. Vaccination is the most effective way to prevent influenza infection. The World Health Organization (WHO) recommends vaccinating those most at risk for severe influenza and its complications, and those who easily transmit influenza to high-risk populations (e.g., healthcare workers). WHO summarizes antigenic variation of influenza virus submitted from the WHO surveillance network with epidemiological data to propose recommendations for influenza vaccine compositions of northern and southern hemisphere influenza vaccines in February and September annually[1].

Most high-income countries in northern and southern hemispheres consistently implement routine annual influenza vaccination, and there is increasing interest in expanding such programs in developing countries. However, several important health intervention programs compete for limited resources in developing countries. Many developing countries are situated in tropical or subtropical zones where influenza seasonality and circulating strains are not well-defined, which poses an additional challenge to implement influenza vaccination programs in those countries.

Thailand is a middle-income tropical country in Southeast Asia with almost a year-round influenza season with a peak usually between July and September. Also, another period of

increased activity is observed between January to March[2-5] (Figure 1)[5]. Health services in Thailand are provided by the government as well as private sector. Health care costs are covered for public providers by the Universal Health Coverage Scheme. Budgeting includes disease prevention measures such as vaccines in the Expanded Program on Immunization (EPI).

While influenza-related complications are difficult to enumerate, efforts have been made to estimate influenza-related morbidity and mortality in the Thai population. Two studies demonstrated the average annual influenza-associated mortality rates were approximately 4 and 6 per 100,000 persons; this rate was more than ten times higher among the elderly (age ≥ 60 years in one study and > 65 years in another)[6,7]. The estimated annual incidence of influenza pneumonia requiring hospitalization was between 18 and 111 per 100,000 population in one study, with children under 5 and adults over 60 having experienced the greatest burden of disease[8]. Another study exploring influenza burden revealed the annual incidence of influenza in young children <5 years was 236 per 100,000 and in persons aged ≥ 75 was 375 per 100,000[3]. Moreover, a study of outbreaks of influenza among healthcare workers reported that the incurred costs of disease and its complications exceeded the costs of healthcare worker influenza vaccination by more than 10-fold[9]. These burden of influenza morbidity and mortality data as well as the increasing outbreaks reported among Thai healthcare institutes and the emergence of the 2009 pandemic influenza have prompted the Thai Ministry of Public Health (MoPH) to begin an annual influenza vaccination program targeting at-risk populations with aims to reduce morbidity and mortality in the groups known to have the greatest incidence of influenza-related complication and deaths.

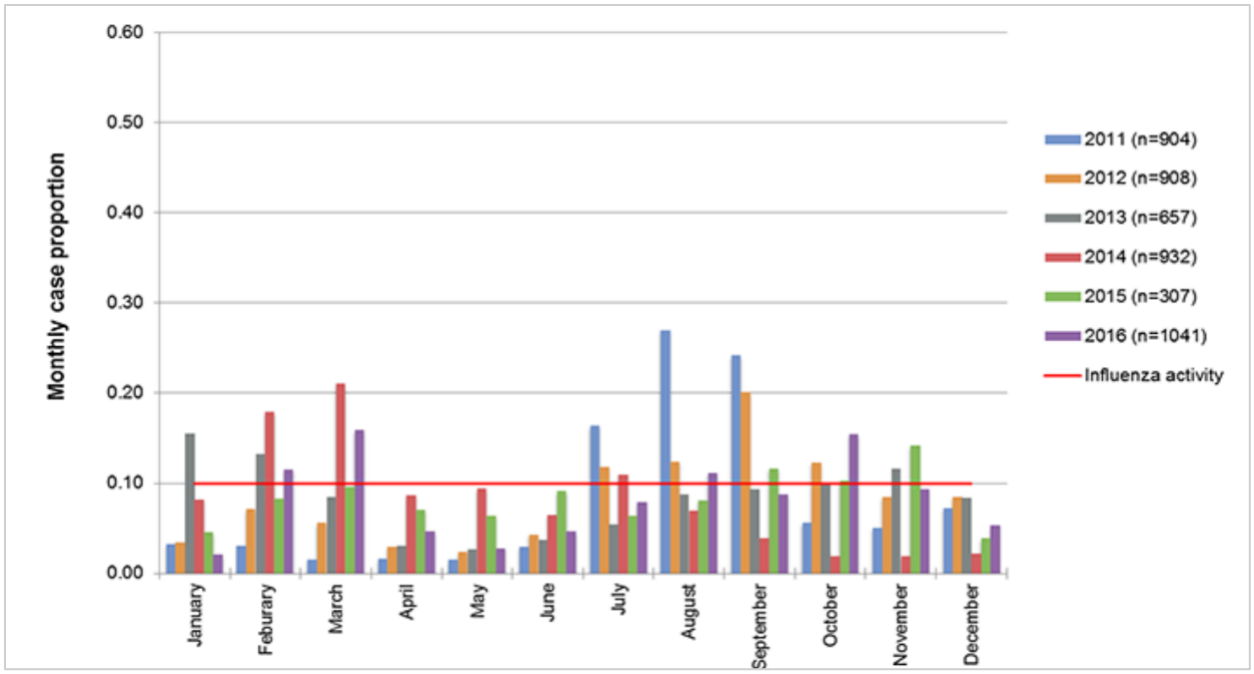


Figure 1 The two peaks of influenza activity in Thailand, 2011-2016, Newman LP, et al. PLoS One (2018)

Since 2009, the Thai MOH launched the National Influenza Immunization program for 5 target populations including: (1) pregnant women (2) children 6 – 36 months of age (3) persons with chronic medical conditions including significant obesity (4) persons > 65 years of age (5) healthcare personnel[10]. The vaccination campaign begins around May to June with vaccination free vaccine of charge from May to August each year[10]. The program provides Southern Hemisphere Trivalent inactivated influenza vaccines (TIV). The Southern Hemisphere strain is selected according to the multi-year surveillance data from the Thai National Influenza Center (http://www.thainihnic.org). Live-attenuated influenza vaccine is not registered in Thailand while Quadrivalent inactivated influenza vaccine (QIV) is registered and provided by some healthcare facilities especially in the private sector with a charge to the patients. The Northern Hemisphere vaccine is also available in Thailand around December to January in the following year but not included in the program. Although the program timing is based on the peak of Thailand influenza

activity, there is no restricted timing recommendation for influenza vaccination in the Thai population due to the year-round circulation as demonstrated in Figure 1.

Using the administrative data from the National Health Security Office (NHSO) and denominators from Thailand national statistics and surveys, the vaccine coverage of the program is still low[10]. Coverage varies among risk groups; higher in the elderly and persons with chronic diseases and lower among children aged 6-36 months and pregnant women; the coverage for young children ranges from 1.5 – 1.6% (Figure 2). While some of the relatively increased coverage of influenza vaccination in the elderly may be due to the "first-come, first-served" vaccine distribution mechanisms, additional data on vaccine effectiveness and safety in other priority groups could encourage the procurement of additional vaccine in Thailand, and the specific allocation of vaccines for specific groups such as children and pregnant women in delivery settings.

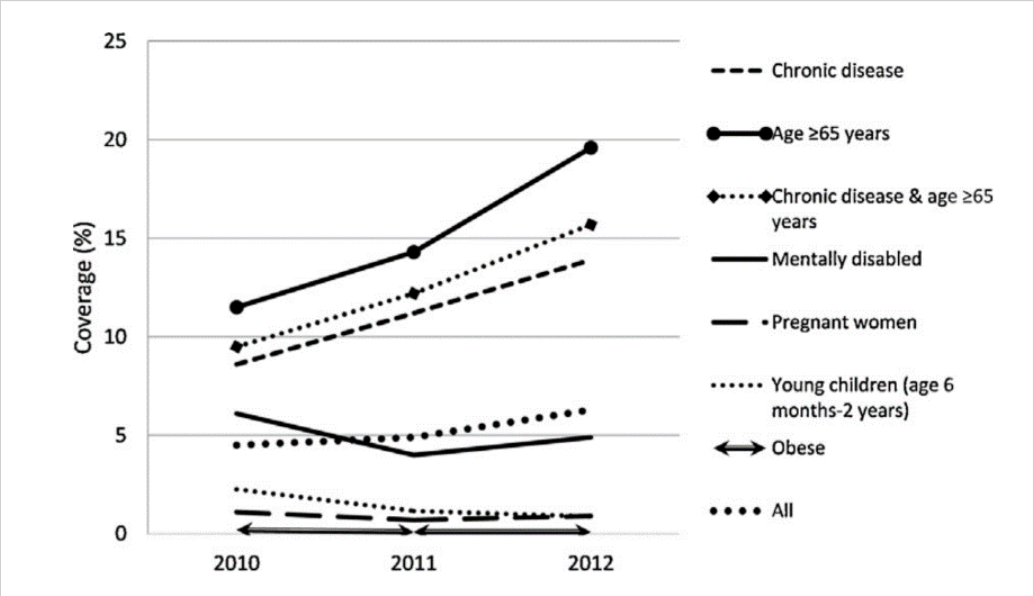


Figure 2 Influenza vaccine coverage from the national program 2010-2012,
J.T. Owusu et al. Vaccine 33 (2015) 742-747.

Published data on influenza vaccine effectiveness evaluation in Thailand are limited; four articles with laboratory-confirmed influenza as an outcome were identified from PubMed in the last decade. One study was conducted in 2 provinces[11], and the rest were conducted in metropolitan Bangkok[12-14] whereas the program administers vaccines in all 76 provinces in 5 regions including North, Northeast, Central, and South of Thailand. Regarding the vaccine, a large variation of effectiveness (VE) is well recognized; such as year to year variation due to the degree to which virus strains included in the vaccine match circulating viruses, or other factors including age, comorbid conditions, the time within the influenza season, and different geographic regions[15].

A multi-sites approach is generally used for evaluation of influenza VE to accommodate the variability of VE estimates and to facilitate timely results for public health communication. Multi-sites network for VE evaluation are well established in temperate climate countries, for example, the U.S. Flu VE Network, I-MOVE (Influenza - Monitoring Vaccine Effectiveness) in Europe, and the FluCAN in Australia. In Latin America, a VE evaluation platform (Relevac-i) has been created based on the existing influenza surveillance system[16]. The VE networks mostly employ the test-negative design to evaluate influenza VE due to the implementation practicality. The network may produce information specifically for each country or region. There is neither a country nor a regional network in Southeast Asia for influenza VE evaluation.

In Thailand, since the seasonal influenza vaccines for specific risk groups have been publicly funded in 2009, a platform for the timely evaluation of influenza VE has not yet been developed. Queen Sirikit National Institute of Child Health, the government referral children hospital in Bangkok, and the Thailand-MoPH US CDC (TUC) Influenza Program are working collaboratively to establish a network of 7 hospitals across five regions of Thailand to serve as a platform for influenza VE estimates. In the starting year, the network will generate influenza VE estimates in young children, one of the influenza vaccine targets during the annual influenza season

using a “test-negative” study design. This evaluation of a public health program is intended to provide information to the Thai MOPH to better understand the benefits of the influenza vaccination program for young children. In combination with efforts to increase influenza vaccine uptake in children, measurement of VE in this age group may help the MOPH in evaluating the impact of vaccination in this age group. Also, the network may potentially serve to assess VE in other risk groups or to enhance other influenza research in the future.

Chapter 2

Literature review

Influenza

Influenza is an acute, febrile illness caused by the influenza virus. Influenza outbreaks of varying severity occur almost every winter in temperate climates, and in tropical climates, influenza occurs year-round. Influenza may cause a pandemic with the greatest pandemic in recorded history having occurred in 1918-1919 when 21 million deaths were estimated worldwide[17].

The viruses

Influenza viruses are enveloped viruses which belong to the family Orthomyxoviridae and can be classified into influenza type A, B, or C[17]. Types A and B may cause severe diseases whereas type C rarely causes disease in humans. Influenza A and B structures comprise 8 RNA genome segments encoding several proteins for the virus. The two important proteins related to immunity are the surface glycoproteins *Hemagglutinin* (H) and *Neuraminidase* (N). There are at least 16 antigenically distinct H and nine antigenically distinct N glycoproteins variation of influenza A[17]. The H and N are assigned by number; they determine the subtype for influenza A, for example, Influenza A(H3N2).

Although influenza B viruses have a similar structure to influenza A. They are not divided into subtypes, but can be further broken down into lineages and strains. Two antigenically distinct lineages of influenza B viruses called the “Victoria” and “Yamagata” lineage co-circulated in human[17].

There are two antigenic changes of the surface glycoproteins called “antigenic drift” and “antigenic shift.” Antigenic drift is a continuous process resulting from limited proofreading of the

viral RNA polymerase causing accumulation of point mutations of H and N protein genes. This process induces minor antigenic changes, yet permits the virus to escape immunity induced through previous exposure to the wild viruses or vaccination; thus, drifted strains of influenza A and B circulate in humans and result in annual seasonal influenza epidemics (or epidemic influenza)[18]. While annual epidemics can occur with undrifted strains, they tend to be less severe than when a drifted strain causes the epidemic. The fact that there are annual epidemics, often with strains not in previous vaccines, leads to the requirement for annual influenza vaccination to mitigate the burden of seasonal influenza.

Antigenic shift arises when viruses with a completely new hemagglutinin (H) to which virtually the entire population is susceptible begins circulating in humans. These new viruses may arise from reassortment in which an animal virus with a new H exchanges genes with a human adapted strain as both strains infect the same cell. Antigenic shift occurs in influenza A, not influenza B, and may cause an influenza pandemic [18,19]. The nomenclature of an influenza strain consists of type, subtype, the city of first identification, the strain number from the isolating laboratory, and the year of virus isolation[19], e.g., A/Hong Kong/4801/2014 (H3N2) virus, A/Michigan/45/2015 (H1N1)pdm09 virus, B/Brisbane/60/2008 virus, B/Phuket/3073/2013 virus, and so on.

Clinical manifestations, diagnosis and treatment

Influenza virus infection may range from asymptomatic infection to severe and fulminant illness. After an incubation period of 1 to 2 days, the patient typically develops fever, myalgia, cough and other respiratory symptoms including runny nose and sore throat. The fever can be as high as 41°C or more in the first few days of the illness[17,18]. Children have higher maximum temperature than do adults. Complications of influenza infection, seen in infants and young children, as well as older age groups may include lower respiratory tract infections such as

laryngotracheobronchitis (croup), bronchitis, bronchiolitis and pneumonia. In addition, children may present with febrile seizures due to the high fever[18]. Children also experience gastrointestinal symptoms such as diarrhea, vomiting, abdominal pain in a higher proportion than adults[19]. Abdominal illness is rare in adults.

The major influenza complication is pulmonary disease. Influenza can cause primary pneumonia or pneumonia secondary to bacterial superimposed infection. Influenza infection may also lead to an exacerbation of the underlying lung disease including asthma, chronic obstructive pulmonary disease, or bronchopulmonary dysplasia[18]. Non-pulmonary complications include myositis, myocarditis, encephalitis, and acute hemolysis in individuals with an underlying hemoglobin disease[18]. Influenza tends to be more severe or with complications when it occurs in persons with underlying chronic conditions.

Clinical diagnosis of influenza is not accurate due to its overlapping manifestations with other respiratory tract viral illnesses. Today, laboratory diagnosis of influenza regularly is done by reverse transcription polymerase chain reaction (RT-PCR) with its sensitivity close to 100% and turnaround time is as short as 1-8 hours[17,18]. In settings like primary care units where RT-PCR is not possible, some clinicians use rapid antigen detection tests (Rapid Test) with lower and variable sensitivity but very short turnaround time at less than 30 minutes[18]. The Rapid Test is used primarily for triage purposes as a point-of-care test and its consistently high specificity may help to support clinical decision before the RT-PCR results available. The lower sensitivity of the Rapid Test has limited its use for disease confirmation and research.

Neuraminidase inhibitors (antiviral drugs) including oseltamivir, zanamivir, and peramivir are specific for the treatment of influenza. In randomized controlled trials of uncomplicated influenza in healthy individuals, the antiviral drugs shorten the disease duration[18] and in a meta-analysis of influenza-confirmed patients antiviral treatment reduced the risk of lower respiratory

tract complications by 44% (relative risk 0.56 [95% CI 0.42-0.75])[20]. It is recommended to give neuraminidase inhibitors to influenza patients who have severe influenza such as hospitalized patients, and the patients who are at risk of influenza complications such as those with underlying diseases regardless of their clinical presentations. Antiviral drugs should be given within the first 48 hours after symptoms onset[17]. The treatment benefit after 48 hours of symptoms onset is controversial but the treatment may also be considered[1,17].

Disease burden

Influenza epidemics are regularly associated with excess morbidity and mortality. In the U.S., influenza is responsible for 14 to 16 million excess respiratory illnesses per year among individuals younger than 20 years of age, and for about 4.5 million excess illnesses in older individuals. The impact is related mostly to the disability from the disease; a study describes 5 to 6 days of restricted activity, 3 to 4 days of bed disability, and about 3 days lost from work or school[17].The U.S. CDC estimates that influenza has resulted in between 9.2 and 60.8 million illnesses, between 140,000 and 710,000 hospitalizations and between 12,000 and 56,000 deaths annually since 2010[21] (Figure 3).

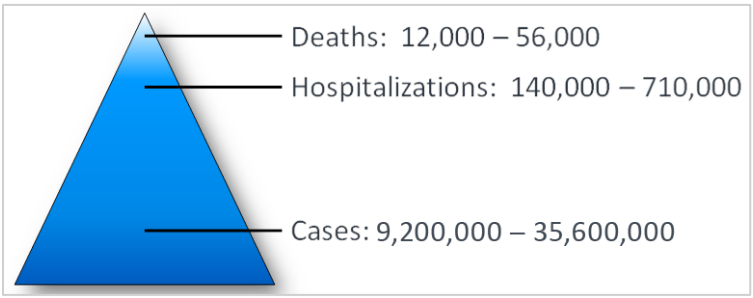


Figure 3 Estimated of influenza infections in the U.S. per year

(https://www.cdc.gov/flu/about/disease/2015-16.htm)

In other temperate climate countries or regions, influenza burdens have also been described; the estimate of excess all-cause mortality from influenza in 17 European countries for

the 2016-2017 season was 217,000 excess deaths, and 7,400 hospitalized cases with half of these associated with hospitalization in intensive care units[22]. Estimates from modeling the national database in the United Kingdom shows the highest risks of influenza-attributable hospitalizations and deaths were among adults aged > 75 years (252/100,000 and 131/100,000 population, respectively). Also, these rates are markedly higher in adults identified as at-risk compared others within the same age range[23].

In tropical climates, increasing evidence also shows the high burden of influenza although data may not be generated from some countries in the regions, and some components of the burden information, such as the contribution of influenza infections to illness and death from underlying diseases, or economic burden and productivity loss from influenza, are still scattered[24]. A study conducted in Latin America, by active community-based surveillance in 4 regions of Peru demonstrated 1 in 10 persons developed influenza each year (100 per 1000 person-year [95%CI 97-104]) with the highest incidence in young children. Hospitalization was 0.7 per 1000 person-year [95% CI 0.4-1.0] and the death rate was 2.8 per 100,000 person-year[25]. Another study in the Americas using a regional mortality database and country influenza virus surveillance data to model the influenza mortality in specific age groups, reported the annual influenza-associated mortality rate was 2.1/100,000 among individuals <65 years, 31.9/100,000 among those 65–74 years, and 161.8/100,000 among those ≥75 years in 35 Pan American Health Organization (PAHO) countries[26].

To measure influenza burden in low and middle countries, a multinational collaboration utilized existing influenza surveillance and ICD-10 cause of death or annual estimates of respiratory death data from 33 countries from different geographic regions including India, Bangladesh, Indonesia and Thailand in South and Southeast Asia, and Kenya and South Africa in sub-Saharan Africa to model country-specific influenza-associated respiratory excess mortality

rates (EMR) for three age groups (<65, 65-74, and ≥75 years)[27]. The EMR for participating countries from South and Southeast Asia and sub-Saharan Africa ranged from 1.3 to 5.1 per 100,000 among individuals < 65 years. The authors extrapolated the existing countries data to model influenza-associated respiratory death of each global region and the results show that influenza contributes to a substantial annual burden of deaths globally which was greater among low-income countries in sub-Saharan Africa and Southeast Asia[27]. For the economic component of influenza burden, the data were scarce from low- and middle-income countries, in a literature review; the findings reveal evidence from sub-Saharan Africa and in pregnant women remain limited while the available information from countries in Latin America and Asia shows influenza results in lower direct costs, but higher productivity losses compared to high-income countries[28].

Vaccination

The most effective method for prevention of influenza infection is vaccination. Four types of Influenza vaccines are licensed: 1) Egg-based inactivated virus 2) Cell-cultured-based inactivated virus, 3) Recombinant hemagglutinin influenza vaccine and 3) Live-attenuated virus vaccine[18]. The egg-based, cell-cultured-based inactivated virus vaccine and recombinant hemagglutinin vaccine are administered by intramuscular injection. The live-attenuated vaccine is administered intranasally[17]. It is available in limited countries including the U.S., Canada, and the E.U[18].

The egg-based inactivated influenza vaccines are used worldwide while the cell-cultured-based and recombinant vaccine are also available in the U.S.[17,18]. In the 2017–18 recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding the use of seasonal influenza vaccines in the United States, egg allergic persons can use any type of influenza vaccine[29]. All types of vaccine in the market contain either three or four influenza virus strains.

The trivalent influenza vaccine (TIV) contains representative strains of 3 major circulating influenza viruses: A (H3N2), A (H1N1) and one of the two lineages of B. The quadrivalent influenza vaccine (QIV) containing representative strains of influenza A (H3N2), A (H1N1) and 2 influenza B lineages[17,30]. Since influenza viruses possess the ability to drift antigenically, the strain composition in the vaccine is evaluated for reformulation annually. Usually at least one of the strains is changed from the preceding year. Seasonal influenza vaccination is recommended annually. The World Health Organization (WHO) convenes technical consultations in February and September each year to recommend strains for inclusion in influenza vaccines for the northern hemisphere (NH) and southern hemisphere (SH) influenza season, based on surveillance, laboratory, and clinical information. The strains recommended may be similar between the NH and SH in some years. An example of strain composition recommendations is as follows: Recommended in September 2017, the 2018-19 SH influenza season vaccine should consist of the following strains[31]:

- A/Michigan/45/2015 (H1N1) pdm09-like virus
- A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus
- B/Phuket/3073/2013-like virus

Influenza vaccine manufacturers produce the vaccine following the recommendation for strain inclusion. Vaccines are made available in the countries using the SH vaccine in April 2018, approximately six months after the WHO consultation meeting, likewise for NH vaccine. The approximate production timetable of NH influenza vaccine production is shown in Figure 4[30].

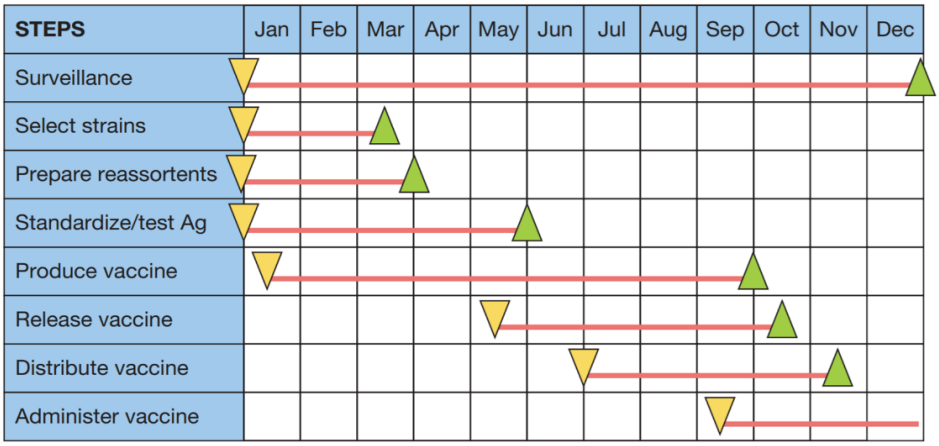


Figure 4 Approximate production timetable for NH influenza vaccine,
Bresee JS, Fry AM, Sambhara S, Cox NJ. In: Plotkin SA, Orenstein WA, Offit PA, Edwards KM, ed. Vaccines. 7th ed. p 469.

The two distinct lineages of influenza B; Victoria and Yamagata lineages may cocirculate in various proportions in different countries. The B/Phuket/3073/2013-like viruses in the example above represent the B/Yamagata lineage. Therefore, a B/Brisbane/60/2008-like strain representing the B/Victoria lineage is the additional strain present in QIV in the 2018 SH vaccine[31]. Both the TIV and QIV are used globally; however, in the U.K. and some European countries, only QIV is recommended in specific groups, particularly children[32,33].

In the U.S. and many developed countries, influenza vaccine is recommended for all individuals 6 months of age or older[17,30]. In contrast, many developing countries still lack national guidelines for influenza vaccine[2,34]. Among countries with national guidelines in the Asia-Pacific region, the recommendation is based on WHO classification of high-risk groups for severe influenza including children age 6 to 59 months, elderly, pregnant women, individuals with chronic illness, and healthcare workers[2].

The decision about which vaccine formulation (NH or SH) to use depends on the antigenic match between the vaccine virus strain and the circulating influenza viruses within the country. The situation is less clear-cut in tropical and subtropical countries; some countries, for example, Thailand and the Philippines in Southeast Asia and El Salvador, Peru, and Paraguay in Latin

America use SH while countries like Mexico and Ecuador use NH vaccine, and some countries like Malaysia, Hong Kong, and Indonesia use both[2,34]; many countries do not state which formulation is to be used in their guidelines[34]. The timing of vaccination is typically before the influenza season in temperate climates, but it is a challenge for tropical and subtropical countries because of the timing of the production cycle of the vaccine formulation the country decides to use and the lack of a clear seasonal pattern in some countries[34]. Most vaccination campaign timings are between October to March and April to July for countries using NH and SH vaccine, respectively. Some countries do not specify such time periods (e.g., vaccination is offered all year) in their recommendations[2,34].

The protection of inactivated influenza vaccine has been assessed in numerous clinical studies both in clinical trials and observational studies. Randomized controlled trials have mostly been conducted in healthy adults with a wide range of efficacy from 40-80% with a lower level of efficacy typically seen in years with apparent antigenic mismatch[17,30]. In a meta-analysis of randomized controlled trials including 8 studies using a PCR-confirmed influenza end-point, in healthy adults aged 18 to 49 years (2 studies included up to 64 years) the pooled efficacy was 59% (95% CI: 51, 67)[35]. Host factors such as age, underlying medical conditions, history of prior infections and vaccinations can affect the response to the vaccine. Studies have demonstrated that vaccine-naive young children require two doses of vaccine administered at least 4 weeks apart to gain adequate protection against influenza[30]. Because of the existing recommendation, few prospective trials of protective efficacy have been conducted in high-risk populations. Only a clinical trial in persons 60 years or older reported a vaccine efficacy of 58% (95% CI: 26, 77) against serologically-confirmed influenza[17,30]. A controlled trial in pregnant women reported a vaccine efficacy of 50% (95% CI: 15, 71) plus 49% (95% CI: 12, 70) efficacy against influenza in their infants. A few randomized controlled trials in children provide widely varying results from

17-91%, largely depending on the circulating strains of each season[30] In the most recent Cochrane Database of Systematic Reviews, inactivated influenza vaccine efficacy was 64% (95% CI: 52, 72%) in children aged 2 to 16 years[36].

Assessing Vaccine Efficacy and effectiveness

Vaccine efficacy and vaccine effectiveness measure the proportionate reduction in cases among vaccinated persons compared to unvaccinated persons. Terms related to vaccine studies may be described as followed[37,38];

Efficacy is the percentage reduction in the disease incidence among those who are vaccinated according to the recommended schedule compared to similar unvaccinated persons, under optimal conditions. This is generally measured in a placebo-controlled randomized trial as the "per protocol" efficacy (i.e., excluding persons who do not follow the schedule).

Effectiveness is the percentage reduction in the outcome of interest (specifically, disease incidence, but may be other outcomes) among vaccinated persons compared to unvaccinated persons, in the context of real-world use of the vaccine (i.e., observational studies). It may be different from the vaccine efficacy because of factors encountered when the vaccine is implemented such as a dosing schedule and cold-chain maintenance. Also, in the real-life use, vaccines are administered universally and thus may include persons with less robust immune responses that would have been excluded in efficacy trials. And probably the major problem with observational studies is the unvaccinated group may be quite different from the vaccinated group in risks of influenza, seeking of care for respiratory illnesses, and other factors, which can bias the effectiveness estimate from what the true efficacy would be.

Impact quantifies the reduction in disease at a population level following the introduction of vaccine. It is determined by a combination of vaccine effectiveness and coverage in the population,

and any herd effect (the disease is reduced, results in less transmission and even unvaccinated persons in the community become protected indirectly because they are not exposed to the virus).

Vaccine efficacy

In a randomized clinical trial (RCT), vaccine efficacy is the percent reduction in the incidence of disease among the vaccinated (I_{vac}) compared to the incidence of disease among the unvaccinated (I_{unv}). The equation can be written as follow[38];

$$\text{Vaccine efficacy} = \frac{I_{unv} - I_{vac}}{I_{unv}} \times 100$$
$$= 1 - \frac{I_{vac}}{I_{unv}} \times 100$$
$$= (1 - RR) \times 100$$

The relative risk (RR) is the ratio of incidence in the vaccinated to unvaccinated groups. In the vaccine efficacy study, we apply a RCT strategy which is designed to maximize internal validity and minimize confounding by randomization. Typically, both observers and subjects are blinded to reduce selection and measurement bias. The outcome measurement usually is an objective outcome, for example, RT-PCR confirmed influenza.

Most vaccine efficacy study is intended to establish the biologic performance capacity of the vaccine. Persons recruited to a vaccine efficacy study are required to strictly follow the assigned vaccination schedules or be eliminated from the efficacy analysis. On the product side, the "experimental" vaccine chain-of-custody and storage at the trial site is well controlled until the vaccine is administered to the subjects.

Efficacy studies are mandatory for vaccine licensing before use in the population, if there are no known correlates of protection such as an antibody level that correlates with immunity. The U.S. FDA requires results from Phase III studies, generally large-scale trials to provide a more thorough assessment of safety and a definite assessment of efficacy[39]. The endpoints of clinical

trials are product specific and may be clinical disease. However, immune response endpoints or "immunogenicity" is considered if efficacy against clinical disease had been previously established and there are immune correlates or surrogates of that protection[39]. For influenza infection, antibody to hemagglutinin (H) can be measured by standard HAI test or the microneutralization (MN) test. A high level of antibody is accepted as protection against influenza; an HAI titer of 1:40 to a specific strain is often referred to as a "protective titer" although it does not guarantee protection for all individuals receiving the vaccine, particularly the elderly[17,30].

The advantages of a vaccine efficacy study include the control for biases through randomization, careful tracking of disease outcome and vaccination status is certain, thus demonstrating the true performance of the vaccine[40]. The disadvantages include resource-consuming from the complexity and expense to conduct. Results may not apply to the population not enrolled in the trials (generalizability). Also, it may be unethical to randomly assign a person to not receiving vaccine when the vaccine is known to be effective.

Vaccine effectiveness

After licensure and a recommendation for use in a given population as the "standard of care", it is not ethical to randomize the population to which the vaccine is recommended into an unvaccinated control group for comparison with the vaccinated group in clinical trials; however; there is still the need to continue to evaluate the vaccine. Vaccine effectiveness assessments demonstrate how well vaccines perform in real-life settings. It is critical to determine the benefit of the vaccination program and to promote immunization, especially for influenza vaccine for which reformulation is necessary every year.

Observational studies are possibly subjected to biases such as unequal exposure to disease or an unequal chance of getting disease diagnosis between systematically selected vaccinees and non-vaccinees, or some specific groups of person may be systematically selected by the providers

to receive the vaccine. Essential components to be considered for vaccine effectiveness studies include the followings[38];

1. Disease case definition should be sensitive and specific.
2. Case finding is at the same degree in both the vaccinated and unvaccinated, which may be a bias for studies relying on passive reporting systems where clinicians might feel it is more important to report disease among those vaccinated (or unvaccinated).
3. Equal effort should be made to confirm the vaccination status of both persons with and without disease, and with reliable methods for vaccination status ascertainment.
4. There is a need to measure covariates that may confound the association between vaccination and the outcome.

Vaccine effectiveness (VE) may be measured in observational study designs, generally cohort or case-control approaches. Another approach is called the "Screening method." It provides a preliminary estimate of VE when the population vaccine coverage is known or can be derived from routine surveillance data. The estimates are only approximate, and there is no control for confounding although an adjustment for age can minimize this bias partially. By comparing the proportion of case vaccinated (PCV) with population-level vaccine coverage (PPV), the VE can be determined in the equation[37,41].

$$VE = \frac{1 - (PCV)(1 - PPV)}{(1 - PCV)(PPV)} \times 100$$

The VE estimation could be inaccurate if the values for PCV and PPV are drawn from different populations. A recent study has adopted this method, also called "Case-cohort" method to estimate the influenza VE against pediatric deaths in the U.S.[42]. The cases were from the Influenza-Associated Pediatric Mortality Surveillance System and cohorts aggregated from 2

National Surveys and an insurance claim database. The authors compared the odds of vaccination among cases with odds of vaccination in comparison cohorts and estimated VE against influenza death was 65% and 51% among children with and without high-risk conditions respectively[42].

The cohort studies estimate effectiveness by comparing the disease incidence among vaccinated and unvaccinated persons, often time in a closed setting, e.g., school or nursing home when a vaccine-preventable disease outbreak occurs or may be designed to measure the secondary case in a household[38].VE is calculated from the relative risk (RR) of disease between vaccinated and unvaccinated, adjustment is made for covariates, and VE is calculated as1 minus the adjusted RR multiplied by a hundred to get the percent[41].

The cohort design has some features of clinical trials, but the vaccinated and unvaccinated are already pre-selected by individuals in the cohort rather than randomly assigned as in RCTs. Therefore, it may subject to a bias called healthy user bias. An example is a cohort study of the elderly on effectiveness of influenza vaccine showed the vaccine recipients were more likely to receive a pneumococcal vaccine, have quit smoking, and be physically independent compared to the unvaccinated elderly[43]. Cohort studies require large samples, may be costly, and vaccination status ascertainment may not be feasible for the entire cohort in countries where electronic vaccine registries have not been fully implied. Also, the cohort design may not be practical for diseases with low incidence[37].

Case-control studies assess VE by enrolling cases (individuals with the target disease) with comparable controls (no disease) and obtaining the vaccine history from both groups. The vaccine effectiveness is estimated by the following equation:

$$VE \approx (1 - OR) \times 100$$

The odds ratio (OR) is the odds of vaccination in the cases relative to the odds of vaccination in the controls, which is used to approximate the RR and calculate VE for the case-control study[38,40]. One advantage of this approach is that data pertaining to multiple factors which can potentially confound the result can be collected and evaluated. Case-control designs require fewer samples than the cohort. Thus resources can be utilized to maximize data quality for confirming cases and vaccination status. They are particularly useful for an uncommon disease outcome[37]. To avoid bias, controls should be selected from the same population as cases and should not significantly differ regarding their probability of vaccination or exposure to infection[38]. In the influenza vaccine VE studies if using case-control typically community controls are selected[44]. Case-control VE studies may be biased in very high vaccine coverage settings because of the strong potential for confounding in the unvaccinated versus vaccinated populations. Also, high coverage may increase the sample size and if the vaccine is very effective may eliminate cases to be enrolled[37].

In the past decade, another case-control design called "test-negative study" has been used increasingly. The test-negative design (TND) compares the prevalence of prior vaccination between individuals seeking care for a defined set of symptoms who tested positive (case) and cases who tested negative (control) for the disease or outcome of interest. The TND has the advantage over traditional cohort or case-control designs of being relatively less expensive and fast to conduct. The use of a laboratory test to confirm the outcome lessens disease misclassification bias. It also reduces the possibility of differential healthcare seeking behaviors among cases and controls because both are enrolled from patients seeking care at the same facility who meet the same clinical case definition for inclusion[45]. Vaccination status may be ascertained using self-report, vaccination records, or registry.

In a test-negative design data may be collected prospectively or retrospectively. The prospective data collection TND is usually embedded in disease surveillance program using sentinel sites while the retrospective data collection is performed through medical records. Both can collect data on potential confounders such as age, medical conditions, and health behaviors. VE is estimated from the odds ratio comparing the odds of testing positive among vaccinated to the odds among unvaccinated patients, usually by means of logistic regression, adjusting for potential confounders[45]. Cautions of TND include some important confounders may not be collected such as prior exposure to the vaccine or the disease, inconsistency in model specifications and different covariate sets among studies[15,45], and individuals' decisions to vaccinate may associate with other factors influencing their susceptibility to disease[46]. Disease misclassification can occur if enrolled subjects present late during illness and the test sensitivity is reduced[45]. Because TND selects only subjects seeking care, it does not measure the effectiveness against symptomatic disease in which diseases occurs in persons who do not seek medical care for that problem. The generalizability of the VE estimates remains a concern for some researchers[45,47], although reasonable validity, speed, and economy result in TND use for evaluation of VE for influenza vaccination programs worldwide[45].

Meng Shi et al., have evaluated the bias and the precision of influenza VE estimates from TND[48]. The TND may produce valid estimates of VE if the vaccine does not modify the risk of non-influenza acute respiratory tract infection (ARI). Also, when influenza vaccine reduces the probability of seeking care against influenza ARI, the VE estimates against medically-attended influenza ARI may be unbiased while estimates against any symptomatic influenza may have a substantial positive bias in a TND-based study[48,49].

Influenza vaccine effectiveness (VE) studies in Southeast Asia

Seasonal influenza VE in the U.S., Europe, Canada, and Australia are estimated annually, applying the TND except in Europe which uses both case-control and cohort-based approaches[15,22,44,50]. The U.S. CDC has been publishing VE estimates against medically-attended laboratory-confirmed influenza to inform the Advisory Committee on Immunization Practices (ACIP), public health officials, providers, and the public[50]. Influenza VE in 2 high-risk groups: the elderly and young children, have also been extensively studied in temperate climate, high-income countries but not in tropical countries. For examples, 5 meta-analyses of VE in the elderly reviewed by the WHO Global Influenza Program only included 3 studies from the tropics, out of 201 total studies in the meta-analysis; 2 studies were from Taiwan and 1 was from Hong Kong. Fourteen systematic reviews in children meta-analyzed up to 47 studies, most of which were from high-income countries[34]. These findings indicate the low numbers of methodologically-accepted influenza VE studies from Southeast Asia until recent years. However, individual studies from some countries in this region have been published.

From a PubMed search with keywords "influenza vaccine" and the country's name, several pandemic and seasonal influenza vaccine trials and immunogenicity studies were identified as having been conducted in this region. Nonetheless, post-licensure effectiveness studies of seasonal influenza vaccine are infrequent. Only studies from Malaysia, Singapore, Lao PDR, and Thailand have been reported.

Mustafa AN, et al[51] conducted a case-control study to estimate TIV effectiveness in preventing influenza-like-illness (ILI) among 820 cases and 600 control Malaysian Hajj pilgrims during February and March 2000. The estimated VE was 77% (95% CI 69-83%). Another study in Malaysian Hajj pilgrims was done in December 2007[52]; a cohort of 65 vaccinated and 41

unvaccinated pilgrims were compared for the occurrence of respiratory symptoms. There was no significant difference in the percentage of pilgrims having cough, runny nose, sore throat and fever and symptoms score between the two groups. In both studies, cases were identified using clinical case definition and did not have laboratory confirmation.

Isahak I, et al[53] conducted a study comparing a TIV vaccinated and unvaccinated cohort of 527 elderly living in 5 old folk homes in Malaysia during June 2003 to February 2004. In this study, the vaccinated group had significantly fewer episodes of ILI than unvaccinated elderly (p<.05). ILI is defined by clinical case definition and did not have laboratory confirmation. The authors did not provide a risk ratio or a statistical value for VE estimates.

Kheok SW, et al[54] studied influenza VE in a cohort of healthcare workers in 2 hospitals in Singapore from April 2004-2005. Influenza vaccine was not effective against self-reported ILI in Singaporean healthcare workers. The authors explained that both NH and SH influenza vaccines were provided in Singapore and the NH vaccine of the study season was mismatched compared to strains that actually circulated. They did a sub-analysis on subjects who only received vaccine which matched the circulating strain and reported VE of 51% against ILI (not laboratory confirmed) (95% CI 34-63%).

Ho HP, et al[55] conducted an influenza subtype-specific VE study in Singapore's Armed Forces camps from 1 June 2009 to 30 June 2012 using TND. From 7,016 military service personnel who presented with febrile respiratory illness during the study period, the TIV effectiveness against laboratory-confirmed influenza A (H1N1)pdm09 and influenza B was 84% (95% CI 78-88%, 79-86%, respectively). The VE estimate against influenza A (H3N2) was markedly lower at 33% (95% CI -4 to 57%).

A study in Laos assessed the effect of influenza vaccination in a cohort of 5,103 pregnant women on birth outcome from April 2014 to February 2015. Olsen SJ, et al[56] demonstrated that

influenza vaccine during pregnancy in Laotian women reduced preterm birth. Although the vaccinated pregnant women belonged to higher socioeconomic status (e.g., higher education and incomes) and attended more antenatal care, the effect remained only for infants born during high influenza virus circulation in Laos (the adjusted RR 0.69, 95% CI .55-.87) . The findings concur with a phase 4 RCT in Nepal[57], in which maternal TIV vaccination reduced low birthweight by 15% (95% CI 3-25). The study in Nepal also shows that vaccine had efficacy in reduction of laboratory-confirmed influenza in their infants at aged 0-6 months by 30% (95% CI 5-48).

In the region, Thailand has the highest numbers of publications on seasonal influenza vaccine effectiveness studies in peer-reviewed journals. Nine influenza VE studies were identified and 6 were published in the last ten years.

Wongsurakiat P, et al[58] determined influenza VE against serological or culture-confirmed influenza-related acute respiratory tract infection (ARI) among 125 adults with underlying chronic obstructive lung disease (COPD) randomized to receive influenza vaccine or placebo in a university hospital in Bangkok from June 1997 to November 1998. The incidences of influenza-related ARI were 28 and 7 per 100 person-years in the vaccine and placebo group, RR 0.24 (p=.005); VE was 76%.

Praditsuwan R, et al[59] evaluated the immune response and effectiveness of influenza vaccine in preventing ILI among 635 adults aged 60 years or above randomized to receive influenza or tetanus toxoid vaccine as a placebo from February to May 1998 and followed-up for one year in Bangkok. The VE against ILI incidence was 56% (95% CI 14-77%).

Plasai V, et al[60] compared the incidence rates of self-reported ILI in a cohort of 519 and 520 influenza vaccinated and unvaccinated healthy adults aged 60 years or above, followed for 1 year from May 2004, in metropolitan Bangkok. The authors reported the 2 groups were similar in most characteristics except for gender, level of education, marital status, and smoking habit. The

complete follow-up rates were 99% and 95% among the vaccinated and unvaccinated groups, respectively. The VE was 47.6% and the adjusted risk ratio of unvaccinated to vaccinated was 1.92 (1.25-2.95).

Phrommintikul A, et al[61] assessed influenza VE in Thai adults aged older than 50 years who presented with acute coronary heart disease syndrome, randomized to receive influenza vaccine or not from November 2007 to October 2008 and were followed for 1 year at Chiang Mai University Hospital. The major cardiovascular events including death, hospitalized from acute coronary heart disease, heart failure, or stroke occurred less in the vaccinated than the unvaccinated group (Unadjusted Hazard Ratio 0.70, 95% CI 0.57-0.86). The incidence of cardiovascular death was similar between the two groups.

Dawood FS, et al[11] used a TND study to estimate trivalent inactivated influenza vaccine (IIV) effectiveness against hospitalization with influenza-associated ARI in adults aged ≥ 50 years, based on the on-going active surveillance for ARI-hospitalization in two provinces of Thailand. Of 279 cases and 1,266 controls during July–December of 2010 and 2011, the adjusted VE was 47% (95% CI 5-71%) for the two seasons.

Jaiwong C and Ngamphaiboon J[62] evaluated VE by comparing 48 asthmatic children receiving two doses of trivalent IIV one month apart (fully vaccinated) and 45 asthmatic children whose parents denied vaccination from June 2012 to August 2013. The outcomes were acute respiratory tract illness and asthmatic exacerbation episodes at one year of follow-up (the authors followed children every 3 months but did not explain how these data were collected). Children in the vaccinated group had significantly lower episodes of respiratory tract illness, asthmatic exacerbation, and all other asthmatic-related events.

Levy JW, et al[14] conducted a TND study to evaluate IIV effectiveness in persons aged 6 months or older with ILI at a military hospital in Bangkok, from August 2009 to January 2013. A

total of 1,059 PCR-positive and 1,940 PCR-negative were analyzed. The May to April (2 months before the beginning of influenza season in Thailand) adjusted VE for the years 2010, 2011, and 2012 were 58% (34-74%), 57% (35-68%), and 38% (4-63%) respectively. The overall VE was highest among the 18-49 years age group (77%) followed by 6-23 months (58%) and 2-17 years (53%).

Kittikraisak W, et al[13] evaluated IIV effectiveness in 968 high-risk and healthy children aged ≤ 36 months enrolled in a 2-year prospective cohort to study influenza burden in Thai children. Subjects were under active surveillance for ARI and were tested by RT-PCR once an ARI had been reported. The incidence of influenza-associated ARI between vaccinated and unvaccinated children in the cohort was compared. The VE for the 2011 and 2012 Thailand influenza season were 55% (95% CI -72 to 88%) and 64% (95% CI 13-85%) respectively. The wide 95% CI in 2011 season was likely due to the low number of children in the cohort during the first year of enrollment.

Kittikraisak W, et al[12] applied a TND to estimate IIV effectiveness against laboratory-confirmed influenza in children aged 7 to 60 months seeking care for ILI at a large referral children hospital in Bangkok from September 2013 to May 2015. There were 490 cases and 887 controls enrolled. The adjusted VE in fully vaccinated children for 2013 and 2014 influenza season were 64% (95% CI 21-84%) and 26% (95% CI -47 to 63%) respectively. The low VE in 2014 was related to the significant antigenic drift of the circulating influenza A/H3N2 strain; similar findings occurred in the NH vaccine 2014-2015[63].

The low numbers of VE evaluation studies in Southeast Asia may be partly explained by the year-round influenza activities and no clear seasonal pattern as seen in Malaysia and Singapore[4] or both NH and SH vaccines are used in countries[2] that challenge the logistics for implementing influenza VE research. Or possibly from the lack of a robust influenza surveillance system in the

country. In Thailand, although public awareness of influenza vaccination is increasing and there are a few studies, yet those studies are still limited to a few areas, mostly in Bangkok.

The Thai Ministry of Health (MOPH) has implemented the Annual National Influenza Vaccination Program which provides trivalent IIV free of charge to the 5 risk groups recommended by WHO. The program distributes IIV to 72 provinces in 5 regions of Thailand approximately in May every year before the peak seasonal influenza activity since 2009. Although the vaccine wastage was small at 9.5% per year, the overall coverage is still low[10]. At the last update in 2018, the MOPH purchased vaccines to cover 26% of eligible risk population[64]. Data on the vaccine effectiveness representing all geographic regions of Thailand will support the expanding of the country influenza immunization program.

Networking to generate VE estimates has several advantages; it increases statistical power and the precision of the estimates, provides timely information as well as more representative geographic regions. Some national and regional networks have been established to generate pooled VE estimates, e.g., US Flu VE Network, FluCAN among adult inpatient populations and WAIVE among pediatric inpatient and outpatient populations in Australia[44]. In tropical countries, REVELAC-i has been initiated and provide data for their region[16,44]. There is no such network in Southeast Asia.

Queen Sirikit National Institute of Child Health, a referral children hospital in Bangkok, and the Thailand-MoPH US CDC Collaboration (TUC) Influenza Program have been working collaboratively on influenza research and experiencing with influenza VE study[12,13]. Together with the Thailand National Influenza Center, which is running influenza surveillance for the country and recognized by WHO as a part of Global Influenza Surveillance and Response System (GIRSP)[65] the TIC has initiated a national network of 7 hospitals across Thailand, with the aim of providing a platform for influenza studies in Thailand particularly influenza vaccine effectiveness.

In the initial year, the network will study influenza VE in young children, one of the high-risk targets for influenza vaccination.

Chapter 3

Materials and methods

The Thailand Influenza Network for Evaluation (TINE) project was initiated in 2016. We have established 7 study sites in 5 regions of Thailand. Names and locations of the sites are shown in Figure 5.

Figure 5 Hospital sites for influenza vaccine effectiveness study

These sites were selected based on their function as a provincial or regional level hospital, the outpatient departments which served patients in influenza vaccination target groups, and locations which were close to the regional laboratories.

In the starting year, the network conducted a study to measure influenza vaccine effectiveness against medically-attended influenza-associated acute respiratory illness (ARI) in young children, one of the target populations of the Thailand Influenza Vaccination Program during the 2017 influenza season in Thailand.

Population and sample

The population for the study was children aged 6 to < 36 months seeking ambulatory care at seven sites in seven provinces, including 1) Nakornping Hospital 2) Khonkaen Hospital 3) Sunpasithiprasong Hospital 4) Queen Sirikit National Institute of Child Health 5) Pranungkloa Hospital 6) Chonburi Hospital and 7) Surat Thani Hospital (Figure 5).

Inclusion Criteria

1. Age 6 months to < 36 months
2. Thai citizen
3. Seeking care at a participating hospital during the enrollment period for ARI onset ≤ 10 days, where ARI is defined as at least two of the following symptoms:
    - measured temperature > 38.0 degree Celsius or parental report of fever
    - cough
    - runny nose or nasal congestion
    - difficulty breathing (characterized by tachypnea or retractions)

Exclusion Criteria

1. Have been previously enrolled in the study and the onset of ARI is less than 10 days after the last day of symptoms of the previous enrollment
2. Caregivers unable to speak or understand Thai
3. Caregivers do not consent the child to participate in the study

Research design

We used a test-negative design case-control study (TND) to evaluate vaccine effectiveness (VE) in this study by comparison of the odds of testing positive for influenza among fully vaccinated versus unvaccinated children. For each enrolled child, we ascertained whether the child

had PCR-confirmed influenza and whether the child had been vaccinated with influenza vaccine of the current season.

Period of Enrollment

The 2017 influenza season in Thailand was June 1, 2017 through May 31, 2018. Determination of enrollment time for this study was based on the level of influenza activity in participating sites. Beginning in mid May 2017, each hospital performed influenza surveillance (see Study Procedure for details) and when the surveillance results reflecting influenza activity from the 7 hospitals combined reached the preset threshold (5% for 2 consecutive weeks), all hospitals started enrollment. Surveillance was ongoing throughout the year. And when influenza activity dropped below the preset threshold for 2 consecutive weeks, all hospitals halted enrollment and began enrolling again when the threshold was reached.

Table 1 Definitions of Key Terms

Term	**Definitions**
Acute Respiratory Illness (ARI)	≥ 2 of the following symptoms: fever >38.0°C or parental report of fever, cough, runny nose or nasal congestion, difficulty breathing
Influenza-like Illness (ILI)	Fever >38.0°C or parental report of fever AND cough
Current season influenza vaccine	An influenza vaccine administered April 1, 2017 – March 31, 2018
Unvaccinated children	1. Did not receive a current season influenza vaccine OR 2. Received only 1 dose of current season influenza vaccine and <14 days before illness onset and no prior doses
Fully vaccinated children	1. Received 2 doses of current season influenza vaccine ≥ 28 days apart and ≥ 14 days before illness onset OR 2. Received 1 dose of the current season influenza vaccine ≥ 14 days before illness onset and received >1 dose of influenza vaccine before the current season
Partially vaccinated children	1. Received 2 doses of the current influenza vaccine but cannot fulfill fully vaccinated criteria (i.e., the 2 doses are <28 days apart or the 2nd dose is <14 days before illness onset) OR 2. Received 1 dose of current season influenza vaccine ≥ 14 days before illness onset and received only 1 dose of influenza vaccine before the current season
Laboratory-confirmed influenza	Children with ARI and have combined nasal and throat swabs that are positive for influenza viruses by real-time RT-PCR

Study Procedure

Surveillance: Starting on the first working day of each week, each hospital conducted influenza surveillance by recruiting children aged 6 to < 36 months who presented at the out-patient department with influenza-like illness (ILI), asked for consent from caregivers for flu screening, collected a nasal swab, and performed influenza testing using QuickVue® rapid test (with no cost to the caregivers). Each hospital performed 10 tests per week. The number tested, and the number positive were aggregated weekly to inform sites of the proportion of influenza positives which corresponded to influenza activity and whether enrollment should begin or pause. The surveillance was continued throughout the 2017 influenza season (May 1, 2017 to April 30, 2018).

Enrollment*:* At enrollment, site staff recruited children who presented at the out-patient department with ARI onset ≤ 10 days earlier, asked for consent and interviewed caregivers on the demographics, breastfeeding practice, smoking exposure, underlying medical conditions, receiving of influenza vaccine and recorded the information on the *Enrollment Form* (see Instrument). After the interview, staff collected a nose and a throat swab, placed the 2 swabs in a Viral Transport Media (VTM) tube, and stored the VTM tube in a refrigerator to maintain the temperature at 2-8°C. If the child had not been in the on-going surveillance, staff also performed a complimentary QuickVue® rapid influenza test to inform the attending physician for the clinical benefit of the child.

Influenza vaccination status verification: Thailand does not have an electronic immunization record. Thai children own a vaccination booklet which normally contains vaccines' names and dates given and often contains manufacturer names and vaccine types. Caregivers usually carry the booklet when bringing their children to hospitals. The vaccination booklet was our primary means of influenza vaccination status ascertainment. Staff abstracted date of

administration, brand, and type of influenza vaccines the child ever received and entered information on the *Vaccine Verification Form* (see Instrument). For children who could not present the booklet, caregivers were asked to send in a copy page later by mail or internet. Finally, for children who did not have the booklet, staff verified influenza vaccination status with hospital influenza vaccination logs and medical records.

***Influenza Laboratory confirmation*:** Stored VTM tubes were sent to each regional laboratory center for influenza real-time RT-PCR (rRT-PCR) within 72 hours of collection. The testing protocol was provided from the Thai National Influenza Center to test influenza type A or B, influenza A subtype and influenza B lineage.

Study Instrument

The Screening, Surveillance, Enrollment Interview, and Vaccine Verification Form were electronic data collection forms formatted to use on mobile devices. Staff entered data into their mobile devices from each site, and the data were synced to a common project database at Thailand MOPH-U.S. CDC collaboration and Queen Sirikit National Institute of Child Health daily. Information collected is shown in figure 6-8.

Figure 6 Enrollment Interview Form

Enrollment Interview Form

Interviewer's initials: ___ ___
Enrollment setting: ☐ 1. OPD ☐ 2. IPD ☐ 3. ER
Was the child referred from other hospital/primary care unit? ☐ 1. Yes ☐ 2. No

INSTRUCTION: Interview parent/caregiver about child's characteristics

1. Date of interview	___/___/___
2. Project identification number	_____
3. Results of rapid test	☐ 1. Negative ☐ 2. Positive for influenza A only ☐ 3. Positive for influenza B only ☐ 4. Positive for both influenza A and B ☐ 5. Indeterminate
4. What is the child's insurance type?	☐ 1. Universal coverage/30 Baht scheme ☐ 2. Social security ☐ 3. Civil service ☐ 4. Private insurance ☐ 5. Out of pocket ☐ 6. Other
5. Was child ever breastfed or fed breast milk?	☐ 1. Yes ☐ 2. No → Skip to question 5.3 ☐ 3. Do not know/not sure → Skip to question 5.3
5.1 Is the child still being fed breastmilk?	☐ 1. Yes → Skip to question 5.3 ☐ 2. No ☐ 3. Do not know/not sure → Skip to question 5.3
5.2 How old was child when child completely stopped breastfeeding or being fed breast milk?	_____ ☐ 1. days _____ ☐ 2. weeks _____ ☐ 3. months
5.3 Was child ever fed formula?	☐ 1. Yes ☐ 2. No → Skip to question 5.5 ☐ 3. Do not know/not sure → Skip to question 5.5
5.4 How old was the child when he/she was first fed formula?	_____ ☐ 1. days _____ ☐ 2. weeks _____ ☐ 3. months
5.5 This next question is about the first thing that the child was given other than breast milk or formula. Please include juice, cow's milk, sugar water, baby food or anything else that the child may have been given, even water. Has the child ever been fed anything other than breast milk?	☐ 1. Yes ☐ 2. No → Skip to question 6 ☐ 3. Do not know/not sure → Skip to question 6
5.6 How old was the child when he/she was first fed anything other than breast milk or formula?	_____ ☐ 1. days _____ ☐ 2. weeks _____ ☐ 3. months
6 Does anyone in the household smoke?	☐ 1. Yes ☐ 2. No ☐ 3. Do not know/not sure
7 Has the child had the following condition? *(read out one by one and pause for answer, explain what the condition is as needed, tick all that apply)* ☐ a. Asthma (for children aged ≥24 months, skip if child is <24 months old) ☐ b. Chronic lung disease ☐ c. Abnormality of the upper airway ☐ d. Heart and circulatory disease (excluding hypertension) ☐ e. Kidney disease ☐ f. Liver disease ☐ g. Neurologic/Neuromuscular disorder (including muscular dystrophy, cerebral palsy) ☐ h. Hemoglobinopathy including thalassemia ☐ i. Metabolic disease (including diabetes) ☐ j. Developmental delay (e.g., Down's syndrome) ☐ k .Immunosuppressive condition (steroids >2 months, chemotherapy) ☐ l. HIV infection ☐ m. History of prematurity (born at <37 weeks gestation) ☐ n. Birth weight <2,500 gm	
8. Has the child received influenza vaccine during the current influenza season (since Songkran 2017)?	☐ 1. Yes, specify # of doses: ____ ☐ 2. No → Skip to question 9 ☐ 3. Do not know/not sure → Skip to question 9
8.1 Did you have to pay for all or some of the cost of the influenza vaccine?	☐ 1. All of the cost ☐ 2. Some of the cost ☐ 3. Did not have to pay

Figure 7 Screening for eligibility form

Surveillance, Eligibility Screening and Project Identification Number Assignment

1. Date of form completion: ___/___/___
2. Project staff's initials: ___ ___
3. Child's sex
   - ☐ 1. Male
   - ☐ 2. Female
4. How old is the child?
   - ☐ 1. If child is 6 to <36 months of age → *Go to 5*
   - ☐ 2. If child is not 6 to <36 months of age → *STOP, Child is NOT ELIGIBLE for participation. Say: "Thank you for your time. We are looking for children who are 6 to <36 months of age for this evaluation."*
5. Are you a Thai citizen?
   - ☐ 1. Yes → *Go to 6*
   - ☐ 2. No → *STOP, Child is NOT ELIGIBLE for participation. Say: "Thank you for your time. We are looking for children who are Thai citizens and therefore eligible to receive free influenza vaccine through the government vaccination program."*
6. On what date did your child become sick with their current respiratory illness? __/__/__

   If ≤10 days ago → Go to 7

   If >10 days ago → STOP, Child is NOT ELIGIBLE for participation. Say: "Thank you for your time. We are looking for children whose illness began within the past 10 days."
7. Has your child had any of the following symptoms as part of their current illness?
   - a. Fever ☐ 1. Yes ☐ 2. No
   - b. Cough ☐ 1. Yes ☐ 2. No
   - c. Runny nose or nasal congestion ☐ 1. Yes ☐ 2. No
   - d. Difficulty breathing (fast breathing or retractions) ☐ 1. Yes ☐ 2. No

Outcome of computer algorithm:

Number of symptoms	Specific symptoms	Surveillance Quota Achieved	Enrollment Status	Outcome
Only one symptom	N/A	N/A	N/A	Not eligible for enrollment or surveillance
Two or more symptoms	At least fever and cough	Surveillance quota not yet achieved	Enrollment is on	Eligible for surveill and enrollment
			Enrollment is paused	Eligible for surveill only
		Surveillance quota achieved	Enrollment is on	Eligible for enrollm only
			Enrollment is paused	No screening shou taking place
	Does not have fever and cough	Surveillance quota not yet achieved	Enrollment is on	Eligible for enrollm only
			Enrollment is paused	Not eligible for surveillance and sh not be enrolled
		Surveillance quota achieved	Enrollment is on Enrollment is paused	Eligible for enrollm only No screening shou taking place

8. The computer will prompt with the correct enrollment status for the child.
   - ☐ 1. Eligible for surveillance only – *request verbal consent only*
   - ☐ 2. Eligible for enrollment only – *request written, informed consent*
   - ☐ 3. Eligible for both – *request written, informed consent*
   - ☐ 4. Not eligible for either - *If child is not eligible for participation, please say: "Thank you for your time. We are looking for children who had at least 2 of these symptoms as part of their illness."*
9. Request consent from the parent/caregiver.
   - ☐ 1. Agreed to participate and gave appropriate consent
   - ☐ 2. Declined to participate
10. If enrolled, project identification number: _____

Figure 8 Vaccine Verification Form

Vaccine Verification

Date of abstraction: ___/___/___
Abstractor's initials: _____
Participant's project ID number: _____

Vaccination Verification

INSTRUCTIONS: This form should be completed for each participant.

1. Source of vaccine information (in order of priority)
☐ 1. Vaccination card seen by project nurse
☐ 2. Vaccination card reviewed from digital photograph
☐ 3. Vaccination card reviewed from photocopy
☐ 4. Vaccination information from medical record
☐ 6. No vaccination information available

Vaccination Card or Medical Record Abstraction

2. Does the vaccination card or medical record indicate that the child ever received an influenza vaccine?
☐ 1. Yes → Proceed to 3
☐ 2. No → STOP

3a. Date of first recorded influenza vaccination: ___/___/_____
3a1. Vaccine manufacturer:_____ ☐ Not recorded
3a2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

3b. Date of second recorded influenza vaccination: ___/___/_____ ☐ No second vaccination
3b1. Vaccine manufacturer:_____ ☐ Not recorded
3b2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

3c. Date of third recorded influenza vaccination: ___/___/_____ ☐ No third vaccination
3c1. Vaccine manufacturer:_____ ☐ Not recorded
3c2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

3d. Date of fourth recorded influenza vaccination: ___/___/_____ ☐ No fourth vaccination
3d1. Vaccine manufacturer:_____ ☐ Not recorded
3d2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)

Vaccination Log of relevant party *(Complete only if the child's vaccination card or medical record is not available)*

4. Does the vaccination log indicate that the child received an influenza vaccine?
☐ 1. Yes → Proceed to 5
☐ 2. No → STOP

5a. Date of first recorded influenza vaccination: ___/___/_____
5a1. Vaccine manufacturer:_____ ☐ Not recorded
5a2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

5b. Date of second recorded influenza vaccination: ___/___/_____ ☐ No second vaccination
5b1. Vaccine manufacturer:_____ ☐ Not recorded
5b2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

5c. Date of third recorded influenza vaccination: ___/___/_____ ☐ No third vaccination
5c1. Vaccine manufacturer:_____ ☐ Not recorded
5c2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

5d. Date of fourth recorded influenza vaccination: ___/___/_____ ☐ No fourth vaccination
5d1. Vaccine manufacturer:_____ ☐ Not recorded
5d2. Vaccine type: ☐ 1. Trivalent inactivated vaccine (intramuscular)
☐ 2. Quadrivalent inactivated vaccine (intramuscular)
☐ 3. Not recorded

Influenza vaccine

Influenza vaccines in Thailand are available from both the publicly-funded immunization program and private healthcare providers. The former provided Southern Hemisphere (SH) inactivated trivalent influenza vaccine (TIV) starting in May until August 2017 whereas the latter provided at a cost, SH TIV or inactivated quadrivalent influenza vaccine (QIV) from late April through November 2017 and shifted to Northern Hemisphere (NH) TIV or QIV from December 2017 through March 2018. The influenza virus strains composition in the SH TIV 2017 were:

- an A/Michigan/45/2015 (H1N1)pdm09-like virus;
- an A/Hong Kong/4801/2014 (H3N2)-like virus; and
- a B/Brisbane/60/2008-like virus

A B/Brisbane/60/2008-like virus represented influenza B Victoria lineage.

The SH QIV 2017 contained the above three viruses and added a B/Phuket/3073/2013-like virus represented influenza B Yamagata lineage[66].

For the 2017 season, the influenza virus strains composition in the NH TIV and QIV 2017-2018 were the same as the SH TIV and QIV for 2017[67].

We collected data on influenza vaccination status of the enrolled children as well as the type (trivalent or quadrivalent) of influenza vaccines (see **Study Procedure** and **Study Instrument**). Only inactivated vaccines are available; as of September 2018, the live-attenuated influenza virus vaccine has not been registered in Thailand.

Sample size consideration and analysis plan

Sample size calculations for estimating VE by TND assume an unmatched Case-Control study. The formula used for sample size calculations based on Fleiss method[68] is:

$$n_1 = \frac{\left(Z_{\alpha/2}\sqrt{(r+1)\bar{p}\bar{q}} + Z_{1-\beta}\sqrt{rp_1q_1 + p_2q_2}\right)^2}{r(p_1 - p_2)^2}$$

$$n_2 = rn_1$$

n_1 =number of cases
n_2 =number of controls
$Z\alpha/2$ =standard normal deviate for two-tailed test based on alpha level
$Z\beta$ =standard normal deviate for one-tailed test based on beta level
r =ratio of controls to cases
p_1 =proportion of cases fully vaccinated and q1=1-p1
p_2 =proportion of controls with fully vaccinated and q2=1-p2

The ratio of cases to controls is determined by the proportion of PCR-confirmed influenza in the enrolled children. We assume the proportion of PCR-confirmed influenza is 10% based on previous studies in Thai children; thus, the ratio of controls to cases is 9 to 1. Assuming 5% of controls are fully vaccinated and assuming the influenza VE is 50%, then the OR equals to 0.5 (VE = 1 – OR x100). At alpha level 0.05 and 1-beta 0.8 (power 80%), calculation using OpenEpi (http://www.openepi.com/SampleSize/SSCC.htm) estimates a needed sample size of 579 for cases and 5211 for controls. However, this sample size was based on the assumption of 5% vaccine coverage, 50% vaccine effectiveness, and 10% influenza positive. If the influenza vaccination campaign in each province could encourage more children to receive influenza vaccination and the coverage is higher the sample size required to achieve statistically significant VE would be lower.

Descriptive statistics were calculated to describe the epidemiology of influenza-associated ARI in the enrolled children. Demographic, clinical characteristics, and other potential confounders were compared between cases (PCR-confirmed influenza) and controls (PCR-

negative) using Chi-square or Fisher's exact test for categorical variables and Student t-test for continuous variables.

We excluded children with inconclusive PCR testing results from the VE analysis. VE was expressed as a percentage and calculated as (1 – aOR) x 100, where aOR is the adjusted odds ratio for influenza positive among fully vaccinated compared with unvaccinated children.

We applied a logistic regression model to determine the odds of PCR-confirmed influenza virus infection in fully vaccinated versus unvaccinated children. The model was adjusted for age group, breastfeeding practice at age < 6 months, smoking in the household, the presence of underlying diseases, and study site as categorical variables, weeks from the beginning of influenza season and days from ARI onset to specimen collection as continuous variables. Using Backward Elimination Strategy, we included only the covariates that significantly contributed at alpha less than or equal to 0.05 in the final model.

Also, we estimated the VE for partial vaccination, for full and partial vaccination combined (any vaccination) against PCR-confirmed influenza virus infection, for full vaccination against each influenza A subtype and against influenza B virus infection, and for full vaccination against PCR-confirmed influenza virus infection during the first and second peaks of the Thailand influenza season using the same method.

All statistical analyses were performed using SAS Software 9.4 (SAS Institute Inc., Cary, NC). For all estimates, p values of less than 0.05 were considered to indicate statistically significant.

Ethical consideration

The research was conducted in Thailand. Before data collection all portions of the study were reviewed by the U.S. CDC IRB (Protocol #6964) and all participating hospitals IRBs.

The analysis was determined by the Emory IRB as not requiring review (IRB0015714) because it was a secondary data analysis of data collected in Thailand under IRB approval and all data were de-identified before analysis.

Limitation and delimitation

One of the potential weaknesses inherent in the TND case-control studies is the possibility of misclassification of cases and controls. Although the real-time RT-PCR is considered the most sensitive and specific test for influenza, the PCR sensitivity may be reduced if performed late in the course of illness[17]. In this study, we restricted enrollment to only children with ARI onset less than 10 days before specimen collection. The 10-days was selected because most young children with influenza have persistent positive PCR 10-12 days after the first symptoms onset[69,70]. We also performed sensitivity analyses to examine influenza VE using only participants with respiratory specimens collected within 5 and 7 days of illness onset threshold.

The VE evaluation in this study is operational research that has been designed for practice in routine outpatient settings. Some information related to influenza exposure such as number of school children in the household, daycare attendance, or a few others were not collected, and may be considered potential confounders for our VE estimates.

Another challenge of the TND for VE evaluation in a developing country is the reliability of vaccination status ascertainment due to an incomplete vaccination registry. Influenza vaccine in Thailand is administered only in health centers or hospitals with paper records available. In this study, staff have put a great effort to identify influenza vaccination status of enrolled children from all possible relevant health records beside the primary mean of abstracting information from vaccination booklets.

Chapter 4

Results

Enrollment

The proportion of nasal specimens testing positive for influenza using the rapid test reached the predetermined threshold (5% for two consecutive weeks) on July 23, 2017 and enrollment for the VE evaluation began. After the proportion dropped below the threshold, on December 23, 2017, enrollment was paused, and resumed when continued surveillance identified positive influenza tests exceeding the threshold again from February 12 through April 14, 2018.

Of the 5,088 children screened, 3,659 fulfilled the enrollment eligibility criteria during the enrollment period and had nasal specimens collected and tested by rRT-PCR. Two specimens from the enrolled children were not tested due to miss-labeling, and 11 were determined to be unqualified because of low human RNase P (RNP) in the samples (Figure 9).

Influenza Type, Subtype and Lineage

Of the 3,646 qualified specimens, 446 (12.2%) tested positive for influenza viruses by rRT-PCR. Of the 446 influenza positive specimens, 310 (69.5%) were influenza A and 136 (30.5%) were influenza B viruses (Figure 9).

The influenza A(H3N2) and influenza A(H1N1)pdm09 subtype comprised 62.3% and 37.7% of influenza A respectively while the influenza B-Yamagata lineage was predominant, representing 92.6% of influenza B identified (Figure 10).

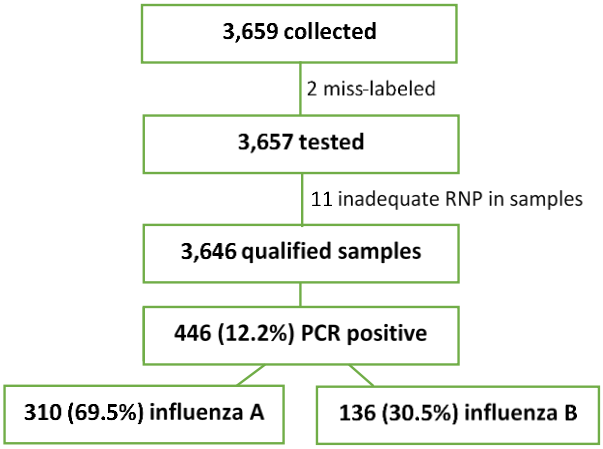


Figure 9 Number of specimens collected and testing positive for influenza virus by rRT-PCR

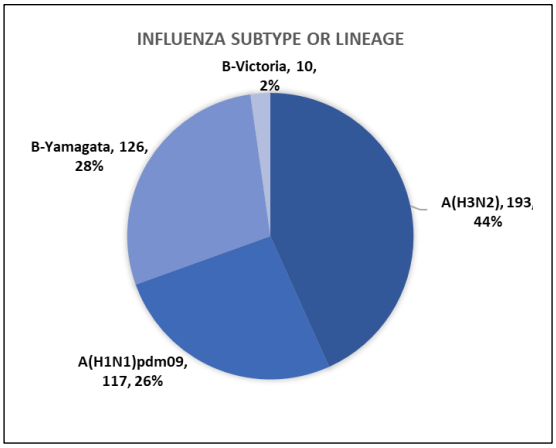


Figure 10 Proportion of influenza A subtype and influenza B lineage

A total of 2,823 children were enrolled during the first enrollment period (July 23 to December 23, 2017) and 836 children during the second period (February 12 to April 14, 2018). The proportion of specimens testing positive for influenza by subtypes and lineage is presented by calendar week and week of the enrollment in Figure 11.

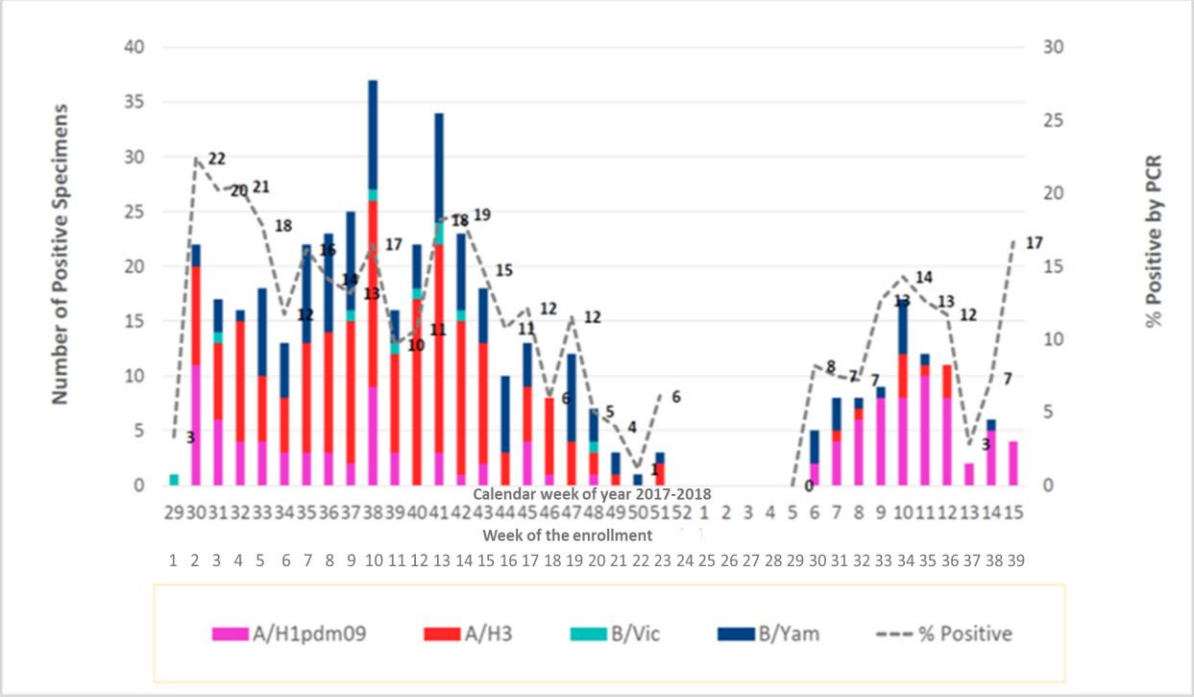


Figure 11 Acute respiratory illness (ARI) specimens tested positive for influenza rRT-PCR by week

Vaccination status

Vaccination status was documented in all except 9 children, who were classified according to caregivers reports as unvaccinated. Of the 3,659 children enrolled, 13 children with no PCR results were excluded. Table 2 shows vaccination status of the 3,646 children included in the VE analysis. Two-hundred and forty-seven children (6.8%) were fully vaccinated, and 170 (4.7%) were partially vaccinated with inactivated influenza vaccine.

Three hundred and forty-four (82.5%) children received influenza vaccines during April to August 2017 (Thailand influenza vaccination campaign in 2017). Among fully vaccinated children, 53.4% received trivalent influenza vaccine, 32% received quadrivalent influenza vaccine, and 14.6% did not have vaccine type recorded (Table 2).

Table 2 Influenza vaccination

Status	**Number** (% within group)	**Percent**
Unvaccinated*	3229	88.6
Fully vaccinated†	247	6.8
• Trivalent	132 (53.4)	
• Quadrivalent¶	79 (32.0)	
• Vaccine type not recorded	36 (14.6)	
Partially vaccinated‡	170	4.7
• Trivalent	88 (51.8)	
• Quadrivalent	48 (28.2)	
• Vaccine type not recorded	34 (20.0)	
TOTAL	**3646**	**100.0**

*Did not receive 2017 influenza vaccine or received < 14 days before the onset of ARI
†Received 2 doses of the 2017 influenza vaccine ≥ 28 days apart or received >1 dose in any previous season plus 1 dose of 2017 influenza vaccine ≥ 14 days before illness onset
‡Received 2017 influenza vaccine but did not fulfill fully vaccinated criteria
¶All two doses, if 2 doses required for fully vaccinated

Characteristics of children

Of 3,646 children, the highest enrollment (908 children) were from the Queen Sirikit National Institute of Child Health (QSNICH) in the metropolitan Bangkok area. Approximately half were male gender, and one-third were less than one year of age. Only 1.8% were exclusively breastfed, and 46.7% had one or more household members who smoked cigarettes (Table 3). There were 19% of children who had underlying medical conditions including prematurity (8.4%), upper airway disease (2.9%), chronic lung disease (1.9%), heart disease (1.9%), neuromuscular disease or developmental delay (1.9%), asthma (1.5%), hemoglobinopathy (1.3%), and others such as kidney or liver disease or immunodeficiency (1.0%). The presenting symptoms included fever (93.4%), cough (94.9%), runny nose (93.5%), and difficulty breathing (15.5%) (Table 3).

Characteristics of children by the percentage testing positive for influenza are also shown in Table 3. The percentage testing positive for influenza differed by study site (p=0.01), with Chonburi having the highest percentage testing positive at 15.4%, followed by QSNICH (14.4%), Pranungklao (12.5%), Nakornping (12.3%), Sunprasittiprasong (10.1%), Surat Thani (9.5%), and Khonkaen (9.5%).

The percentage testing positive for influenza also differed significantly by age group (p<0.01) as children aged 2 to 3 years had the highest percentage testing positive for influenza at 16.6% whereas the percentage testing positive for influenza in children aged 1 to 2 years and <1 year were 11.1 and 10.0% respectively. There was no difference in the percentage of children testing positive for influenza by sex (p=0.27), breastfeeding practice at age <6 months (p=0.16), and household smoking (p=0.27).

The median time from ARI onset to enrollment was 4 days and was not statistically significantly different between children with and without influenza (p=0.06).

The presence of underlying medical conditions was less frequent among children with influenza than those without influenza (12.6 versus 19.9%, $p<0.01$).

The presenting symptoms of ARI were generally similar between children with and without influenza virus infection. Fever was observed more frequently ($p<0.01$) and difficulty breathing less frequently ($p<0.01$) among children testing positive for influenza virus infection (Table 3).

Characteristics of children by vaccination status

The proportion of fully vaccinated children differed significantly by study site, age group, smoking in the household, time from ARI onset, and underlying medical conditions (Table 3). The percentage of fully vaccinated children varied from 2.1% at Khonkaen to 13.9% at Surat Thani study site. Children aged <1 year had the lowest proportion fully vaccinated (3.1%) compared to 8.5% and 8.4% among children aged 1-2 year and 2-3 year respectively ($p<0.01$), (Table 3).

Smoking in the household was less frequent among fully vaccinated than unvaccinated children (38.9% versus 47.4%, $p<0.01$). The median time from ARI onset to enrollment among fully vaccinated children was 3 days (Interquartile Range (IQR) 2, 5) versus 4 days (IQR 3, 5) in unvaccinated children ($p<0.01$). The proportion of fully vaccinated children did not differ by sex, breastfeeding practice at age < 6 months and presenting symptoms (Table 3).

Table 3 Characteristics by influenza test and by vaccinated status

Characteristic		No. total	Influenza positive	Influenza negative	P Value *	Fully vaccinated †	Not vaccinated	P Value †
			No. (%)	No. (%)		No. (%)	No. (%)	
Overall		**3646**	**446 (12.2)**	**3200 (87.8)**		**247 (7.1)**	**3229 (92.9)**	
Hospital site, n (%)								
Chonburi		403 (11.1)	62 (13.9)	341 (10.7)	0.01	17 (6.9)	368 (11.4)	<0.01
Khonkaen		388 (10.6)	37 (8.3)	351 (11.0)		8 (3.2)	369 (11.4)	
Nakornping		374 (10.3)	46 (10.3)	328 (10.3)		17 (6.9)	350 (10.8)	
Pranungklao		583 (16.0)	73 (16.4)	510 (15.9)		19 (7.7)	543 (16.8)	
QSNICH		908 (24.9)	131 (29.4)	777 (24.3)		103 (41.7)	759 (23.5)	
Surat Thani		503 (13.8)	48 (10.8)	455 (14.2)		65 (26.3)	403 (12.5)	
Sunprasitthiprasong		487 (13.4)	49 (11.0)	438 (13.7)		18 (7.3)	437 (13.5)	
Sex, n (%)								
Male		1986 (54.5)	232 (52.0)	1754 (54.8)	0.27	144 (58.3)	1751 (54.2)	0.22
Female		1660 (45.5)	214 (48.0)	1446 (45.2)		103 (41.7)	1478 (45.8)	
Age group (years)								
< 1		1146 (31.4)	115 (25.8)	1031 (32.2)	<0.01	35 (14.2)	1072 (33.2)	<0.01
1 – 2		1526 (41.9)	169 (37.9)	1357 (42.4)		130 (52.6)	1314 (40.7)	
2 – 3		974 (26.7)	162 (36.3)	812 (25.4)		82 (33.2)	843 (26.1)	
Breastfeeding at age <6 months, n (%)								
None		215 (15.9)	32 (7.2)	183 (5.7)	0.16	16 (6.5)	191 (5.9)	0.40
Breastfeeding and other foods		3364 (92.3)	402 (90.1)	2962 (92.6)		224 (90.7)	2983 (92.4)	
Exclusive breastfeeding		67 (1.8)	12 (2.7)	55 (1.7)		7 (2.8)	55 (1.7)	
Smoking in household								
Yes		1701 (46.7)	219 (49.1)	1482 (46.3)	0.27	96 (38.9)	1531 (47.4)	<0.01
No		1944 (53.3)	227 (50.9)	1717 (53.7)		151 (61.1)	1697 (52.6)	
Days from onset to enrollment (Median, IQR)		4 (3, 5)	4 (2, 5)	4 (3, 5)	0.06	3 (2, 5)	4 (3, 5)	<0.01
Underlying medical conditions								
Yes		692 (19.0)	56 (12.6)	636 (19.9)	<0.01	62 (25.1)	584 (18.1)	<0.01
No		2954 (81.0)	390 (87.4)	2564 (80.1)		185 (74.9)	2645 (81.9)	
Symptoms, n (%)								
Fever	Yes	3405 (93.4)	440 (98.7)	2965 (92.7)	<0.01	3029 (93.8)	225 (91.1)	0.09
	No	241 (6.6)	6 (1.3)	235 (7.3)		200 (6.2)	22 (8.9)	
Cough	Yes	3460 (94.9)	421 (94.4)	3039 (95.0)	0.61	3069 (95.0)	231 (93.5)	0.29
	No	186 (5.1)	25 (5.6)	161 (5.0)		160 (5.0)	16 (6.5)	
Runny nose	Yes	3410 (93.5)	423 (94.8)	2987 (93.3)	0.23	3022 (93.6)	230 (93.1)	0.77
	No	236 (6.5)	23 (5.2)	213 (6.7)		207 (6.4)	17 (6.9)	
Difficulty breathing	Yes	565 (15.5)	44 (9.9)	521 (16.3)	<0.01	500 (15.5)	30 (12.1)	0.16
	No	3081 (84.5)	402 (90.1)	2679 (83.7)		2729 (84.5)	217 (87.9)	

*The Chi-square test was used to assess the differences in the percentage testing positive for influenza in the distribution of the enrolled children.

†The Chi-square test was used to assess the differences between the percentage of fully vaccinated and unvaccinated in the distribution of the enrolled children (i.e.,170 partially vaccinated children were excluded).

Vaccine Effectiveness (VE) estimates against laboratory-confirmed medically-attended influenza virus infection for full and partial vaccination

Children who received full, partial, or any vaccination were all significantly less likely to test positive for influenza virus infection. The crude odds ratios (OR) were 0.47 (95% confidence interval (CI): 0.28, 0.78), 0.56 (95% CI: 0.31, 0.99), and 0.50 (95% CI: 0.34, 0.75) for full, partial, and at any vaccination respectively, (Table 4). Controlling for age group, presence of underlying diseases, study site, weeks from the beginning of influenza season, and days from ARI onset to specimen collection, the adjusted OR for full, partial, and at all vaccination are shown in Table 4.

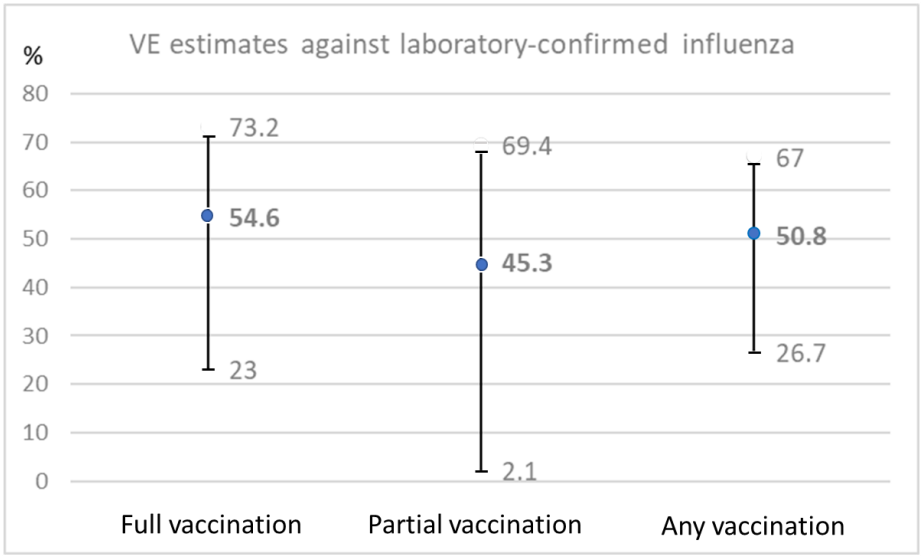
The VE for PCR-confirmed medically-attended influenza-associated ARI for full vaccination was 54.6% (95% CI: 23, 73.2). The VE were also statistically significant for those partially vaccinated and those who received any vaccination. VE estimates for partial and any vaccination were 45.3% (95% CI: 2.1, 69.4) and 50.8% (95% CI: 26.7, 67) respectively (Figure 12).

Table 4 Influenza virus infections by influenza vaccination status, TINE network, 2017 Thailand influenza season

	Influenza-positive		**Influenza-negative**		**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
	Total	**No. (%) vaccinated**	**Total**	**No. (%) vaccinated**		
Full vaccination† (n=3476)	433	16 (3.7)	3043	231 (7.6)	0.47 (0.28, 0.78)	0.45 (0.27, 0.77)
Partial vaccination†† (n=3399)	430	13 (3.0)	2969	157 (5.3)	0.56 (0.31, 0.99)	0.55 (0.31, 0.98)
Any Vaccination* (n=3646)	446	29 (6.5)	3200	388 (12.1)	0.50 (0.34, 0.75)	0.49 (0.33, 0.73)

†Exclude partially vaccinated children
††Exclude fully vaccinated children
*Fully and partially vaccinated children combined

Figure 12 Vaccine effectiveness against PCR-confirmed medically attended-influenza-associated ARI in children receiving full, partial and any vaccination



VE estimates against influenza A viruses by subtype and influenza B viruses

Children with medically-attended ARI who tested positive for influenza A(H1N1)pdm09 viruses were significantly less likely to have received an influenza vaccination (Adjusted OR 0.16, 95% CI: 0.04, 0.65) (Table 5). The VE estimate for full vaccination against influenza A(H1N1)pdm09 virus infection was 84% (95% CI: 34.6, 96.1). The VE estimate was 50.4% against influenza A(H3N2) virus (95% CI: -8.4, 77.4) (Figure 13).

Of 136 specimens tested positive for influenza B viruses, only 10 specimens (7.4%) were Influenza B Victoria lineage; the lineage contained in the trivalent influenza vaccine of 2017 influenza season. The VE estimate for full vaccination against all influenza B viruses was 15.4% (95% CI: -87.1, 61.8) (Figure 13).

Table 5 Influenza virus infection by influenza type and subtype in fully vaccinated and unvaccinated children

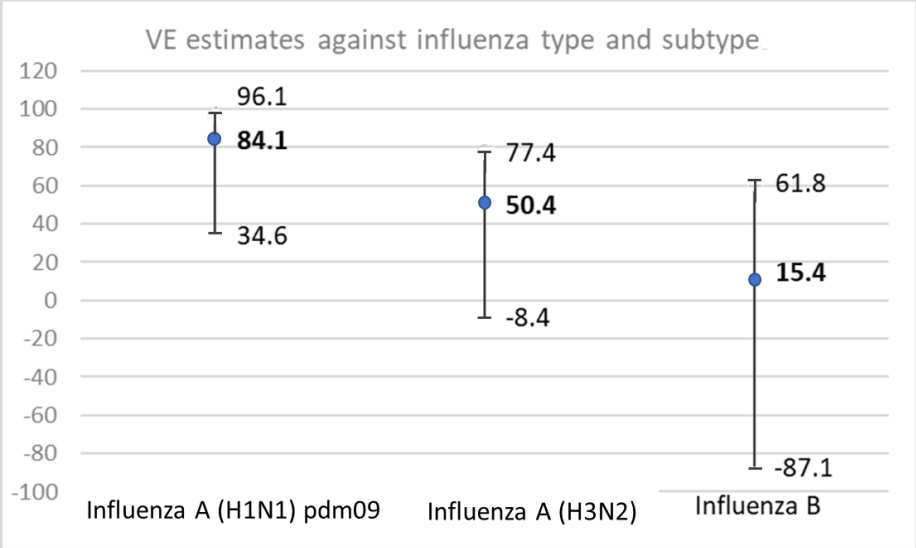
	Influenza-positive		Influenza-negative		Crude OR (95% CI)	Adjusted OR (95% CI)
	Total	No. (%) vaccinated	Total	No. (%) vaccinated		
Influenza A(H1N1) pdm09	113	2 (1.7)	3043	231 (7.6)	0.22 (0.05, 0.88)	0.16 (0.04, 0.65)
Influenza A(H3N2)	189	7 (3.7)	3043	231 (7.6)	0.47 (0.22, 1.01)*	0.50 (0.23, 1.08)*
Influenza B	129	7 (5.4)	3043	231 (7.6)	0.70 (0.32, 1.51)*	0.85 (0.38, 1.87)*

†Exclude Partially vaccinated children

††Exclude other influenza type/subtype for each type/subtype analysis

*Not statistically significant at the p<0.05 level

Figure 13 Vaccine effectiveness against laboratory-confirmed medically-attended influenza-associated ARI by influenza type and subtype



VE estimates during the first and the second peaks of influenza activity

Our first enrollment period approximately corresponded to the first peak (July to December 2017) whereas the second enrollment period was associated with the second peak (February to April 2018) of influenza activity in Thailand. We estimated the VE for full vaccination against PCR-confirmed medically-attended influenza ARI. The VE estimates were 44.2% (95% CI: 1.2,

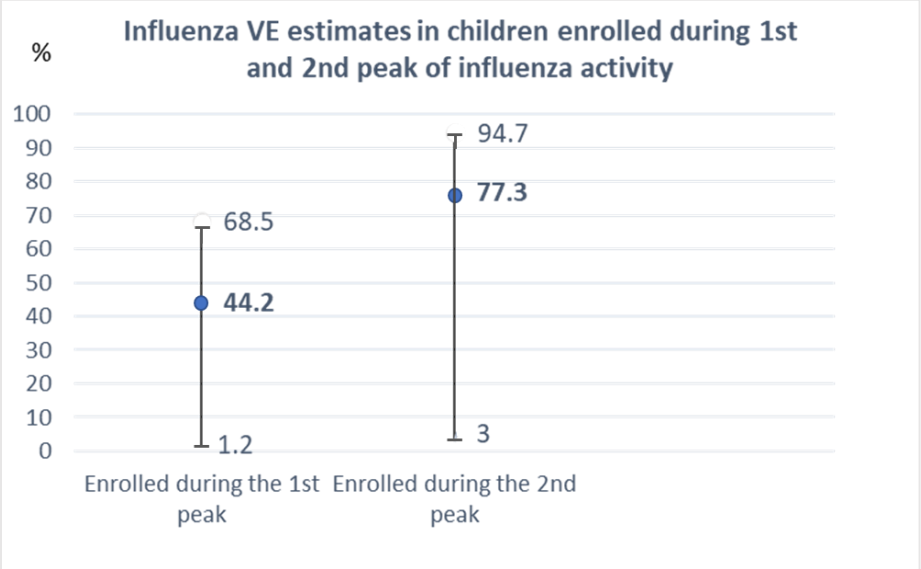
68.5) and 77.3% (95% CI: 3.0, 94.7) for the first and second peak of influenza activity, respectively (Table 6 and Figure 14).

Table 6 Influenza virus infection by peak of influenza activity, Thailand 2017-2018 in fully vaccinated and unvaccinated children

	Influenza-positive		Influenza-negative		Crude OR (95% CI)	Adjusted OR (95% CI)
	Total	No. (%) vaccinated	Total	No. (%) vaccinated		
First peak (July-Dec.)	353	14 (4.0)	2319	168 (7.2)	0.53 (0.30, 0.92)	0.56 (0.32, 0.99)
Second peak (Feb.- Apr.)	80	2 (2.5)	724	63 (8.7)	0.27 (0.07, 1.12)	0.23 (0.05, 0.97)

†Partially vaccinated children excluded

Figure 14 Vaccine effectiveness against laboratory-confirmed influenza-associated ARI by seasonal peak of influenza activity, Thailand 2017-2018



Sensitivity analyses with thresholds of influenza testing 5 and 7 days post ARI onset

Of 3,646 enrolled children who had influenza testing within 10 days post-ARI onset, 2,722 children (74.7%) and 3,185 children (87.4%) had the testing performed within 5 and 7 days post ARI onset respectively. The VE against medically attended-influenza ARI in children testing

within 5 and 7 days post-onset were 55.9% (95% CI: 20.8, 75.5) and 54.0% (95% CI: 20.5, 73.3) respectively. Indicating a minimal change (<3%) from the original VE estimate (54.6%, 95% CI: 23.0, 73.2) when restricting the analyses to children testing within 5 and 7 days post-ARI onset thresholds.

Chapter 5

Discussion and Conclusion

The first year VE study from the TINE (Thailand influenza network for evaluation) suggests moderate effectiveness at 55% (95% CI: 23, 73) of inactivated influenza vaccine against medically-attended laboratory-confirmed influenza illness in children 6-36 months during Thailand 2017/2018 influenza season.

Two peaks of influenza activity characterized the 2017/2018 influenza season in Thailand. We observed a high proportion of children who presented with acute respiratory illness (ARI) tested positive for influenza from late-July through mid-December with a peak in late-September 2017 and lower influenza activity from mid-February through mid-April 2018. The findings are concordant with multi-year data reported by Newman LP, et al[5] and support the timing of influenza vaccination in Thailand before July each year[4]. Although the Thai publicly funded influenza vaccination campaign is from May to July annually, there is no guideline of the appropriate timing of influenza vaccine for Thai children. Healthcare providers may offer the vaccine after influenza seasons have passed and routinely advise the one-year interval for the next season vaccine. While influenza vaccine may be given at any time of the year, the Thai MoPH should educate medical practitioners to choose the preferred timing between May to July for a better benefit of influenza prevention.

Vaccine coverage remains a challenge for VE evaluation. Influenza vaccine uptake in our network differed by site with 5 of 7 sites having coverage less than 5%. The overall uptake in our study population was approximately 10% with only 7% having full vaccination. The low coverage of influenza vaccine in young children is a consistent finding with the other studies in Thailand[13,14]. However, a study conducted in a cohort of Thai children aged 6-36 months who regularly visited

the study hospital during 2011-2013 demonstrated a higher influenza vaccination coverage of 30%[12]. Repeated exposure to influenza communication may help to increase the vaccine uptake in young children. In addition, limited numbers of influenza vaccines are provided free of charge for individuals with an increased risk of severe influenza including young children from May to August in Thailand every year[10]. Parents may also pay for the vaccine which costs about 12-15 USD from healthcare providers. While the overall coverage for all target groups of the free influenza vaccine has been less than 10%, the proportion administered to young children was only 1% compared to 77% among persons with chronic disease and the age group ≥ 65 years combined[10]. During the establishment of the TINE network, we also found that some healthcare providers misunderstood the young children as a non-risk group unless having comorbidity; thus, they were not eligible for free vaccine. Increasing the vaccine supply and allocation to each specific risk group as well as educating healthcare providers before the influenza vaccination campaign will help to expand influenza vaccine coverage in young children.

Both Southern hemisphere (SH) and Northern hemisphere (NH) influenza vaccines are available in Thailand. The SH vaccine is used from April to November and NH vaccine from December to March of the following year[12]. The recommended influenza virus strains composition for SH 2017 and NH 2017-2018 influenza vaccine were the same[66,67]. Regarding circulating influenza virus, the Thai National Influenza Center (NIC) disseminates results from their sentinel surveillance regularly. The gene sequencing study of circulating influenza virus strains and the vaccine strains for Thailand 2017-2018 season are shown in Table 7[71].

Table 7 Comparison of the Southern and Northern hemisphere influenza vaccine strains recommended by WHO and the circulating strains during the study period

Influenza strain	**SH and NH 2017-2018 vaccine strain (same strains composition)**	**Circulating strain in Thailand**
A(H1N1)	A/Michigan/45/2015(H1N1)pdm09-like virus	A/Michigan/45/2015(H1N1)pdm09-like virus
A(H3N2)	A/Hong Kong/4801/2014 (H3N2)-like virus (clade 3C.2a)	A/Hong Kong/4801/2014 (H3N2)-like virus (subclade 3C.2a1)
B/Victoria	B/Brisbane/60/2008-like virus	B/Brisbane/60/2008-like virus (10% of influenza B)
B/Yamagata*	B/Phuket/3073/2013-like virus	B/Phuket/3073/2013-like virus (90% of influenza B)

*Additional influenza B virus composition in quadrivalent influenza vaccine

The VE estimates from the network indicate that influenza full vaccination is moderately effective and superior to partial vaccination in young children during the 2017/1018 season. The sample size could not reach the statistical power to estimate VE against specific influenza type or subtype except for influenza A(H1N1) due to the low influenza vaccination coverage in some participating sites, and the low VE against influenza A(H3N2) and against influenza B. Approximately two-thirds of the identified influenza virus was influenza A, and one-third was influenza B. An influenza A(H3N2) comprised approximately 60% of influenza A whereas an influenza B-Yamagata lineage comprised more than 90% of influenza B. The very high predominant influenza B-Yamagata lineage virus circulating when most vaccines used in our setting were trivalent inactivated influenza vaccine (TIV) containing an influenza B-Victoria lineage may skew the overall VE. The point estimate of VE against influenza B virus in our study is very low at 15% even though it is not statistically significant (95% CI, -87 - 62).

The good match between circulating influenza A(H1N1)pdm09 virus strain and vaccine composition explains the high VE of 84% (95% CI, 35-96) against influenza A(H1N1)pdm09 in our study. We observed lower VE against influenza A(H3N2) which is possibly explained by the ongoing issue of antigenic drift. Although the circulating influenza A(H3N2) virus is A/Hong

Kong/4801/2014 virus which is the virus strain in the vaccine, but the drift to subclade 3C.2a1 that is antigenically different from the clade 3C.2a virus in the vaccine[71] could blunt the VE against influenza A(H3N2) in our study (Table 7). Sullivan SG, et al describe a similar concern in a VE study during the 2017 influenza season in Australia[72]. Melidou A and Broberg E, and Valenciano M, et al also report that circulating antigenic drifted influenza A(H3N2) viruses resulted in reduced VE in European influenza surveillance and I-MOVE Case-Control VE study[73,74]. Although not statistically significant, our VE estimate against influenza A(H3N2) of 50% (95% CI: -8, 77) is close to the predicted VE of 48% against influenza A(H3N2) from an antigenic diversity study in Thailand[75].

Most tropical countries experience several months or all year round of influenza activity[4]. As such, the influenza VE estimates may change over the season. We have an opportunity to generate VE for children enrolled during the first and second peaks which shows a much lower VE against laboratory-confirmed medically-attended influenza illness during the first peak than the second peak. A study in Bangkok by Levy J, et al found VE became lower as the time interval between vaccination and illness became longer suggesting a reduction of strain-specific vaccine-induced immunity overtime[14]. But the higher VE estimate during the second compared to the first peak in our study may be explained by the co-circulating influenza virus predominance during the first six months of the study period were Influenza A(H3N2) (50%) and Influenza B-Yamagata lineage (30%) which were antigenically drifted from the vaccine strains during the first peak. In contrast, the influenza A(H1N1)pdm09 virus strain which predominated during the second peak of our enrollment period was well matched to the strain in the vaccine.

To date, a few studies on 2017/2018 influenza vaccine effectiveness evaluation are published. Although almost all studies use TND, it is difficult to compare the VE results because they are evaluated from different settings, using a different target group or an outcome or both,

and influenza vaccines administration such as live-attenuated or inactivated, trivalent or quadrivalent influenza vaccine is of different proportions in each country. An interim analysis of the winter 2017/2018 influenza season from the U.S. Flu Network and I-MOVE network in Europe show overall low to moderate VE against medically-attended influenza illness for patients of all age with the lowest VE against influenza A(H3N2)[76,77]. Another study from the 2017 Australia influenza season (May to September 2017) also shows low VE against medically-attended influenza illness skewed by a poorer VE estimate against influenza A(H3N2)[72], like our study during the first enrollment period when influenza A(H3N2) virus was predominant. The VE against laboratory-confirmed influenza illness in hospitalized children from the PAEDS-FluCAN network in Australia during the same season is also low[78]. In Hong Kong, influenza circulates for most of the year while the influenza vaccination campaign is in October through December[79], the VE estimates against laboratory-confirmed influenza in hospitalized children and all age patients enrolled from the primary-care providers network for 2017/2018 winter are moderately good at 66% and 59% respectively[80,81]. The influenza predominant influenza B/Yamagata during the season and the use of QIV vaccine in Hong Kong , which contained an influenza strain of that lineage led to good protection.

Because of the diversity of circulating influenza viruses in different geographic regions, each country should attempt to collaborate within the country and the region for continuous influenza surveillance and VE monitoring to optimize influenza prevention by means of vaccination. The TINE network is the first collaboration to evaluate VE against laboratory-confirmed influenza illness in Thailand by a multi-site approach. Our first year data shows overall statistically significant VE since the first period of enrollment even though we could not achieve enough statistical power for specific influenza type, subtype, and lineage. The major challenge is a low vaccine coverage of 10% of any vaccination compared to 18-51% among children in

Australia, Hong Kong, U.S., and Latin American countries[16,76,78,80]. Although some studies evaluate hospitalized children and vaccine coverage may differ from ours using outpatient settings, the influenza vaccine uptake in Thai children is still insufficient arguing for additional efforts to promote influenza vaccination.

In conclusion, we established a network of 7 hospitals in Thailand to provide information on influenza VE against laboratory-confirmed influenza as well as a platform for influenza research in the country. In our first year of establishment, we generated a VE for young children at the outpatient setting, showing the recommended influenza vaccine is protective during the 2017/2018 influenza season. The effectiveness depends on the degree of matching between the circulating and the vaccine influenza virus strains requiring continuing effectiveness monitoring, together with strategies to improve vaccination coverage in Thai children.

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