Six-monthly versus annual influenza vaccination in older adults: an observer-blinded, active-comparator controlled, randomised superiority trial

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Abstract

Background: Antibody titres and vaccine effectiveness decline within six months after influenza vaccination in older adults. Biannual vaccination may be necessary to provide year-round protection in the tropics where influenza is present throughout the year.

Methods: Tropical Influenza Control Strategies (TROPICS1) was a single-centre, 1:1 randomised, observer-blinded, active-comparator controlled, superiority study in 200 community-resident adults aged ≥65 years. Participants received standard-dose trivalent inactivated influenza vaccination (IIV3) at enrolment, and either tetanus-diphtheria-pertussis vaccination or IIV3 six months later. Primary outcome was the proportion of participants with haemagglutination-inhibition (HI) geometric mean titre (GMT) ≥1:40 one month after the second vaccination (Month 7). Secondary outcomes included GMTs to Month 12, the incidence of influenza-like illness (ILI), and adverse reactions after vaccination. This study is registered with ClinicalTrials.gov: NCT02655874.

Findings: At Month 7, the proportion of participants with a HI tire ≥1:40 against A/H1N1 increased by 21·4% (95% CI 8·6-33·4) in the six-monthly vaccination group. This proportion was not significantly higher for A/H3N2 (4·3, 95% CI -1·1-10·8) or B (2·1, 95% CI -2·0-7·3). Six-monthly vaccination significantly increased GMTs against A/H1N1 and A/H3N2 at Month 7, but not B. Participants receiving repeat IIV3 reported a significantly lower incidence of ILI in the six months after the second vaccination (relative vaccine efficacy of 57·1%, 95% CI 0·6-81·5). The frequency of adverse events was similar after first or second influenza vaccination.

Interpretation: Six-monthly influenza vaccination in older residents of tropical countries is a simple intervention with the potential for improving protection against infection.

Funding: National Healthcare Group

Research in context

Evidence before this study

The TROPICS1 study was developed following the findings of a systematic review of haemagglutination-inhibition (HI) antibody persistence after standard-dose inactivated influenza vaccination in older adults. The influenza virus circulates year-round in tropical regions, and year-round persistence of antibodies against infection is expected to be advantageous. MEDLINE, EMBASE, Global Health and other relevant databases and the reference lists of all included studies were searched for studies which measured strain specific HI titres up to 360 days after influenza vaccination. Databases were searched with the keywords ("influenza" or "flu"), ("immune" or "vaccine"), ("haemagglutination or hemagglutination" and "inhibition" or "assay"), ("HI" or "HAI" or "HIA") in September 2015. This search yielded 2864 articles, which were screened to identify studies meeting the selection criteria. 16 articles met these criteria and were reviewed. The overall conclusion from this review was that HI antibody responses following single dose influenza vaccination do not reliably persist year-round in older adults. Titres fell significantly from the first six months after vaccination, to the second six months. By one year after vaccination, HI titres were similar to pre-vaccination. No studies of the serological or clinical outcomes following six-monthly vaccination were uncovered.

Added value of this study

This trial was designed to compare the serological outcomes following annual versus six-monthly standard-dose influenza vaccination in older adults in a randomised manner. Study participants were otherwise healthy, community-resident adults aged 65 years or older. The study was conducted in Singapore, a tropical country which typically experiences year-round influenza virus activity and biannual epidemics coinciding with Northern and Southern hemisphere winters. The proportion of subjects with a HI titre ≥1:40, which traditionally is used to indicate seroprotection, increased by 21% against A/H1N1. This proportion was not significantly higher for H3N2 or B. HI titres against

Influenza A/ H1N1 and A/H3N2 were significantly higher following repeat vaccination, though no different for B. The group receiving repeat influenza vaccination also experienced 57% less symptomatic respiratory illness and less utilization of health services. Adverse events following repeat vaccination were numerically lower than after the first vaccine.

Implications of all the available evidence

Our results indicate that in older adult residents in the tropics, biannual vaccination is able to offer better year-round protection compared with single-dose vaccination. They also suggest that the immune responses to repeat influenza vaccination are reduced in older adults, which may be due to interference from pre-vaccination immunity. Studies performed over multiple seasons, and which include changes in vaccine strains are needed. If serological advantages are confirmed, large studies to determine if this translates to reduced influenza infection would be needed.

Background

The inactivated seasonal influenza vaccine in current use contains purified surface antigens from two influenza virus types: influenza A and B. Antigens with two components of influenza A virus subtypes (H1N1 and H3N2), and either one or two components of influenza B viruses (B/Victoria and/or B/Yamagata-lineages) are included in trivalent and quadrivalent vaccines respectively. Inactivated vaccine-induced protection against influenza infection is primarily conferred via antibody to the haemagglutinin (HA) surface antigen.[1]

Both the HA and NA proteins for influenza A and B undergo frequent change through antigenic drift, and new antigenic strains emerge. Influenza virus surveillance data reported to the World Health Organisation (WHO) global network of national influenza centres (GISRS; Global Influenza Surveillance and Response) is reviewed by the WHO, and every six months recommendations for the composition of strains in the upcoming seasonal vaccine are updated.[2] These recommendations are published in September for the next Southern hemisphere winter, and in February for the next Northern hemisphere winter. Between hemisphere winters, a change in the strain composition of the trivalent vaccine has been recommended approximately half the time.

A close match between the viral antigens (HA and NA) in the vaccine and the circulating strain is important to maximise protection against infection.[3] Vaccine effectiveness (VE) also depends on an adequate immune response to the vaccine. Immune responses to the influenza vaccine are reduced in older adults due to an age-related decline in the immune system, termed immunosenescence. This results in lower antibody titres immediately after vaccination, and a significant fall in antibody titres in older adults within six months.[4]

A consequence of these limitations is the failure of vaccination to maintain clinical protection over the course of a typical, temperate-climate winter season of four to six months. Observational studies have consistently reported lower VE in the second quarter after vaccination compared with the first quarter for all age groups.[5] To improve vaccine effectiveness in older adults, a number of alternative

vaccines have been developed. This includes the Sanofi Fluzone® High-Dose vaccine, with four-times the regular amount of HA; adjuvanted vaccines such as the Seqirus FluAd®, which includes MF-59 to form an oil in water emulsion; and the recombinant influenza vaccine Flublok®, which contains three times the regular amount of HA.[6–8] While these have been shown to reduce virologically confirmed infections in older adults, the benefit over standard-dose vaccine is small, with a relative reduction in infection rates of only 24-30%. In addition, long-term antibody data is lacking, and it is not known if these newer vaccines will extend protection.

The duration of vaccine-induced protection is a critical issue in the sub-tropics and tropics, where influenza transmission often occurs year-round, with biannual peaks.[9] Twice-annual vaccination coinciding with those peaks might be beneficial, where each vaccination can be synchronised with both the waning of antibody titres and biannual WHO vaccine strain recommendations. Current vaccine recommendations remain for annual administration, however, as there is little clinical or public health evidence available to determine whether one or two doses per year is the best strategy.

Repeat vaccination within three months following the first injection has been previously investigated with mixed results. Studies in residents of long-term care facilities and individuals with end-stage renal failure found no clinically significant improvement in haemagglutination-inhibition (HI) antibody titres with repeat vaccination, whereas a recent study in recipients of a solid organ transplant observed significantly higher antibody titres when an influenza vaccine was re-administered after five weeks.[10–12] These studies, however, were conducted with the aim of improving protection for the duration of a temperate winter season, rather than year-round protection.

An observational study of antibody persistence conducted over 2016-17 among older adults in Hong Kong proposed that six-monthly vaccination improved protection against circulating seasonal

influenza strains.[13] Similar to observations following repeated annual vaccination, the results also raised the concern that more frequent vaccination may actually impair immune responses.[14]

Singapore is a tropical city-state, where influenza epidemics typically occur biannually, coinciding with the Northern and Southern hemisphere winters, and endemic virus activity persists year-round. The TROPICS1 study is a clinical trial of six-monthly versus annual influenza vaccination in older adults, with the aim of determining if a repeat influenza vaccine at six months is able to improve the year-round immune markers of protection against influenza.

Methods

Study design and setting

Tropical Influenza Control Strategies (TROPICS1) was a single-centre, 1:1 randomised, observer-blinded, active-comparator controlled, parallel-group superiority study of a repeat influenza vaccine administered six-months after initial vaccination. Study procedures were conducted at Tan Tock Seng Hospital in Singapore.

The study protocol was approved by the Institutional Review Board with oversight of Tan Tock Seng Hospital, the Domain Specific Review Board (DSRB 2015/01047). The study was conducted without significant amendment to the published protocol.[15]

Participants

Study participants were adults aged 65 years or over, living independently in the community who were able to comply with the study protocol. All participants provided written informed consent. Participants were mainly enrolled from senior activity centres and community centres, with no restrictions based on gender or race. For inclusion in the study, all participants must not have received any influenza vaccine in the preceding ten months nor have been diagnosed with a virologically confirmed influenza infection.

Major exclusion criteria included known systemic hypersensitivity to any of the vaccine components, or prior life-threatening reaction to influenza vaccination including a history of Guillain-Barré syndrome (GBS) within six weeks following previous vaccination. Other exclusion criteria included a known or suspected congenital or acquired immunodeficiency, or receipt of immunosuppressive therapy within the previous six months, such as anti-cancer chemotherapy, radiation therapy, or long-term systemic corticosteroid therapy. Participation in the study was delayed if the individual had an acute respiratory illness, fever ≥37·5°C, or moderate or severe acute illness/infection on the day of enrolment.

Intervention

Following enrolment, all participants received an injection of a standard-dose, inactivated trivalent influenza (IIV3) vaccine (Fluzone®, Sanofi-Pasteur, PA, USA). Six months after enrolment, participants allocated to the control group received an injection of Tetanus-diphtheria-pertussis (TDaP) vaccine (Boostrix®, GSK, Brentford, UK), while participants allocated to the experimental group received a repeat IIV3 injection (Influvac®, Abbott Laboratories, IL, USA). The vaccines were provided in ready-to-use 0·5-ml syringes and administered intramuscularly, in the deltoid muscle.

Influenza vaccine manufacturer changed between first and second influenza vaccination due to a countrywide replacement by Sanofi of the trivalent Fluzone® with a quadrivalent formulation. To ensure the intervention group received a trivalent formulation, Influvac®, Abbott Laboratories, IL, USA was used for the second dose. Strains included in both vaccines did not change, reflecting the contemporaneous World Health Organisation recommendations for the Southern hemisphere winter 2016 and Northern hemisphere winter 2016/7, and comprised: A/California/7/2009 (H1N1)pdm09-like virus, A/Hong Kong/4801/2014 (H3N2)-like virus, and B/Brisbane/60/2008-like virus.[2]

Procedures

Venous blood samples were obtained from participants for immunogenicity assessment at enrolment (Month 0, pre-injection), Month 1 (21-28 days after enrolment), Month 6 (166-194 days after enrolment), Month 7 (21-28 days after second vaccine), and Month 12 (346-376 days after enrolment).

Participants were monitored for 30 min after vaccination for immediate adverse events and reactogenicity. Diary cards were distributed on the day of vaccination to record solicited local (pain, redness, and induration) and systemic (fever, headache, fatigue, arthralgia, chills, malaise, myalgia, and vomiting) reactions for seven days after vaccination.

All other adverse events (AEs) were recorded for seven days after each vaccination, while adverse events considered to be serious (SAEs) were recorded for 28 days. The relation of AEs and SAEs to study vaccines was determined by study investigators according to ICH guidelines and protocol-specified safety considerations.

Participants were contacted every two weeks till final visit at Month 12 by either text message, phone call or clinic visit as surveillance for clinical endpoints. At each contact, participants were asked about any symptoms of an influenza-like Illness (ILI), using the European Centre for Disease Prevention and Control (ECDC) clinical case-definition: the sudden onset of systemic symptoms (fever or feverishness, malaise, headache or myalgia) and respiratory symptoms (cough, coryza, shortness of breath or sore throat).[16] Subjects with the sudden onset of respiratory symptoms, with or without accompanying systemic symptoms, were recorded as having an acute respiratory illness (ARI). Laboratory confirmation of influenza infection was not pursued.

In addition to symptoms, participants were also asked regarding unscheduled doctor (e.g. general practitioner, polyclinic) and hospital visits (e.g. emergency department, hospitalisation) since the previous contact.

Outcomes

The pre-specified primary outcome for this study was the percentage of participants with a haemagglutination-inhibition (HI) titre ≥1:40 for each of the influenza strains present in the administered influenza vaccine at Month 7, one month after the second vaccination. Other serological outcomes include a comparison by intervention group of geometric mean titres (GMT) and the geometric mean fold-rise (GMFR) pre- and post-vaccination from Month 7 to 12. Seroconversion was also measured using the standard definition of a pre-vaccination HI titre <10 and post-vaccination HI titre ≥40 or at least a 4-fold increase in HI titres from a pre-vaccination HI titre ≥10.

Clinical outcomes included the number of participants reporting an influenza-like-illness, and the number of participants reporting healthcare utilisation (emergency rooms visits and hospitalisations in each group) across vaccine groups from Month 6 to 12.

Sample size

A sample size of 200 (100 per group) was estimated to offer more than 80% power to detect a 20% difference in the proportion of participants with a HI titre ≥1:40 at Month 7. This assumed a 60% seroprotection rate in the control group, a loss to follow up rate of 15%, and a two-sided significance level (α) of 5%.

Randomisation and blinding

Participants were assigned to intervention groups through a computer-generated randomisation sequence created using the 'blockrand' package on the R statistical software program.[17] The randomisation sequence was generated by a statistician with no clinical involvement in the trial. Randomisation was stratified by participant age (≥65-74, ≥75 years) and used random block sizes of 2, 4 and 6.

Participant group allocation was performed by an unblinded vaccine team who were solely responsible for preparing and administering the vaccines at the Month 6 study visit. This team also securely stored vaccination documents revealing participant group assignment. Blinded study personnel were not allowed to be present during preparation and administration of the vaccines. Contents of prepared syringes were masked with an opaque tape, and participants were asked to look away during vaccine administration to ensure participant blinding to group allocation. The unblinded team was not involved in collecting safety data or performing any other study procedures.

Participants, investigators, study staff members and laboratory personnel were all blinded to group assignment until after serological studies for the study primary endpoint were completed.

Laboratory methods

Blood samples were processed immediately after collection, and serum stored at -80°C, before being shipped frozen to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia for the haemagglutination-inhibition (HI) assay.

HI assays were performed on serum samples following standardized protocols using the egg-derived vaccine viruses strains and turkey red blood cells.[18] HI titres were expressed as the reciprocal of the highest dilution of serum where hemagglutination was prevented (from 1:10 to a maximum of 1:20,480) and analysed on a $\log_2$ scale (titres <10 and ≥20,480 were assigned a value of 5 and 20,480 respectively).

Statistical methods

Continuous variables were compared between groups with unpaired *t* tests, and the paired *t* test was used to compare GMTs within study groups at baseline and after vaccination. For categorical variables, we assessed differences between vaccine groups with Fisher's exact tests. Linear regression was used to identify predictors of HI response to vaccination, and regression lines compared by analysis of covaraince.

As per the study protocol, analysis for serological and clinical endpoints were performed in the intention-to-treat population, while safety data was analysed based on the actual vaccine received. All analyses were performed using the R statistical software, version 3·4·1.[17]

This study is registered with ClinicalTrials.gov, identifier NCT02655874.

Role of the funding source

TROPICS1 was an investigator-initiated study, funded by the National Healthcare Group. The study sponsor was not involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Results

200 participants were enrolled, with 100 randomly assigned to repeat IIV3 vaccination at six months, and 100 assigned to the control group. All 200 participants received influenza vaccination at study enrolment (Figure 1). 192 (96%) received a repeat vaccine after six-months and were included in the intention-to-treat (ITT) and safety analysis. 189 (95%) participants received the allocated vaccine and were included in the per-protocol (PP) analysis.

Participants were enrolled and received the first study vaccine from May to November 2016. The final study visit was in October 2017. The epidemiological week of vaccination visits and the weekly incidence of PCR-confirmed influenza infections in Singapore from WHO surveillance data is shown in Figure 2.

Baseline characteristics of the participants in each group were similar (Table 1).

Immunogenicity

HI antibody responses to the first influenza vaccine administered met traditional licensing criteria for older adults set by the Center for Biologics Evaluation and Research (CBER) for all three strains at Month 1 (Figure 3).[19]

Antibody titres declined significantly from Month 1 to 6 for all three influenza strains (all $p<0.0001$, paired t-test). However, only for A/H1N1 did the proportion of subjects with a HI titre ≥1:40 decline significantly (from 83.1% to 58.5%), with this proportion >95% at Month 6 for both A/H3N2 and B.

Following repeat vaccination, there was a statistically significant increase in the proportion of participants with a HI titre ≥1:40 at Month 7 for A/H1N1, with an absolute increase of 21.4% (95% CI 8·6-33·4, p=0·0011). A non-statistically significant increase in this proportion was observed for A/H3N2 (4·3%, 95% CI -1·1 to 10·8, p=0·088) and B (2·1%, 95% CI -2 to 7·3, p=0·152).

Repeat IIV3 vaccination significantly improved the HI Geometric Mean Titre (GMT) against the influenza vaccine subtypes A/H1N1 (63·9, 95% CI 52·7-77·4 vs 35·7, 95% CI: 28·8-44·4, p<0·0001) and A/H3N2 (238·7, 95% CI 195·3-291·8 vs. 174·0, 95% CI 141·1-214·6, p=0·030) at Month 7. This significantly higher GMT following repeat vaccination did not persist to Month 12 for either influenza A subtype, while no significant difference in GMT was observed for influenza B at either time point. From Month 6 to 12, GMTs declined significantly for A/H1N1 and A/H3N2 in participants receiving annual vaccination (p<0·0001 and 0·0002 respectively). In the six-monthly vaccine group antibody titres were not significantly different between these two timepoints. The immune responses to influenza B again formed an outlier, with no significant change in GMT for either group from Month 6 to 12. See appendix for full GMTs prior to Month 7 (pp1-3).

Using the GMT at enrolment (Month 0) as baseline, the geometric mean fold rise (GMFR) was significantly higher in the six-monthly vaccination group at Month 7 and 12 for A/H1N1 and at Month 7 for A/H3N2, but not significantly different for B (Figure 4). Similarly, a significant increase in GMFR was evident at Month 7, compared with Month 6 in the six-monthly vaccination group.

GMTs were significantly lower in the six-monthly vaccination group following the second vaccination compared with first (Month 7 vs Month 1, p<0.01 for all). Linear regression of the change in $\log_2$ titre following first and second influenza vaccination, indicated increases in HI titre were smaller following the second vaccination, even when the pre-vaccination titre was zero (Figure 5). As a result, the proportion of participants with a four-fold or greater increase in HI titre in the repeat vaccination group was substantially lower compared with the first vaccination: 75·5% vs. 21·6% to A/H1N1, 76·0% vs. 12·4% to A/H3N2 and 47·4% vs 3·1% to B (p<0·0001 for all three influenza strains). The proportion of participants meeting the protocol definition for seroconversion was also significantly lower (see appendix p4).

Linear regression of the baseline characteristics, including age, sex, co-morbidity index or previous vaccination identified no significant predictors of GMT at Month 1 or 7. GMT pre-vaccination was a

significant correlate of post-vaccination titres at all time points (p<0·0001 for all 3 influenza types/subtypes at Month 1).

Serological results were not significantly different when analysed by ITT or PP (see appendix pp1-3 for the PP analysis).

Vaccine Efficacy

A significant reduction in the proportion of participants reporting an acute respiratory infection (ARI) or influenza-like illness (ILI) was observed in the group who received the repeat influenza vaccine (Table 2). From Month 6 to 12, seven (7·2%) of participants in the experimental group reported an ILI, and 16 (16·8%) participants in the control. Vaccine efficacy against ILI was 57·1% (0·6-81·5%, p=0·047). Consistent with this reduced ILI incidence, a reduction in emergency department attendances and hospital admission was observed in the six-monthly vaccination group with an estimated VE of 34·7%. Of the three attendances attributable to an acute respiratory condition, all were in the control group. Diagnoses were of an upper respiratory tract infection, a lower respiratory tract infection and an infective exacerbation of chronic airways disease.

The incidence of ARI, ILI and healthcare attendances were not significantly different between groups from Month 0 to 6 (see appendix p4)

Safety

197 participants returned a diary card after the first vaccination, and 32 (16·2%) participants reported 57 solicited adverse events (AE) in the seven days after vaccination. Ten (10·0%) of 100 participants who received a second influenza vaccine reported 19 solicited adverse events (see appendix pp4-5 for a full description of the nature and duration of AEs). This lower incidence of adverse events was not significantly different (p=0·162). The majority of reported adverse events were short-lived and of only mild severity. Seven (3·6%) AEs were reported as moderate or more severe after the first vaccination, and one (0·5%) of participants reported an AE persisting for more

than seven days. Following the second influenza vaccination, two (2·0%) of AEs were reported as moderate or more severity, while none persisted for more than seven days.

Some 28 (30·4%) of participants receiving the TDaP vaccine reported a solicited AE. This was significantly more common than either the first (p=0·0078) or second (p=0·0005) influenza vaccination and probably reflects reactogenicity from the alum adjuvant. Eight (5·4%) of all solicited AEs were reported as of moderate or more severity, while six (6·5%) persisted for more than seven days.

Two serious adverse events were recorded in the month after the second vaccination, one in each vaccination group. Neither were assessed by study investigators as related to vaccination.

Discussion

TROPICS1 is the first randomised clinical trial of six-monthly versus annual influenza vaccination. Our results show that a repeat standard-dose influenza vaccine administered after six months in older adults can significantly increase strain-specific antibody titres. Repeat vaccination also correlated with a significant reduction in symptomatic respiratory illness and utilization of health services.

This finding has important implications for influenza vaccination strategies in tropical and sub-tropical climates where influenza virus activity is not confined to a short winter season. With aging demographics and developing economies resulting in an increasing demand for healthcare across much of the tropics and sub-tropics, a biannual vaccination schedule could have a substantial public health impact. It also suggests repeat vaccination may have utility in temperate climates in seasons when the virus circulates for longer than is typical.

Further study with different populations, vaccines, vaccine strains and over more seasons are required to confirm that these study findings are reproducible, and a number of points warrant further consideration.

The major concern with a six-monthly vaccination schedule, is whether repeat vaccination might result in lower vaccine effectiveness compared with an annual schedule. Reduced vaccine effectiveness with sequential years vaccination has been reported from a number of test-negative design case-control studies. We observed a significantly reduced antibody response at the second influenza vaccination, suggesting interference from pre-vaccination immunity. While the motivation for six-monthly vaccination is to overcome the problem with waning of immunity in older adults, this study finds data to support the notion that waning is itself important to stimulate a rise in HI titre.

The reduced HI response between first and second vaccination across a range of baseline titres implies, however, that it is not simply the *quantity* of pre-vaccination antibody which predicts vaccine response. Instead, qualitative differences in the post-vaccination antibodies may be more

important. In the TROPICS1 cohort, prior vaccination was uncommon (11% in the past two years). Pre-vaccination antibody hence largely reflects previous infection. Compared with infection, the breadth of the antibody repertoire following vaccination is reduced, and appears to be more focused towards epitopes of the HA head which are variable between strains.[20] Repeat vaccination with the same influenza strains may result in rapid neutralisation from circulating antibody, but poorly boost memory responses in a phenomenon known as antigen trapping.[21] Possible solutions to this problem include vaccinating with strains that have drifted sufficiently from the previous vaccinating strain, and so are neutralised less effectively by circulating antibody.[22] Alternatively, in the absence of vaccine strain change, memory responses may be more effectively stimulated with adjuvants or a higher antigen dose.

As even at low or undetectable pre-vaccination titres the HI response to repeat vaccination was also reduced compared with first vaccination, other components of the immune response are also likely to be important. For example, neuraminidase (NA) inhibiting antibodies are stimulated by vaccination and provide an independent measure of protection against infection.[23] The time-course of these and other vaccine-induced antibodies in the months after vaccination is not known. While cell-mediated immunity is typically poorly stimulated by the inactivated influenza vaccine, repeat vaccination has been associated with diminished T-cell responses.[13]

Despite this interference, there was clear evidence of an immune response to repeat influenza vaccination and reduced waning of titres by Month 12 compared with the control group – most clearly described by the difference in GMFR between the two vaccination groups. This immune response may explain the unexpected finding of a significant reduction in the incidence of influenza-like-illness (ILI) and healthcare utilisation. The observed ILI attack rate of 12%, is similar to conventional estimates of a 5-15% annual influenza infection rate, but is a non-specific endpoint, and other respiratory viruses produce similar clinical symptoms.[24] Detecting a reduction in

virologically confirmed influenza infection rates, while necessary to conclude clinical benefit, would require much larger sample sizes.

A reduction in the observed incidence of ILI with six-monthly vaccination may appear surprising given the nominal 'seroprotection' rate (HI≥1:40) remained high in the control group even at Month 12. However, this threshold has been estimated to offer only ~50% protection against infection, and higher titres are associated with a reduced risk of infection.[25] Furthermore, the limitations of HI titres as a catch-all for protection are well understood, and while they are long established, a better correlate of protection is desirable. Study of other immune responses from samples collected during the TROPICS1 study are currently under way.

The optimal timing of influenza vaccination in Singapore and other tropical countries has not been determined by randomised clinical trials. Based on epidemiological studies of the typical pattern of virus activity, Singapore influenza vaccination is recommended annually, and in clinical practice is administered at any time of the year.[26] Similar to the difficulties with predicting which influenza strains to include in vaccines, variation in the timing of influenza outbreaks in tropical countries hampers prospectively determining the optimal time for vaccination. The period when the TROPICS1 study was conducted – mainly the northern hemisphere winter of 2016/17 and the southern hemisphere winter of 2017 – recorded higher influenza virus activity than usual. This pattern was observed in other countries in the region, with a record number of infections notified in Australia, and an unusually prolonged season in Hong Kong.[27,28] Similar findings have also been reported from the Northern hemisphere 2017/18 winter.[29] These seasons have been dominated by A/H3N2 and estimates of vaccine effectiveness against this subtype have been low. As vaccine effectiveness (particularly for A/H3N2) declines significantly 3 months after vaccination, some of the apparent clinical benefit observed in this trial may thus reflect better timing of vaccination for the epidemic peak in Singapore, as the majority of repeat influenza vaccines were administered between Jan-Apr 2017.

The impact of six-monthly vaccination also needs to be considered in other populations to the TROPICS1 cohort, which may have different responses to vaccination. For example, similar to the local population, only 10% of study participants had received an influenza vaccine in the two years prior to study enrolment, and findings may be different in a more frequently vaccinated population. Participants in this study were also relatively well older adults. Adults at more advanced age, frailty and with more health-limiting co-morbidities are expected to have reduced immune responses vaccination and may be more likely to benefit from six-monthly administration. These characteristics were probably not identified as significant predictors of post-vaccination HI titres in the TROPICS1 study due to a lack of statistical power.

In addition to the issues discussed above, data collection and analysis were performed by study personnel blinded to participant group allocation, however, there is a possibility of loss of allocation concealment due to the reactogenicity of TDaP. An active-comparator was used in the control group to avoid an 'empty' injection in an under-vaccinated population. In future studies a saline injection may be preferable to reduce this risk. The switch in vaccines manufacturers between first and second influenza vaccination may also have interfered with vaccine responses – either positively or negatively - but is a pragmatic consideration and would not have impacted ARI/ILI incidence. The immune response is thought to be similar across inactivated vaccine types (split or subunit), and this switch reflects common practice in healthcare institutions.[30]

In conclusion, six-monthly vaccination in tropical and sub-tropical countries offers promise as an important public health intervention for tropical and sub-tropical to reduce the burden from influenza infection. Further studies exploring the effects of repeat vaccinations and strain changes are important to perform before concluding the immune and patient benefits of such an approach.

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Potential conflicts of interest

BY has received honorarium from Roche. All other authors have no conflicts of interest to disclose

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