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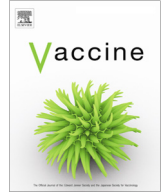
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Review

Do antibody responses to the influenza vaccine persist year-round in the elderly? A systematic review and meta-analysis



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ABSTRACT

Introduction: The influenza vaccine is less immunogenic in older than younger adults, and the duration of protection is unclear. Determining if protection persists beyond a typical seasonal epidemic is important for climates where influenza virus activity is year-round.

Methods: A systematic review protocol was developed and registered with PROSPERO [CRD42015023847]. Electronic databases were searched systematically for studies reporting haemagglutination-inhibition (HI) titres 180–360 days following vaccination with inactivated trivalent seasonal influenza vaccine, in adults aged ≥ 65 years. Geometric mean titre (GMT) and seroprotection (HI titre $\geq 1:40$) at each time point was extracted. A Bayesian model was developed of titre trajectories from pre-vaccination to Day 360. In the meta-analysis, studies were aggregated using a random-effects model to compare pre-vaccination with post-vaccination HI titres at Day 21–42 ('seroconversion'), Day 180 and Day 360. Potential sources of bias were systematically assessed, and heterogeneity explored.

Results: 2864 articles were identified in the literature search, of which nineteen met study inclusion/exclusion criteria. Sixteen studies contained analysable data from 2565 subjects. In the Bayesian model, the proportion of subjects seroprotected increased from 41–51% pre-vaccination to 75–78% at seroconversion. Seroprotection subsequently fell below 60% for all serotypes by Day 360: A/H1 42% (95% CI 38–46), A/H3 59% (54–63), B 47% (42–52). The Bayesian model of GMT trajectories revealed a similar pattern. By Day 360, titres were similar to pre-vaccination levels. In the meta-analysis, no significant difference in proportion of subjects seroprotected, 0 (–0.11, 0.11) or in \log_2 GMT 0.30 (–0.02, 0.63) was identified by Day 360 compared with pre-vaccination. The quality of this evidence was limited to moderate on account of significant participant dropout.

Conclusions: The review found consistent evidence that HI antibody responses following influenza vaccination do not reliably persist year-round in older adults. Alternative vaccination strategies could provide clinical benefits in regions where year-round protection is important.

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1. Introduction

Influenza is a common viral respiratory infection, which worldwide causes substantial morbidity and mortality, particularly at extremes of age [1,2]. Influenza vaccination is the primary tool available for disease control but immune responses to vaccination are reduced in the elderly compared with younger, healthy adults [3]. Persistence of vaccine-induced immunity over periods longer than a typical winter season have not been widely investigated, but similar to short term responses a reduced duration of persistence and hence protection against infection may be expected [4].

The primary immune response to the standard inactivated influenza vaccine is strain-specific antibody to surface haemagglutinin (HA) [5]. These antibodies mediate protection against infection by interfering with virus binding to host-cell receptors, and are measured with standardised Haemagglutination-Inhibition (HI) Assays [6]. Currently, age specific immunogenicity criteria based on the HI titre are used by the regulatory committees of the European Medicines Agency (EMA) and Federal Drug Administration (FDA) [7]. For example, a HI titre of $\geq 1:40$ is conventionally considered 'seroprotection', and more than 70% of younger adults, or 60% of older adults must reach this threshold for licensing.

A literature review published in 2008 studied antibody persistence in the elderly (>60 years) [8]. Up to 16 weeks post-vaccination the authors did not find evidence for substantial waning of seroprotection. This review was primarily qualitative and did not attempt to apply statistical methods to reported outcomes. Beyond this review, antibody persistence in the elderly has not been systematically assessed and a number of new studies providing data on antibody persistence have since been published.

Waning of vaccine effectiveness over the course of a winter season has been reported from a number of surveillance studies in Europe and Australia [9–11]. For example, in a study in Spain, vaccine effectiveness declined from 61% in the first 100 days after vaccination to 42% between days 100–119, and no protection after 120 days. This decline in effectiveness was most significant in the elderly aged over 65 years. These studies used the test-negative case-control design, and so do not include accompanying serological data. It is not clear to what extent this decline in effectiveness reflects loss of vaccine-induced immune responses, or reduced vaccine-strain matching from antigenic drift in circulating strains.

Limited data is available from studies of antibody persistence after influenza infection. An observational study monitored titre trajectories in subjects who were assessed to be infected with A/H1N1 during the 2009 pandemic (seroconversion without vaccination) [12]. In 71% haemagglutination-inhibition (HI) antibody titres were $\geq 1:40$ immediately after the epidemic peak. This declined to 25% of subjects at 6 months, and only 14% at 1 year after the pandemic. In a sub-group analysis of the small number of elderly

subjects in the cohort, the rate of antibody decline was significantly faster.

The duration of protection following vaccination is of particular public health importance in countries which report more than a single annual influenza season. Biannual epidemics, triannual epidemics and year round virus activity are described in tropical countries, from Indonesia and Malaysia to Peru and Mexico [13,14]. Despite the difference in seasonality, the burden of disease from influenza in countries with tropical, sub-tropical and temperate climates has been reported to be similar [15]. The implication of this differing epidemiology for vaccination schedules is yet to be understood. For example, recommendations for influenza vaccine timing from the World Health Organization (WHO) are based the pattern of influenza virus activity rather than prospective studies of year-round vaccine effectiveness [16,17].

With year-round influenza virus activity in the tropics, year-round seroprotection is expected to be beneficial, but is least likely to be attained in populations such as the elderly with impaired immune responses. This study is a systematic review and meta-analysis of the available evidence for year-round persistence of vaccine-induced antibody following trivalent, inactivated, seasonal influenza vaccination in the elderly.

2. Materials and methods

An abbreviated study protocol is available from the National Institute for Health Research International Prospective Register of Systemic Reviews (PROSPERO), registration number CRD42015023847 [18]. The Preferred Reporting Items for Systematic Reviews and meta-Analyses (PRISMA) checklist for reporting of systematic review was also followed [19].

2.1. Search strategy and study selection

A search strategy was developed using the PICOST framework. Study inclusion and exclusion criteria are presented in Table 1.

Population: Elderly ≥ 65 years

Intervention: Trivalent inactivated seasonal influenza vaccination administered by intra-muscular injection

Comparison: No comparative group (e.g. healthy younger adults) will be included. HI antibody responses at selected time points will be compared with the pre-vaccination results.

Outcome: HI geometric mean titre (GMT) from 180 to 360 days after vaccination and proportion with GMTs $\geq 1:40$ per Centre for Biologics Evaluation and Research (CBER) criteria [20]

Situation: For immunologic studies, the country in which the study is performed is not important

Table 1
Study inclusion and exclusion criteria.

Inclusion criteria

Study performed in elderly humans (≥ 65 years)
 No restriction is placed on study population place of residence (e.g. community or long-term care facility), vaccination history or presence of chronic diseases
 Studies of standard dose (15 mcg hemagglutinin per strain) trivalent inactivated seasonal influenza vaccine, without adjuvant
 Studies using inactivated whole virion, split virion or subunit influenza antigen preparations
 Studies reporting seroprotection and/or GMT at pre-vaccination, 21–42 days ('seroconversion') and at least one additional time point between 180 and 360 days
 No restriction is placed on HI antibody assay methodology
 Where seroprotection data is reported, only studies which use thresholds recommended by CBER will be included ($\geq 1:40$)
 No restriction is placed on study design – for example RCT or cohort study
 Multi-year studies will be included if vaccine and/or strain composition do not change during the first year of the study
 Full text of manuscript is published in English, after 1977

Exclusion criteria

Studies which include subjects due to the presence of a specific comorbidity (e.g. haemodialysis or post-solid organ transplant)
 Studies using monovalent/bivalent/quadrivalent or live-attenuated seasonal influenza vaccines
 Studies administering vaccine by non-parenteral or intra-dermal routes
 Studies using virosomal or virus-like particle vaccines, and experimental/novel preparations
 Studies using vaccines targeting non-seasonal strains, such as avian influenza
 Uncontrolled trials of experimental interventions (e.g. day 28 boosters, novel adjuvants)

Type of Study: Any study type - observational or randomised controlled trial (RCT).

A publication date limit of 1977 was chosen to reflect the year when a third co-circulating seasonal influenza strain (H1N1) was recognised and WHO recommendations switched from a bivalent to trivalent influenza vaccine [21]. The search construct developed for MEDLINE is available in the [Supplementary Material](#). This was subsequently adapted for other information sources as required. Results were limited to human subjects and English language records.

Information sources searched to identify relevant literature included the MEDLINE, EMBASE and Global Health databases, the Cochrane Database of Systematic Reviews, [Clinicaltrials.gov](#) and reference lists of all included studies. After removal of duplicates, a three-stage screening process was used: reviewing title, then abstract and finally full text where possible.

2.2. Data collection

Data was extracted by a single reviewer using a template adapted from [Cochrane.org](#). Data was collected as presented, with estimates of numerical values made where only graphical data was supplied. Verification of entered data was performed by an independent recollection of data from the source manuscript. Corresponding authors were contacted to request for further data or clarifications where necessary. Data that was not published in peer reviewed literature, but available in [clinicaltrials.gov](#) study results or study sponsor trial registries were included. Further systematic search for grey or unpublished literature was not performed. Seroprotection and GMT outcome data were collected for each influenza strain subjects were vaccinated with. Retention rates were calculated using the GMT data presented for A/H1.

2.3. Risk of bias and quality of evidence assessment

Risk of bias was assessed using the Cochrane Collaboration's tools for assessing RCTs and non-randomised studies [22]. Each study was assessed as at either low, high or unclear risk for bias. The terminology for assessing risk of bias was harmonised for cohort and RCTs, however, bias assessments are not equivalent between scales. Studies were not excluded from the systematic review or meta-analysis as a result of this assessment.

The GRADE approach was used to evaluate the overall quality of evidence for each outcome [23]. Because the study compared pre- and post-vaccination results in the same subjects, both RCTs and

observational studies started as 'high quality'. They were downgraded by one level for serious (or by two for very serious) study limitations identified in the risk of bias assessment such as issues that may limit the generalisability of the reported results, inconsistency of results between studies, or potential publication bias.

2.4. Synthesis of results

Seroprotection and GMT were analysed by influenza subtype A/H1, A/H3 and type B at available time points. Number seroprotected ($HI \geq 1:40$) was estimated from provided data, rounded to integers. Where GMT confidence intervals (CI) were available, the sample standard deviation was calculated from the \log_2 transformed values and a Student's t distribution assumed if not stated. Where only sub-group data was available from a study each sub-group was entered and analysed separately.

To account for both inter- and intra-study variability, Bayesian mixed-effect models were developed to determine an estimate of seroprotection and GMT for each subtype. We assumed a normal distribution for the GMT on a base-2 logarithmic scale and a binomial distribution for seroprotection for each subtype. The likelihood function of the GMT and seroprotection data could be then derived using the following models:

For GMT data, we assumed a log-Normal distribution

$$\log_2(GMT_{ij}) \sim N(\mu_{ij}, \sigma_i^2)$$

$$\mu_{ij} = \alpha_i + \beta_j + \epsilon_i$$

where GMT_{ij} is the j th observed GMT in study i , μ_{ij} controls the location of GMT_{ij} and ϵ_i is the random effect term. Both α_i and β_j are model parameters that need to be estimated where α_i determines the study effect and β_j captures the variation in time.

For seroprotection data, we assumed a binomial distribution:

$$x_{ij} \sim \text{Bin}(p_{ij}, n_{ij})$$

$$\text{logit}(p_{ij}) = a_i + b_j + \epsilon_i$$

where x_{ij} is the j th observed number of subjects with GMTs $\geq 1:40$ in study i , n_{ij} is the j th observed sample size in study i , p_{ij} gives the estimate of seroprotection and ϵ_i is the random effect term. Again, both a_i and b_j are model parameters that need to be estimated where a_i determines the study effect and b_j captures the variation in time.

Non-informative priors were adopted for model parameters. Parameters were estimated using Markov chain Monte Carlo

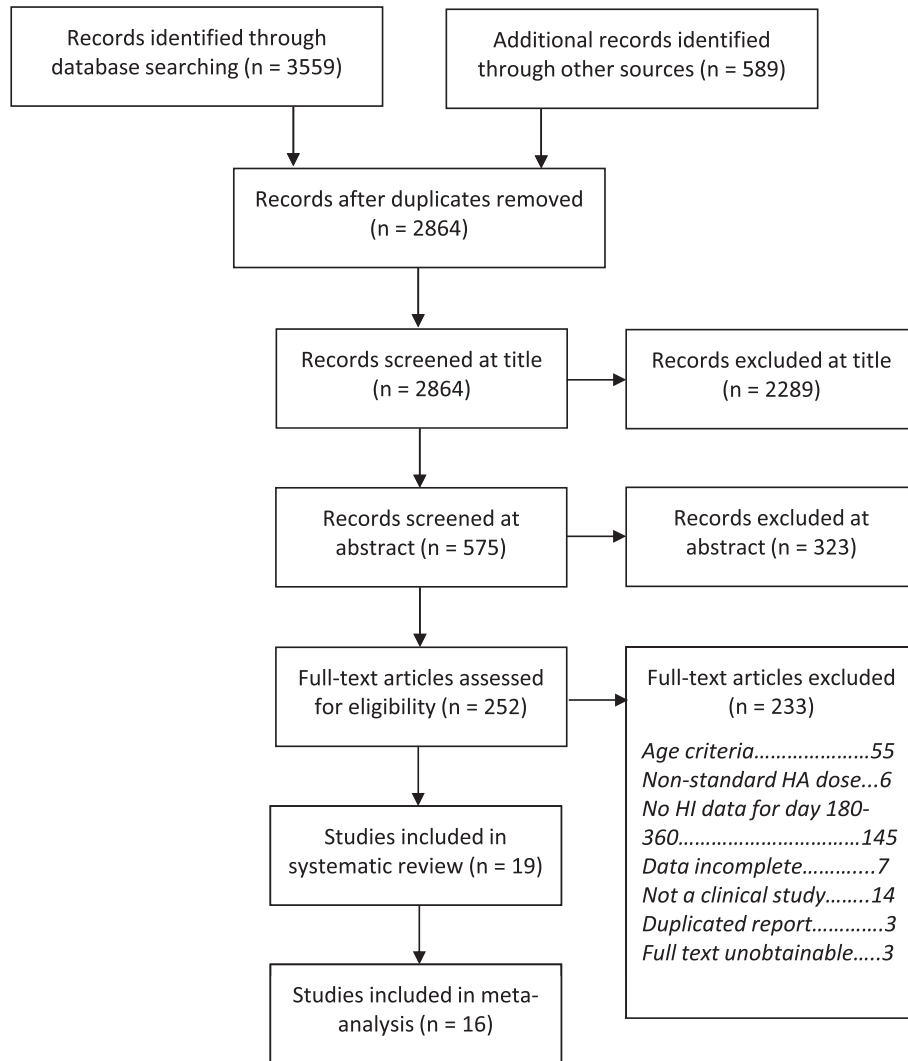


Fig. 1. PRISMA flow diagram of study selection and reasons for study exclusion at screening of full text.

with 100,000 Metropolis-Hastings iterations with a thin of 10 iterations after a burn-in period of 50,000. Point estimates are posterior means and uncertainty intervals are 95% credible intervals (CrI).

The proportion of subjects seroprotected and \log_2 GMT were compared at baseline (pre-vaccination) with Day 21–42, Day 180 and Day 360 in the meta-analysis. The Mantel-Haenszel method was used to calculate the risk difference in seroprotection. Mean differences in \log_2 GMT were aggregated by inverse-variance weighting. Random-effects models were used for all meta-analyses to reflect the assumption that the effects being estimated in the different studies are not identical. Sub-group analyses by influenza subtype were also performed (A/H1, A/H3, B).

Variability of outcomes between studies was assessed using the heterogeneity statistic I^2 . Possible publication bias was assessed by visual interpretation of funnel plots.

Descriptive statistics were calculated using Microsoft Excel 2016 (Microsoft Corporation, Richmond, USA). The Bayesian binomial/normal model was implemented in R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Meta-analyses were calculated using RevMan v5.3 (The Cochrane Collaboration, Copenhagen, Denmark). Statistical significance was assumed with a 2-sided alpha of 0.05.

3. Results

3.1. Study selection and characteristics

Database searches were performed in September 2015. The PRISMA flow diagram for the study selection process and reasons for exclusion of studies at full-text screening are shown in Fig. 1. Summary characteristics of the included studies are shown in Table 2. There were fifteen randomised control trials (RCTs) and 4 cohort studies.

Fourteen of the RCTs compared standard dose inactivated trivalent influenza vaccine with alternative formulations (adjuvanted, high-dose vaccine, intra-dermal vaccine or quadrivalent vaccine). One RCT compared the effect of an oral adjuvant with placebo. Three RCTs included an immunogenicity analysis as a subset of a larger analysis with clinical endpoints, while the others were designed with immunological primary endpoints. One of the cohort studies was designed to investigate antibody persistence in the elderly and other age groups as the primary objective. Two cohort studies investigated serological responses to influenza vaccination after first and subsequent annual vaccination, while one was an observational study of booster influenza vaccine at 12 weeks in elderly travellers.

Table 2
Summary of study characteristics.

	Author, publication year [Ref.]	Vaccine year	Principal country	Study design	Comparative arm	Number of subjects (IIV3 group)	Male (%)	Average age (year)	Vaccine type	Vaccine in previous year (%)	Retention rate (% day 180/1)	Living situation	Source of data
1*	Minutello 1999 [24]	1992	Italy	RCT, observer-blind	allIV3	46	39.1	73	SU	72	NA	C	P
2*	Van Hoecke 1996 [25]	1994	Slovakia	Cohort	–	495	29.5	80	SV	–	NA	LTCF	P
3	Buxton 2001 [26]	1998	Canada	Cohort	IIV3 booster	32	–	74	–	–	96.4 (27/28)	C	P
4	Iorio 2007 [27]	1998	Italy	Cohort	–	580	10.8	81	SU/SV	–	NA	LTCF	P
5*	Ben-Yehuda 2003 [28]	2000	Israel	RCT, open-label	Liposomal vaccine	33	30.3	88	SV	69.7	100 (33/33)	LTCF	P
6	NCT00386113 [29,30]	2005	Belgium	RCT, open-label	allIV3	50	62	69	SV	–	100 (49/49)	C	R
7	NCT00377585 [31,32]	2006	US	RCT, observer-blind	allIV3	737	46.8	73	SV	–	91.8 (657/716)	C	R
8	Song 2010 [4]	2007	South Korea	Cohort	–	350	32.4	72	SV	90.1	NA	C	P
9	Rumke 2013 [33,34]	2007	Germany	RCT, observer-blind	allIV3	200	55	73	SV	–	96.3 (181/188)	C	P, R
10	Couch 2014 [35,36]	2008	US	RCT, observer-blind	allIV3	73	41	71.3	SV	78	100 (66/66)	C	P, R
11	McElhaney 2013 [37–39]	2008–10	15 countries	RCT, observer-blind	allIV3	NA	43.0	73.5	SV	72.3	100 (267/265)	C	P, R
12	Song 2013 [40]	2009	South Korea	RCT, open-label	allIV3	50	51.6	73.3	SV	–	75 (36/48)	C	P
13	Tinoco 2014 [41,42]	2010	Canada	RCT, double-blind	IIV4	NA	NA	NA	SV	–	100 (38/38)	C	P
14	Frey 2014 [43]	2010	US	RCT, observer-blind	allIV3	NA	34	72	SU	–	NA	C	P, A
15	Scheifele 2013 [44]	2011	Canada	RCT, observer-blind	allIV3, intradermal IIV3	307	40.7	74	SU	94.5	99 (301/304)	C	P, R
16	Saiki 2013 [45]	2011	Japan	RCT, open-label	Oral adjuvant allIV3,	46	29.4	86	–	–	97.8 (44/45)	LTCF	P
17	Seo 2014 [46]	2011	South Korea	RCT, open-label	intradermal IIV3	118	38.9	73	SU	–	100 (113/113)	C	P
18	Nace 2015 [47]	2011–12	US	RCT, observer-blind	High dose IIV3	102	32	87	–	–	84.7 (83/98)	LTCF	P
19	Chan 2014 [48]	2013	Hong Kong	RCT, open-label	Intra-dermal IIV3	50	44	84	SV	90	96 (48/50)	LTCF	P, A

* Not included in quantitative analysis. N/A, not available, Randomised Control Trial, RCT; Subunit, SU; Split-Virion, SV; Community, C; Long-term Care Facility, LTCF; Publication, P; Author, A; Clinical Trial Registry, R.

For three studies (numbers 1, 2 and 5 in Table 2) data was not evaluable in the Bayesian model or meta-analysis.

3.2. Risk of bias within studies

Overall nine RCTs were considered at low risk for bias, three at high risk and for three the risk was unclear (see Supplementary Material for full assessment). One cohort study was considered at low risk of bias, two at high risk and for one the risk was unclear.

Six RCTs were conducted as open-label, eight observer-blinded, and one double-blind. Although open-label studies have a greater potential for bias when compared to blinded studies, open-label studies were not assessed as high-risk on the basis of this because of the immunological nature of outcomes in this review. Most of the RCTs failed to report their allocation concealment methodology, but as this review is not comparing outcomes between groups, this limitation was not considered likely to cause significant bias.

For both RCT and cohort studies the major potential source of bias was subject dropout. For three RCTs and two cohort studies, results were selectively reported, excluding subjects who did not have HI data for Day 180–360, or the enrolled sample size was not clear. Two of the fourteen evaluable studies reported loss of $\geq 10\%$ of subjects from Day 1 vaccination to Day 180. The overall retention rate from these thirteen studies at Day 180 was 95.1%.

Retention fell to 68.2% from Day 1 to 360 for two of the five studies reporting this data.

In sixteen studies the number of subjects enrolled was available. 20.7% (677/3269) of subjects were excluded with no serological data presented. It could not be evaluated if exclusion of these subjects introduced a systematic bias in results.

3.3. Systematic review and Bayesian models of antibody persistence

The pattern of GMT and seroprotection rise and decline after vaccination was similar across all studies. Raw data extracted is available in the Supplementary Material. The majority of individual studies demonstrated a good initial serological response to vaccination with waning from seroconversion to Day 360. The degree of pre-vaccination immunity and magnitude of post-vaccination responses varied considerably. From individual studies, only one of 11 seroprotection and three of 14 GMT comparisons demonstrated statistically significant higher levels at Day 360 compared with pre-vaccination.

Summary data from the Bayesian model of antibody persistence is presented in Table 3. The estimate of population seroprotection was just above the CBER licensure threshold of 60% at Day 180, and by Day 360 fell below this threshold for all three serotypes.

Table 3
Estimates of population GMT and seroprotection (HI ≥ 1:40) at each time point by subtype.

	Pre-vaccination	Day 21–42	Day 180	Day 360
<i>GMT (95% Credible Intervals)</i>				
A/H1	20.3 (18.8–22.0) n = 2537	65.8 (60.5–71.0) n = 2536	33.6 (31.1–36.5) n = 2327	22.6 (19.8–26.4) n = 734
A/H3	23.6 (21.7–25.6) n = 2538	83.3 (76.1–90.5) n = 2537	45.3 (41.6–49.5) n = 2327	36.0 (30.9–41.4) n = 734
B	32.0 (29.2–35.3) n = 2470	78.2 (71.5–86.2) n = 2469	54.9 (49.9–60.1) n = 2327	28.2 (23.1–34.1) n = 666
<i>Seroprotection rate (95% Credible Intervals)</i>				
A/H1	0.41 (0.39–0.44) n = 2155	0.78 (0.76–0.80) n = 2157	0.61 (0.59–0.64) n = 1950	0.42 (0.38–0.46) n = 481
A/H3	0.45 (0.43–0.47) n = 2155	0.78 (0.76–0.79) n = 2157	0.62 (0.60–0.64) n = 2003	0.59 (0.54–0.63) n = 481
B	0.51 (0.50–0.53) n = 2088	0.75 (0.73–0.77) n = 2090	0.66 (0.64–0.68) n = 1950	0.47 (0.42–0.52) n = 481

3.4. GMT meta-analysis

Fifteen studies reported the pre-vaccination GMT and were included in the meta-analysis. All fifteen provided data for comparison at Day 21–42, fourteen for comparison at Day 180, and four for Day 360.

In the overall analysis, combining estimates for influenza A/H1, A/H3 and B, log₂ GMT difference declined linearly from Day 21–42 to Day 360, implying an exponential decay in GMT titres (Fig. 2). GMTs at Day 360 were not significantly different from pre-vaccination (0.30, 95% CI –0.02, 0.63). This result was consistent when analysed by subtype A/H3 (0.47, 95% CI –0.17, 1.15) and type B (–0.27, 95% CI –0.51, –0.04). While a significant difference at Day 360 was detected for A/H1 (0.57, 95% CI 0.23, 0.91), the overall trend was similar.

3.5. Seroprotection meta-analysis

Thirteen studies which reported the proportion of subjects who were seroprotected pre-vaccination were included in the meta-analysis. All provided data for comparison at Day 21–42, twelve for comparison at Day 180, and three for Day 360.

Similar to the analysis of GMTs, when combining influenza A/H1, A/H3 and B, seroprotection risk difference compared with pre-vaccination declined linearly from Day 21–42 to Day 360 (Fig. 2), and no significant difference in seroprotection compared

with pre-vaccination was detected by Day 360 (0, 95% CI –0.11, 0.11). Analysis by individual influenza subtypes A/H1, A/H3, and type B were consistent, with no significant difference from the pre-vaccination level for each at Day 360: A/H1 (0.09, 95% CI –0.15, 0.33), A/H3 (–0.08, 95% CI –0.11, 0.12), B (0, 95% CI –0.13, 0.13).

3.6. Risk of bias between studies

Substantial heterogeneity between studies was evident in the main pooled analysis from the heterogeneity statistic I² (89–98%). This estimate of heterogeneity was generally lower for the Day 360 and individual subtype analyses.

No asymmetry was evident from examination of the funnel plots for seroprotection risk difference at all time points. For GMT mean difference, asymmetry was evident in the Day 21–42 funnel plots (Fig. 3). Asymmetry was primarily a result of a larger than expected mean difference (MD) from four data points, all provided by A/H1 and A/H3 from study 13 (Tinoco et al.). Funnel plots were similar for Day 180, while no asymmetry was apparent at Day 360 (see Supplementary Material).

3.7. Quality of evidence

Estimates of differences between GMT and seroprotection pre-vaccination and Day 360 were assessed to be of moderate-

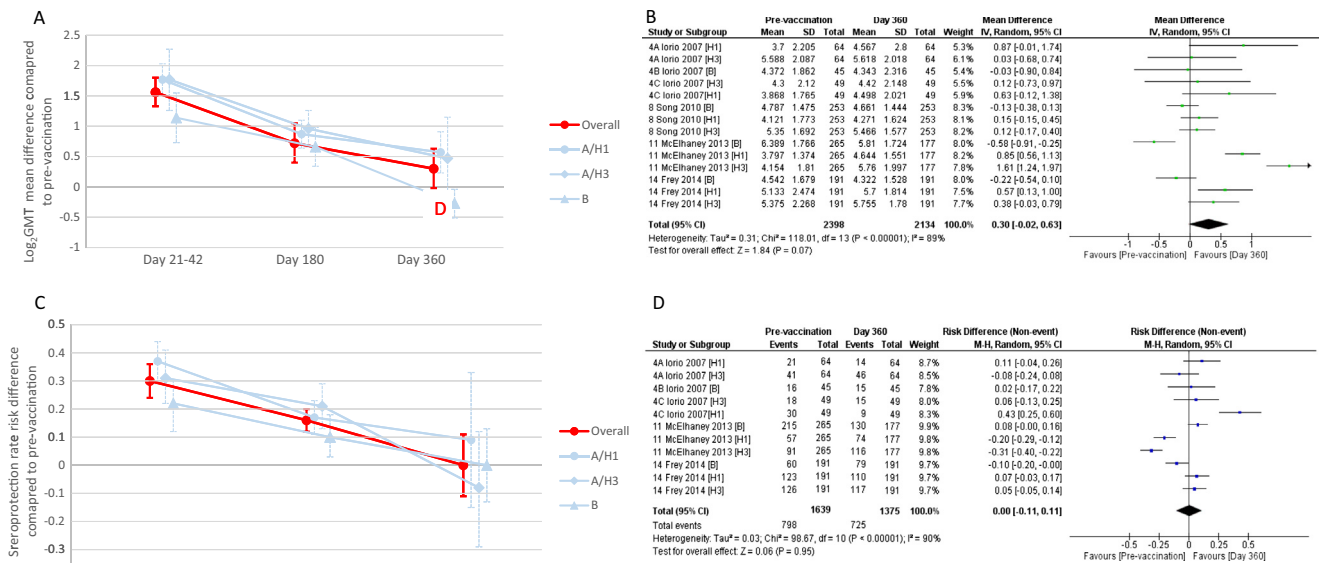


Fig. 2. Overall and sub-group analysis by influenza subtype of mean difference in log₂ GMT and seroprotection pre- and post- vaccination at each time point (A + C), and the forest plots for Day 360 (B + D).

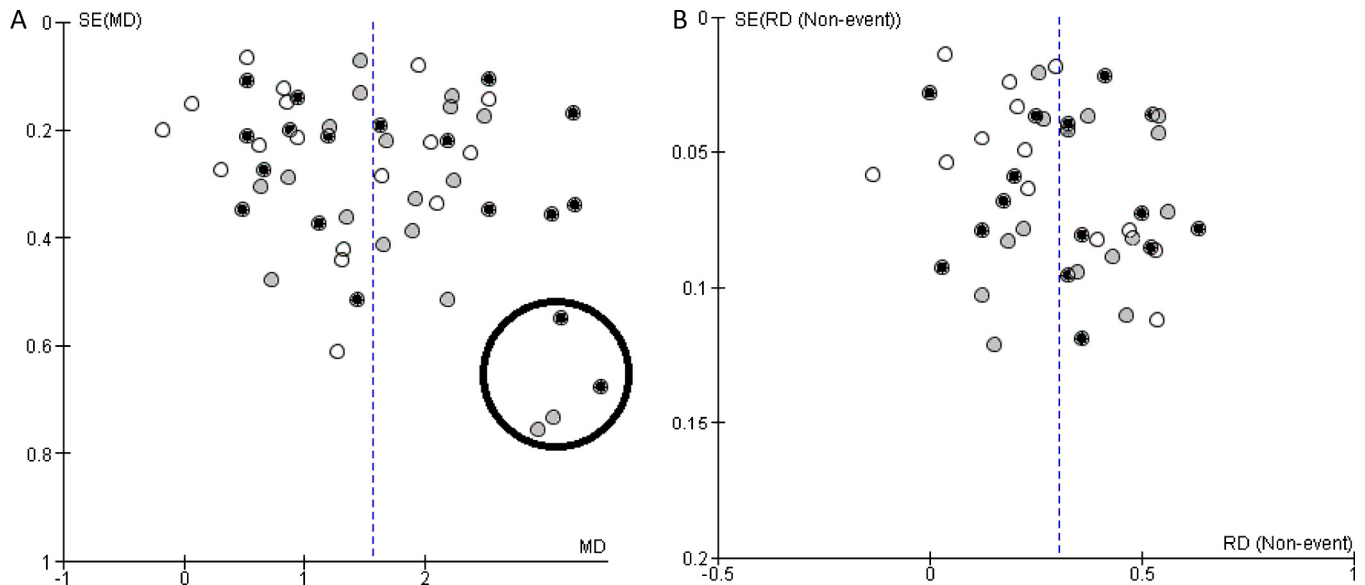


Fig. 3. Funnel plots at Day 21–42 versus pre-vaccination for (A) mean difference (MD) in \log_2 GMT and (B) risk difference (RD) in seroprotection. Ringed outliers are from study 13 (Tinoco et al.). Grey-filled circles represent subtype A/H1, Black A/H3, White B. Dashed line is the effect estimate.

quality. Despite heterogeneity in vaccine responses initially, study results were consistent by Day 360. This assessment was considered as only moderate-quality evidence because of problems with incomplete reporting of outcome data following subject dropout. The quality of evidence for the effect size estimates of the change in GMT and seroprotection compared between pre-vaccination at Day 21–42 and Day 180 were assessed as low due to heterogeneity.

4. Discussion

This meta-analysis finds robust evidence to support the conclusion that in the elderly older than 65 years, haemagglutination-inhibition (HI) antibody responses following influenza vaccination does not reliably persist year-round. The findings were consistent between the Bayesian model and the meta-analysis, and consistent when using seroprotection ($\text{HI} \geq 1:40$) or GMT as the endpoint. Overall, an exponential decay in antibody titres was evident from Day 21–42 to Day 360 after vaccination, as reflected in a linear decline in the estimates of mean difference in \log_2 GMT. By Day 360, GMTs and the proportion of subjects seroprotected were not significantly different from pre-vaccination levels, and for seroprotection, below the CBER licensing threshold.

There is a lack of clinical studies on vaccine efficacy or effectiveness from elderly populations in tropical countries to confirm the clinical significance of this finding. However, the immunological evidence presented here suggests that the elderly in countries with biannual and year-round influenza epidemics would benefit from an alternative to the currently recommended annual standard dose trivalent inactivated influenza virus (IIV3). Newer vaccine formulations which include an immunological adjuvant - MF59 or AS03 - or a higher antigen dose are more immunogenic than standard IIV3 [49,50]. Over a winter season these have been reported to reduce infection rates compared with IIV3 in double-blind RCTs, but it is not clear if this would translate into longer term benefits [37,51]. For IIV3 with adjuvant, serological outcome data is mixed at Day 180, with reports of significantly higher HI titres compared to standard IIV3 in some studies [40,44,46]. Any serological benefit, however, does not appear to persist to Day 360 [24,37,43]. Antibody persistence data beyond Day 180 is not currently available for the high dose vaccine. An alternative approach to changing

the formulation may be to vaccinate more regularly – for example before each season in countries with biannual epidemics. The outcomes of such a strategy has not been investigated.

Despite the established use of a HI titre of $\geq 1:40$ to define seroprotection, this threshold is associated with only 40–70% protection from infection in the elderly and younger adults [52,53]. Using this threshold will underestimate the changes in proportion of subjects who become susceptible to infection when GMTs decline. Coudeville et al. developed a protection curve to model the probability of protection at different HI titres [54]. As the HI titre increases from 1:40 to 1:100, the probability of protection increases to approximately 90%, with marginal benefits beyond this. Thus, even if vaccine responses meet the ‘seroprotection’ cut-off of 1:40 at Day 180, decay in titres in the elderly may result in a substantial proportion of the population becoming susceptible to infection, including before the end of a seasonal epidemic in temperate countries. Clearly, if following seroconversion significant susceptibility to infection develops by Day 180, this is only likely to increase further by Day 360 and offer inadequate efficacy with an annual vaccination strategy for elderly individuals in the tropics.

The persistence of other immune responses to vaccination also needs to be considered. Microneutralization (MN) assays may be a more accurate measure of humoral immunity than the HI assay, and MN titres also correlate with protection against influenza infection [55,56]. Cell-mediated immunity reduces the severity of disease after infection, and while this is only weakly induced by IIV3 it may still have a clinically significant role [57]. Vaccine-induced T-cell responses have been reported to be better correlates of protection than HI titres in the elderly [58]. This review did not seek to identify studies which explored persistence of these immune responses in the elderly after influenza vaccination, but this would be useful to investigate.

There was substantial variability in baseline GMTs, seroprotection levels, and the magnitude of vaccination responses between studies. Some of this variability is influenza strain dependent, and reflects the immunogenicity of individual strains, and host immunological memory from previous infection or vaccination exposure. The influence of pre-existing immunity on vaccine responses is complex. An observational study of mainly younger adults and children suggested interference in vaccine responses

from previous vaccination [59]. However, a meta-analysis identified high pre-vaccination titres and previous vaccination as predictors of higher vaccine responses [60]. Influenza vaccination rates in the year prior to study enrolment were high in those studies that reported this (70–100%), while information on recent influenza infections were not available.

Influenza independent mechanisms which will affect vaccine response in the elderly include participant health, concomitant medications, and in particular the impact of immunosenescence. RCTs generally excluded participants with unstable health conditions or immunosuppression due to the presence of specific comorbidities or treatment with cytotoxic chemotherapy or high-dose corticosteroids. Most studies also recruited volunteers from the community rather than long-term care facilities. It is not clear if the prevalence and severity of common chronic diseases such as diabetes mellitus and chronic obstructive pulmonary disease in recruited subjects is similar to the general population. While these conditions are associated with increased morbidity and mortality after influenza infection, their impact on vaccine responses is less certain [61–63]. In a study of antibody persistence after vaccination among subjects ≥ 50 years neither age nor presence of chronic disease were significantly associated with maintenance of seroprotection at 8 months [64]. Song et al. also did not find the presence of chronic diseases as a significant risk factor for reduced antibody persistence at 12 months [4].

Immunosenescence describes the complex process of immune dysregulation that develops with aging, but defining immunosenescence by a single variable – age – as is traditional in vaccination guidelines, is an over-simplification [65]. The pace of immunosenescence development is the result of numerous interactions between host genetic and environmental processes which also includes biological sex, nutritional status, chronic stress, chronic infection, and other co-morbidities. An important systemic cause and consequence of immunosenescence may be the chronic release of inflammatory cytokines such as TNF, IL-1 and IL-6 [66]. This syndrome of ‘inflammaging’ correlates with progression of a number of chronic diseases and contributes to functional decline and frailty in the elderly. *In vitro* studies have also implicated inflammaging as associated with reduced influenza vaccine response [67].

It is debatable what age cut-off is most appropriate for this review – the older adult immunogenicity criteria used by the FDA apply when age is ≥ 65 years, while for the EMA the cut-off is >60 years. A number of studies were excluded from this review because they enrolled younger subjects. Lowering the inclusion criteria to >60 years would probably not have significantly altered its findings, although we can expect that vaccine responses would have been better and more durable with the addition of younger individuals. Deeper understanding of the impact of age and other risk factors for immunosenescence was beyond the scope of this review, but could be further explored if patient level data were available.

The findings of this meta-analysis were consistent when analysed by GMT or seroprotection, and in both the overall analysis or by influenza subtypes. The variability between subjects and studies places limits on applying the estimates of the magnitude of changes in GMT and seroprotection to individual patients or influenza strains. Some of this variability is mitigated by the matched design of the meta-analysis, with control and experimental outcomes measured in the same population. There was a significant loss of participants by Day 360 in all the studies included in the meta-analysis. Reasons for dropout were generally not available, but participant morbidity and mortality is likely to be an important contributor. This is likely to bias study results towards more sustained immune responses than the general elderly population.

Identification of studies in this review may have missed relevant studies, through human error, imperfections in the literature databases or search methodology, or a failure to identify unpublished studies. As relatively few studies included evaluable data at Day 360, this could impact findings of the statistical analysis. However, more than 2000 individual comparisons were available for analysis at Day 360, and so new data is less likely to impact the overall trend of antibody decline after seroconversion.

In conclusion, the decline from Day 21–42 to 360, in GMTs and the proportion of subjects seroprotected suggests that clinical protection is not likely to persist year-round in the elderly. Confirmation of this finding with studies of vaccine effectiveness is necessary, but identifying alternative vaccination schedules which provide year-round protection against influenza infection is likely to offer substantial public health benefit, particularly in tropical countries with year-round virus activity.

Conflicts of interest

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2016.11.013>.

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