Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies

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See Comment page 874

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Summary

Background Influenza vaccine effectiveness (VE) can vary by type and subtype. Over the past decade, the test-negative design has emerged as a valid method for estimation of VE. In this design, VE is calculated as 100%×(1−odds ratio) for vaccine receipt in influenza cases versus test-negative controls. We did a systematic review and meta-analysis to estimate VE by type and subtype.

Methods In this systematic review and meta-analysis, we searched PubMed and Embase from Jan 1, 2004, to March 31, 2015. Test-negative design studies of influenza VE were eligible if they enrolled outpatients on the basis of predefined illness criteria, reported subtype-level VE by season, used PCR to confirm influenza, and adjusted for age. We excluded studies restricted to hospitalised patients or special populations, duplicate reports, interim reports superseded by a final report, studies of live-attenuated vaccine, and studies of prepandemic seasonal vaccine against H1N1pdm09. Two reviewers independently assessed titles and abstracts to identify articles for full review. Discrepancies in inclusion and exclusion criteria and VE estimates were adjudicated by consensus. Outcomes were VE against H3N2, H1N1pdm09, H1N1 (pre-2009), and type B. We calculated pooled VE using a random-effects model.

Findings We identified 3368 unduplicated publications, selected 142 for full review, and included 56 in the meta-analysis. Pooled VE was 33% (95% CI 26–39; I^2=44·4) for H3N2, 54% (46–61; I^2=61·3) for type B, 61% (57–65; I^2=0·0) for H1N1pdm09, and 67% (29–85; I^2=57·6) for H1N1; VE was 73% (61–81; I^2=31·4) for monovalent vaccine against H1N1pdm09. VE against H3N2 for antigenically matched viruses was 33% (22–43; I^2=56·1) and for variant viruses was 23% (2–40; I^2=55·6). Among older adults (aged >60 years), pooled VE was 24% (–6 to 45; I^2=17·6) for H3N2, 63% (33–79; I^2=0·0) for type B, and 62% (36–78; I^2=0·0) for H1N1pdm09.

Interpretation Influenza vaccines provided substantial protection against H1N1pdm09, H1N1 (pre-2009), and type B, and reduced protection against H3N2. Vaccine improvements are needed to generate greater protection against H3N2 than with current vaccines.

Funding None.

Introduction

Influenza vaccines are licensed on the basis of findings from immunogenicity studies or randomised clinical trials (RCTs) showing efficacy and safety. In a previous meta-analysis[1] of RCTs in healthy adults, we found that pooled vaccine efficacy was 59% against all strains. Although the RCT is the optimal design to minimise bias and confounding, it has important limitations. RCTs are often limited to one or two seasons, enrol healthy individuals, have low power to measure efficacy by subtype, and are not feasible to do annually. Placebo-controlled trials are not ethical in populations for whom vaccination is routinely recommended, and results from a single season might not predict efficacy in subsequent seasons.

Over the past decade, the test-negative design (TND) has emerged as a valid approach for estimation of influenza vaccine effectiveness (VE). In this design, VE is calculated as 100%×(1−odds ratio [OR]) for vaccine receipt in influenza cases versus test-negative controls. The first TND study[2] was published in 2005 by Canadian investigators who reported VE in British Columbia during the 2004–05 season. Since then, multiple TND studies have been done to estimate VE in both the northern and southern hemisphere. The TND is similar to a case-control study, but cases and controls are not identified at the time of enrolment. Instead, patients seeking medical care for an acute respiratory illness are enrolled and respiratory tract samples tested for influenza with RT-PCR. Findings from TND simulation studies[3,4] suggest that this method yields a valid estimate of VE in the source population under most scenarios.

Investigators of an increasing number of TND studies are reporting VE estimates separately by type and subtype. We did a systematic review and meta-analysis of published TND studies to estimate seasonal VE against illness caused by H3N2, H1N1pdm09, H1N1 (pre-2009), and type B.

Methods

Search strategy and selection criteria

In this systematic review and meta-analysis, published studies were eligible for inclusion if they met all of the following criteria: original analysis of influenza VE with the

Research in context

Evidence before this study

In March, 2014, we did an informal review of the literature by searching PubMed for original reports of influenza vaccine effectiveness (VE) published in English from 1990 to 2013. We restricted our review to studies that reported single-season VE against H3N2, H1N1, H1N1pdm09, or type B. To minimise potential bias, we further restricted our preliminary review to studies using the test-negative design with outpatient recruitment based on predefined criteria, those that had confirmation of influenza with RT-PCR or culture, and those that had age adjustment. We identified 43 publications that met these criteria, leading to a decision to do a formal meta-analysis. Our preliminary review indicated that the earliest test-negative design study of influenza VE was conducted in 2004–05, and the formal meta-analysis was therefore restricted to the period from Jan 1, 2004, to March 31, 2015.

Added value of this study

Findings from this study show substantial variation in VE across influenza types and subtypes. Influenza vaccine provided moderate to high protection against H1N1pdm09, H1N1 (pre-2009), and type B, and substantially lower protection against H3N2. Differences across age groups were minimal for H1N1pdm09 and type B. VE against H3N2 was highest in paediatric age groups and lowest in older adults. VE against H3N2 was low regardless of reported antigenic match, but this comparison was limited by the absence of standardised antigenic characterisation and information about antigenic distance. In this systematic review and meta-analysis, we found that relevant information about patient recruitment, symptom eligibility, and vaccine ascertainment was inconsistently reported, and we have made recommendations to optimise VE methods in the outpatient setting. These recommendations are consistent with draft recommendations being developed by WHO.

Implications of all the available evidence

H3N2 is associated with higher morbidity and mortality than are other subtypes, and vaccine improvements are needed to generate greater protection against H3N2 than against other subtypes. Alternatives to egg-based manufacturing should be pursued since egg-induced mutations in H3N2 vaccine strains contribute to antigenic mismatch.

test-negative design; used RT-PCR to confirm influenza; reported VE (or corresponding OR) for one or more individual seasons against H3N2, H1N1, H1N1pdm09, or type B; recruited patients on the basis of predefined illness criteria; and reported results from age-adjusted logistic regression models or age-stratified VE estimates. We excluded the following types of studies: studies restricted to hospitalised patients or special populations (eg, chronic care or military), duplicate reports, interim reports superseded by a final report, studies of live-attenuated vaccine, and studies of prepandemic seasonal vaccine against H1N1pdm09. We accepted studies that enrolled both outpatients and inpatients because hospitalised patients represent a small proportion of medically attended influenza and VE estimates from these studies should largely reflect outpatient illness. To substantiate this assumption, we did a secondary analysis that excluded studies with combined outpatient and inpatient enrolment.

A preliminary review of the literature showed that the first study[2] of influenza VE using the TND was published by Canadian investigators in 2005. We contacted the authors of this study and they confirmed that they did originally develop the TND for influenza VE evaluation (Skowronski D, British Columbia Centre for Disease Control, personal communication). We searched MEDLINE (PubMed) and Embase from Jan 1, 2004, to March 31, 2015, for articles on influenza vaccine efficacy and effectiveness, published in English, Spanish, French, or German. The search was implemented on three dates: Oct 7, 2014, Jan 22, 2015, and April 17, 2015. For PubMed, the following terms were searched in various combinations within titles, abstracts, and medical subject headings: "influenza", "vaccines", "effectiveness", "treatment outcome", and "case-control studies". The specific PubMed search syntax is shown in the appendix. We used the same search terms for Embase. Additionally, we searched for publications by selected investigator groups who have published influenza VE studies. The search did not include conference abstracts or unpublished studies because detailed methods were needed to assess study eligibility. The search strategy was reviewed by experienced librarians at the Marshfield Clinic Research Foundation (Marshfield, WI, USA) and University of Minnesota (Minneapolis, MN, USA). Titles and abstracts were independently reviewed by two authors to identify publications that potentially met the eligibility criteria and required full review. Discrepancies in article eligibility were adjudicated by consensus. Selected articles were independently reviewed by two abstractors.

See Online for appendix

Data analysis

We abstracted data for eligibility criteria, study characteristics, and VE estimates using a structured electronic data collection form. Discrepancies in VE estimates and inclusion and exclusion criteria were adjudicated by consensus. Discrepancies in non-essential data were reviewed and corrected by one author (EAB). Abstracted study characteristics were study season, hemisphere, country, report type (final or interim), patient recruitment method, enrolment setting (outpatient only or combined outpatient and inpatient), respiratory sample type, maximum interval from illness onset to sample collection, source of vaccination data, exclusion of individuals vaccinated less than 14 days before illness onset, and

	n (%)
Hemisphere	
Northern	45 (80%)
Southern	11 (20%)
Continent	
Europe	23 (41%)
North America	19 (34%)
Australia	10 (18%)
Asia	3 (5%)
Africa	1 (2%)
Publication year	
2007	1 (2%)
2009	2 (4%)
2010	1 (2%)
2011	14 (25%)
2012	5 (9%)
2013	10 (18%)
2014	16 (29%)
2015*	7 (13%)
Report type	
Interim	8 (14%)
Final	48 (86%)
Recruitment method	
Research staff	8 (14%)
Physicians	47 (84%)
Not specified	1 (2%)
Enrolment setting	
Outpatient only	45 (80%)
Outpatient and inpatient	11 (20%)
Respiratory sample type	
Nasal or nasopharyngeal swab	13 (23%)
Oral swab	1 (2%)
Combined nasal and oral	17 (30%)
Other	12 (21%)
Not specified	13 (23%)
Maximum swab interval†	
<5 days	7 (13%)
5 days	3 (5%)
7 days	30 (54%)
>7 days	5 (9%)
Not specified	11 (20%)
Source of vaccination data	
Self-report	9 (16%)
Medical records	19 (34%)
Both	10 (18%)
Not specified	18 (32%)
	(Table 1 continues in next column)

	n (%)
(Continued from previous column)	
Exclusion of patients vaccinated <14 days before onset	
Yes	48 (86%)
No	5 (9%)
Not specified	3 (5%)
Calendar time adjustment	48 (86%)
Other covariates included or assessed‡	
Comorbidity	38 (68%)
Sex	26 (46%)
Geographical location	25 (45%)
Previous season vaccination	6 (11%)
Swab interval	16 (29%)

*Studies published up to March 31, 2015. †Maximum interval from illness onset to sample collection. ‡Among 55 publications that reported model covariates.

Table 1: **Characteristics of 56 published studies that reported type-specific or subtype-specific influenza vaccine effectiveness using the test-negative design**

antigenic characterisation for H3N2. We abstracted analytic methods, including adjustment for calendar time, restriction of the analysis to periods of local influenza circulation, and assessment or inclusion of potential confounders. We abstracted VE estimates and 95% CIs for H3N2, H1N1pdm09, H1N1, and type B; we assessed monovalent VE against H1N1pdm09 for the 2009–10 pandemic. We abstracted the number of cases, vaccinated cases, controls, and vaccinated controls. If missing, this information was often provided by study authors. We preferentially abstracted VE estimates on the basis of the entire population rather than of a target population for maximum comparability across studies because VE on the basis of the entire population was reported in nearly all studies and the definition of target population was variable.

We abstracted information about antigenic match for studies reporting VE against H3N2. A virus is considered vaccine like if the haemagglutination inhibition titre is within fourfold of the homologous titre against the reference vaccine strain.[5] However, considerable test-to-test variability exists, and haemagglutination inhibition assay methods are not standardised. For most studies, antigenic characterisation methods were not reported and antigenic similarity was simply categorised as a binary variable (antigenic match or antigenic variant). We therefore classified H3N2 viruses as predominately matched, predominately variant, or mixed on the basis of the authors' interpretation. We also included analyses based on genetic sequencing of viruses if viral clades were used as surrogates for antigenic groups. We restricted this analysis to antigenic data from viruses obtained from study participants rather than from national surveillance samples.

We defined influenza VE as the relative reduction in the odds of laboratory-confirmed, medically attended influenza after vaccination: 100×(1−adjusted OR) for vaccine receipt in cases (influenza positive) versus controls (influenza negative). We did a separate meta-analysis for each outcome: H3N2, H1N1pdm09 (seasonal vaccine and monovalent vaccine), H1N1 (pre-2009), and type B. We assessed heterogeneity among studies using the χ^2-based Q test and I^2 statistic.[6] We used a simple random-effects

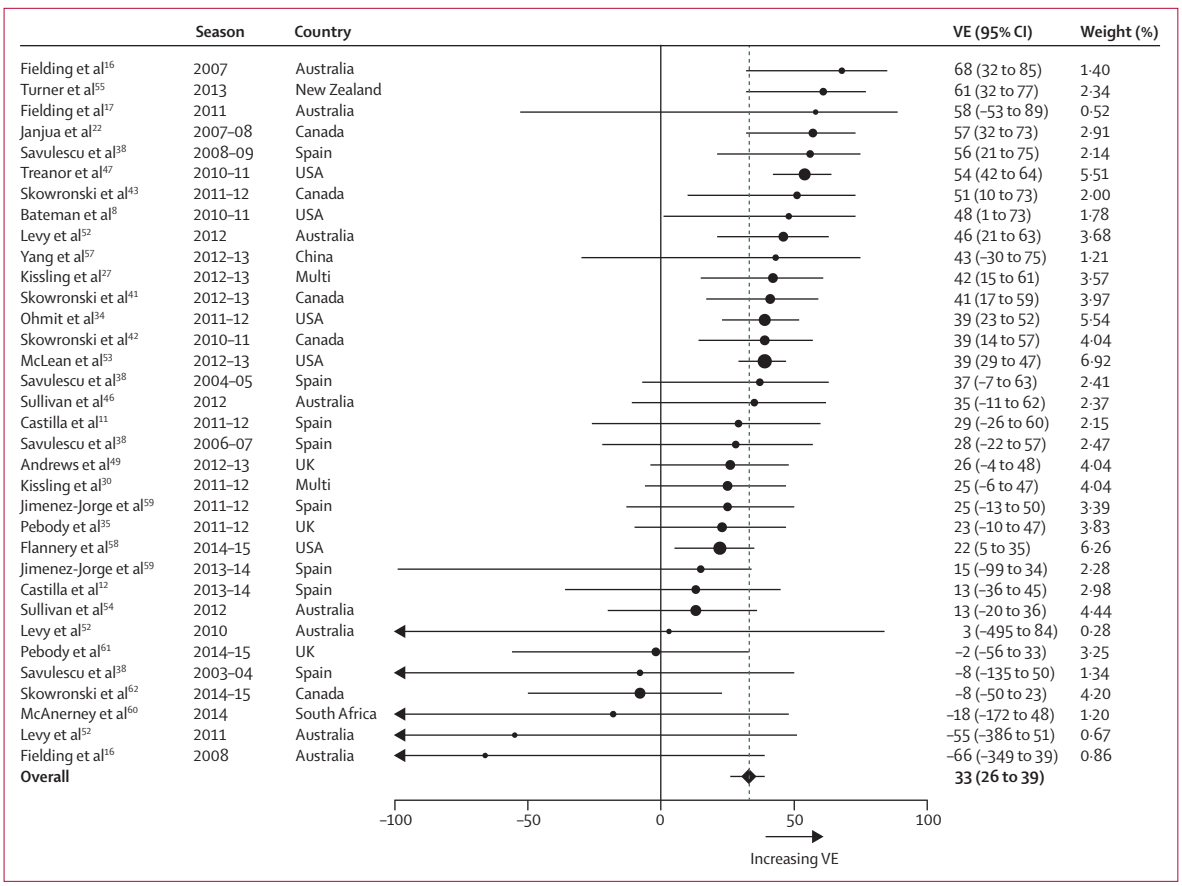

Figure 1: **VE for H3N2 in studies without age restriction**
The numbers of cases and controls for each VE estimate are provided in the appendix. VE=vaccine effectiveness.

model to calculate the weighted pooled log OR, 95% CI, and corresponding VE.[7] We used inverse variances that incorporated an estimate of the between-study variance to calculate the weights for the model.[6,7] We used funnel plot regression to assess publication bias. We did all analyses with SAS version 9.4.

Our primary analysis included studies that enrolled patients with no age restriction beyond infancy. We also did analyses stratified by age group: paediatric (any age group <20 years old), older adults (any age group >60 years), and working-age adults (any age group 20–64 years old). We did secondary analyses of pooled VE by season and antigenic match for H3N2 viruses. For the season analysis, we grouped each southern hemisphere season with the preceding northern hemisphere season.

We did a sensitivity analysis by calculating pooled VE for the highest-quality studies with the least potential for bias and confounding. These studies met all of the following criteria: restricted to outpatient setting, illness onset to swab interval 7 days or less, medical record confirmation of all vaccinations, exclusion of patients vaccinated within 14 days before illness onset, and calendar time included as a covariate in the logistic regression model. We repeated the analysis of high-quality studies using less restrictive criteria than these ones for confirmation of vaccination status (ie, a combination of medical records and self-report).

Role of the funding source

There was no funding source for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We identified 3368 unduplicated publications, selected 142 for full review, and included 56 that met eligibility criteria in the meta-analysis (appendix).[8–63] Most studies originated in the northern hemisphere, with a similar number of studies originating from Europe and North America (table 1). The earliest eligible study was published in 2007,[16] and 52 (93%) were published after 2010. 11 (20%) studies enrolled both outpatients and inpatients, eight (73%) of which adjusted for enrolment location (outpatient *vs* inpatient). Of the 45 (80%) studies that specified a maximum interval from illness onset to sample collection, 40 (89%) restricted the analysis to patients who were swabbed within 7 days of illness onset.

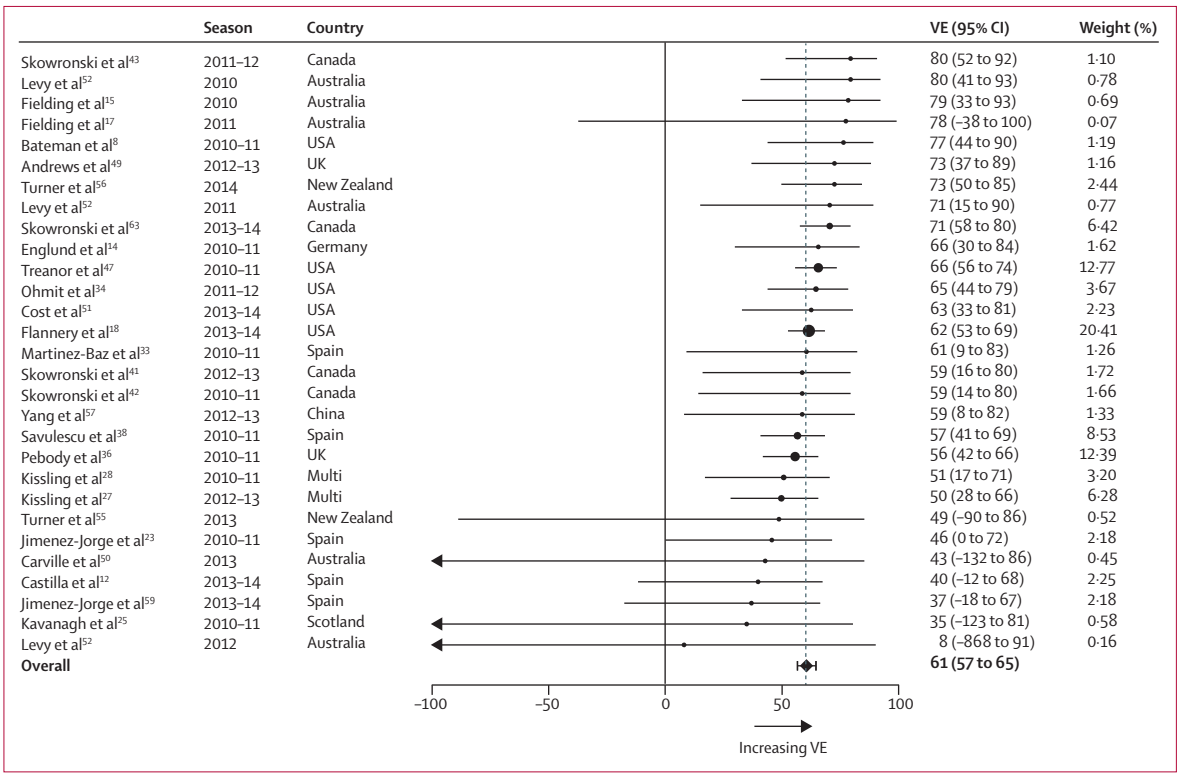

Figure 2: **VE for H1N1pdm09 (seasonal vaccine) in studies without age restriction**
The numbers of cases and controls for each VE estimate are provided in the appendix. VE=vaccine effectiveness.

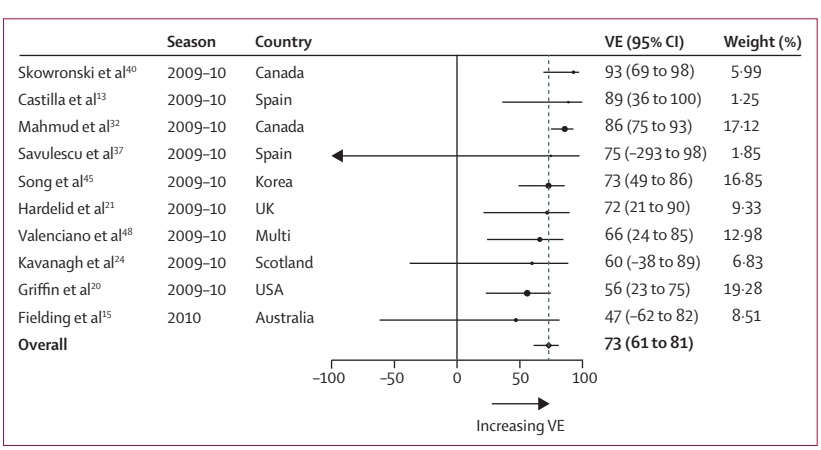

Figure 3: **VE for H1N1pdm09 (monovalent vaccine) in studies without age restriction**
The numbers of cases and controls for each VE estimate are provided in the appendix. VE=vaccine effectiveness.

The 56 publications reported 114 VE estimates based on unrestricted age enrolment, consisting of 34 (30%) for seasonal vaccine against H3N2, 36 (32%) against type B, 29 (25%) against H1N1pdm09, five (4%) against H1N1 (pre-2009), and ten (9%) for monovalent vaccine against H1N1pdm09 (figures 1–4). Additionally, we found 33 VE estimates for paediatric age groups, 28 for working-age adults, and 13 for older adults. A list of all included VE estimates is provided in the appendix.

In the age-unrestricted analysis, we found high heterogeneity for VE against H3N2 (p=0·005; I^2=44·4) and type B (p<0·0001; I^2=61·3) and low heterogeneity for VE against H1N1pdm09 (p=0·783; I^2=0·0). The high heterogeneity against type B was driven by a single outlier study,[31] and heterogeneity was not significant (p=0·598) when this study was excluded. Funnel plot regression analysis showed no evidence of publication bias for VE estimates stratified by type or subtype. Egger's p values were 0·5 for H3N2, 0·08 for type B, 0·2 for H1N1pdm09 (seasonal vaccine), and 0·7 for H1N1pdm09 (monovalent vaccine).

Pooled VE estimates were significant with lower confidence limits of more than 0 for each type or subtype in the age-unrestricted analyses. Pooled VE was highest (73% [95% CI 61–81]) for monovalent vaccine against H1N1pdm09 and lowest (33% [26–39]) against H3N2 (table 2). Seven (70%) of ten monovalent vaccine studies were based on adjuvanted H1N1pdm09 vaccine,[13,21,32,37,40,45,48] and pooled VE was higher for the adjuvanted vaccine studies (79% [68–86]) than for the three non-adjuvanted pandemic vaccine studies (55% [28–72]). In the age-stratified analyses, pooled VE against H1N1pdm09 and type B exceeded 50% and was similar across age groups (table 3). Pooled VE against H3N2 was highest in paediatric age groups and lowest in older adults. The VE CI included 0 for monovalent H1N1pdm09 vaccine in paediatric age groups and seasonal H3N2 in older adults.

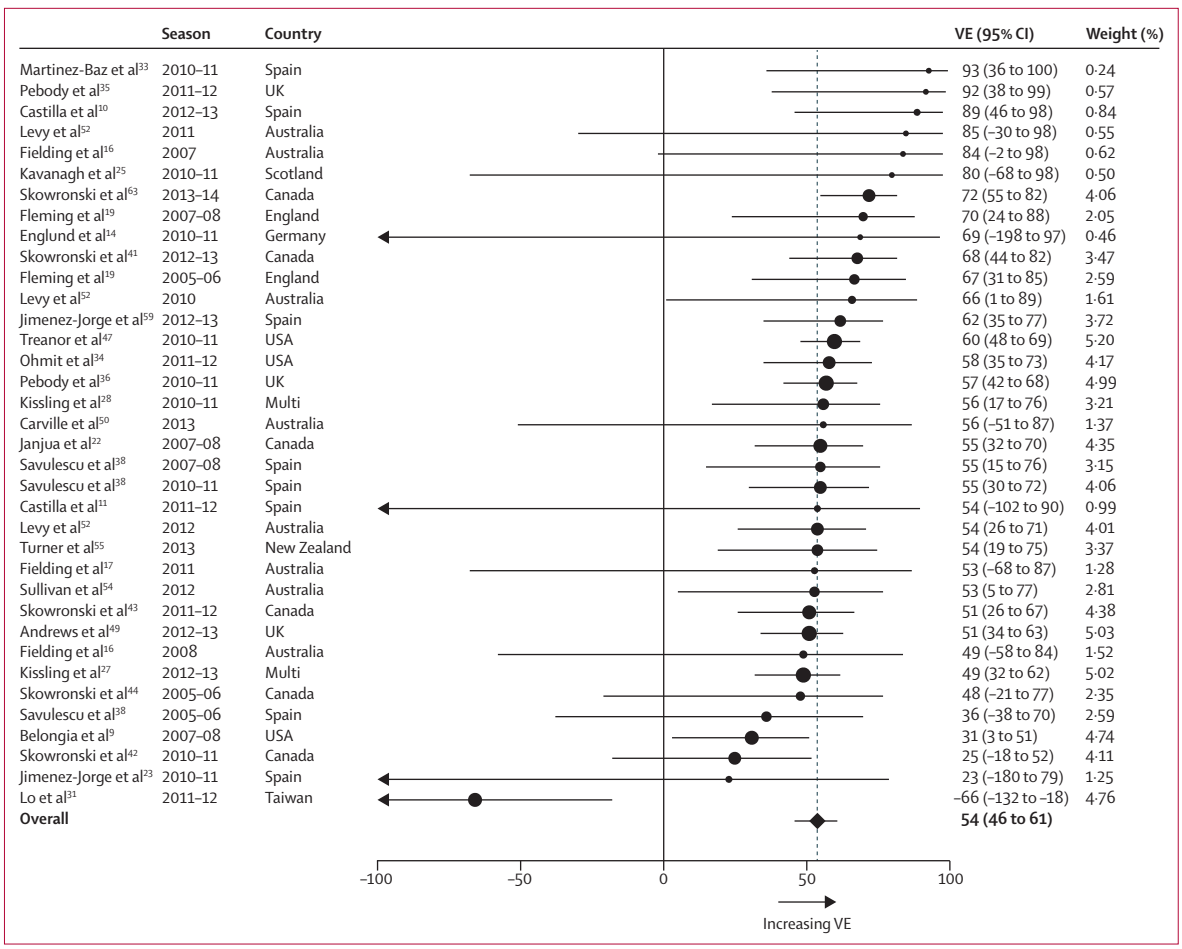

Figure 4: **VE for type B in studies without age restriction**
The numbers of cases and controls for each VE estimate are provided in the appendix. VE=vaccine effectiveness.

Pooled VE against H1N1pdm09 and type B was relatively stable across four to five seasons, but VE against H3N2 was 32–46% before 2013–14, and 10% or less in the subsequent two seasons (table 4). In a secondary analysis restricted to studies that enrolled patients exclusively in the outpatient setting, pooled VE estimates were nearly identical to overall pooled VE for each type or subtype (data not shown).

The sensitivity analysis was restricted to the small number of high-quality studies that met stringent quality criteria.[34,53,55,56] This analysis included three VE estimates for H3N2 and three for seasonal vaccine against H1N1pdm09. Pooled VE for high-quality studies was 41% (95% CI 31–50) against H3N2 and 67% (53–77) against H1N1pdm09. We further analysed a larger pool of high-quality studies than the previous pool of studies by using less restrictive criteria for documentation of vaccination status.[15,34,52,53,55,56] These studies included six VE estimates for H3N2, seven for H1N1pdm09, and five for type B. Pooled VE was 41% (32–49) against H3N2, 57% (44–67) against type B, and 69% (58–78) against H1N1pdm09.

Antigenic or genetic characterisation results were reported for 19 (56%) of 34 VE estimates for H3N2 in the primary analysis. 12 estimates for H3N2 viruses were predominately similar to the vaccine reference strain.[35,41,42,46,47,49,52–54,61] We found six VE estimates for variant H3N2 viruses.[11,22,58–60,62] For one of the studies with variant viruses,[59] antigenic results were abstracted from a previous publication[64] by the same authors. Investigators of one additional study[43] reported mixed antigenic similarity and we excluded it from this analysis. Pooled VE was low against both antigenically similar and variant H3N2 viruses (table 4). Investigators of one antigenically similar

	Vaccine type	Pooled VE (%)	Pooled standard error	VE estimates (n)	p value for heterogeneity	I^2
Type B	Seasonal	54% (46–61)	0·083	36	<0·0001	61·3
H3N2	Seasonal	33% (26–39)	0·050	34	0·005	44·4
H1N1pdm09	Seasonal	61% (57–65)	0·048	29	0·783	0·0
H1N1pdm09	Monovalent	73% (61–81)	0·188	10	0·217	31·4
H1N1 (pre-2009)	Seasonal	67% (29–85)	0·397	5	0·093	57·6

Data in parentheses are 95% CIs. VE=vaccine effectiveness.

Table 2: **Pooled VE by type and subtype in studies without age restriction**

	Vaccine type	Pooled VE (%)	Pooled standard error	VE estimates (n)	p value for heterogeneity	I^2
Paediatric age groups*						
Type B	Seasonal	56% (38 to 69)	0·179	11	0·279	24·4
H3N2	Seasonal	43% (28 to 55)	0·119	10	0·251	28·2
H1N1pdm09	Seasonal	69% (49 to 81)	0·253	7	0·054	56·7
H1N1pdm09	Monovalent	62% (−5 to 87)	0·525	3	0·207	56·2
Working-age adults						
Type B	Seasonal	54% (16 to 75)	0·308	7	0·005	70·7
H3N2	Seasonal	35% (14 to 51)	0·146	9	0·078	48·4
H1N1pdm09	Seasonal	73% (52 to 84)	0·290	5	0·159	49·6
H1N1pdm09	Monovalent	74% (44 to 88)	0·391	3	0·852	0·0
H1N1 (pre-2009)	Seasonal	64% (29 to 82)	0·343	4	0·541	3·2
Older adults†						
Type B	Seasonal	63% (33 to 79)	0·295	3	0·989	0·0
H3N2	Seasonal	24% (−6 to 45)	0·166	6	0·416	17·6
H1N1pdm09	Seasonal	62% (36 to 78)	0·267	3	0·906	0·0

VE=vaccine effectiveness. *Pooled VE was not calculated for two studies reporting VE against H1N1 (pre-2009) in paediatric age groups. †One VE estimate for monovalent vaccine in older adults is not shown.

Table 3: **Pooled vaccine effectiveness in paediatric age groups, working-age adults, and older adults**

	Pooled VE (%)	Pooled standard error	VE estimates (n)*	p value for heterogeneity	I^2
H3N2 by season					
2010–11	46% (30 to 58)	0·131	5	0·368	26·1
2011–12	32% (23 to 40)	0·063	9	0·626	0·0
2012–13	40% (32 to 46)	0·059	6	0·644	0·0
2013–14	10% (−25 to 35)	0·164	3	0·913	0·0
2014–15	7% (−32 to 34)	0·179	3	0·051	74·3
H3N2 by antigenic similarity					
Variant	23% (2 to 40)	0·126	6	0·081	55·6
Similar	33% (22 to 43)	0·080	12	0·014	56·1
H1N1pdm09 by season					
2010–11	60% (54 to 65)	0·071	12	0·894	0·0
2011–12	68% (50 to 80)	0·239	3	0·541	7·2
2012–13	55% (41 to 66)	0·142	6	0·930	0·0
2013–14	62% (52 to 70)	0·117	6	0·260	35·2
Type B by season†					
2005–06	52% (25 to 70)	0·231	3	0·648	0·0
2007–08	50% (29 to 64)	0·172	5	0·235	41·2
2010–11	55% (48 to 62)	0·080	11	0·554	0·0
2011–12	49% (0 to 74)	0·343	7	<0·0001	89·7
2012–13	55% (46 to 62)	0·087	7	0·566	0·0

Data in parentheses are 95% CIs. VE=vaccine effectiveness. *Seasons with fewer than three VE estimates for a given subtype were not included. †2009–10 is not shown because only one estimate for type B during that season existed.

Table 4: **Pooled VE estimates by season and reported antigenic similarity of H3N2 viruses to the vaccine strain**

study[61] reported discrepant results between antigenic characterisation and genetic clade for the 2014–15 season. Exclusion of this study yielded a pooled VE of 36% (95% CI 27–45) for antigenically similar viruses.

Discussion

In this systematic review and meta-analysis, we found substantial variation in VE across types and subtypes. In the primary analysis that was not restricted by age, influenza vaccine provided moderate to high protection against H1N1pdm09, H1N1 (pre-2009), and type B, and substantially lower protection against H3N2. Monovalent pandemic vaccine yielded the highest pooled VE estimate. Pooled VE was higher for adjuvanted monovalent vaccines than for non-adjuvanted pandemic vaccines, but the small number of studies limits this comparison. VE against type B and H1N1pdm09 exceeded 50% in every age category. Pooled VE against H3N2 was highest in paediatric age groups and lowest in older adults.

H3N2 seasons are associated with increased influenza morbidity and mortality, and antigenic drift of H3N2 viruses contributes to reduced VE.[65–67] Antigenic drift might also contribute to the high heterogeneity that we observed for VE against H3N2. Pooled VE against H3N2 was only 33% for studies reporting antigenically matched viruses and 23% for those reporting mismatched viruses. This modest difference in VE could reflect the limitations of measurement and reporting of antigenic similarity. Most studies reported a crude measure of antigenic similarity without quantifying the antigenic distance, and methods for establishment of antigenic similarity were not standardised. Antigenic drift can cause a substantial reduction in VE, as shown in the 2014–15 season when there was widespread circulation of H3N2 viruses that were antigenically distinct from the A/Texas/50/2012 vaccine virus. Authors of a study by the US Flu VE Network found that VE against H3N2 was nearly zero for the 3C.2a genetic group viruses that were antigenically drifted and 44% against 3C.3b viruses that were antigenically similar to the vaccine strain.[68] A previous meta-analysis of VE studies using the TND reported pooled VE of 52% for matched viruses and 36% for mismatched viruses during epidemic seasons.[69] However, these results are difficult to interpret because VE was not analysed by type or subtype.

The vaccine manufacturing process can also contribute to low VE against H3N2 by generating egg-induced mutations in the haemagglutinin that affect antigenicity. Before vaccine production, the mammalian cell-passaged reference virus is reassorted and propagated in eggs to generate a high-growth reassortant virus.[70] Mutations that occur during replication in eggs can affect antigenic characteristics,[71] and Canadian investigators reported suboptimal VE that they attributed to egg-induced mutations in the H3N2 vaccine strain during the 2012–13 season.[41] During 2014–15, the same group identified aminoacid mutations in egg-adapted viruses that might have amplified the effect of antigenic drift in circulating viruses.[62] The antigenic similarity between circulating viruses and egg-adapted vaccine viruses has not been routinely reported during most influenza seasons, and this absence of reporting complicates interpretation of antigenic match results.

This systematic review and meta-analysis has several limitations, including few eligible studies being done before 2009 and few VE estimates existing for older adults. Additionally, evidence is increasing that VE might be influenced by vaccines received in previous seasons,[43,72,73] and this factor was not assessed in most studies. We did not assess VE for prevention of serious outcomes such as admission to hospital. We chose to exclude hospital-based studies because of the potential for different VEs in outpatients versus inpatients and the absence of consensus regarding the optimal control group and analytical approach for these studies. Although we included studies with combined outpatient and inpatient enrolment, most of these studies adjusted for enrolment location and our sensitivity analysis did not suggest that VE estimates were biased by including them.

This analysis was also limited by variability in study methods and reporting, despite the restriction to TND studies meeting specific eligibility criteria. Symptom eligibility criteria, recruitment methods, and vaccine ascertainment methods were not adequately reported in many studies, and consistency regarding the specific covariates that were included in the models was low. Our findings are consistent with a review,[74] authors of which found substantial variation in methods across TND studies of influenza VE. However, the major findings of this meta-analysis are unlikely to be due to bias or confounding because the magnitude and direction of any bias should be similar for each influenza subtype, allowing valid comparisons to be made across them. Although few studies met our stringent criteria for quality, the pooled VE from high-quality studies was similar to the overall VE in the primary analysis.

We have identified several factors that can be addressed to optimise VE methods in the outpatient setting and facilitate pooling of VE estimates (panel). Additionally, WHO has developed a draft field guide for evaluation of influenza VE.[75] The guide describes the role of VE for assessment of influenza vaccination programmes and provides a framework for development and implementation of VE studies. This guide will be an important tool for public health and programme assessment, particularly in low-income settings.

In this systematic review and meta-analysis, we have shown that influenza vaccines provide substantial protection against H1N1pdm09, H1N1 (pre-2009), and type B, and reduced protection against H3N2. An accumulating body of evidence suggests that egg-based manufacturing is not optimal for H3N2 influenza viruses that are poorly adapted for growth in eggs. A crucial need exists for alternative vaccine technologies that generate greater protection against H3N2 than do current vaccines, and product-specific VE studies will be needed to assess their effect after licensure. The European Medicines Agency has already embarked on this path by releasing a draft framework[76] that calls for manufacturers to routinely do postlicensure studies to assess product-specific VE. The TND will play a key role in these assessments.

***Panel:* Recommendations for implementation and reporting of influenza vaccine effectiveness studies using the test-negative design**

- Require and report specific symptom eligibility criteria corresponding to influenza-like illness or acute cough illness. VE analyses based on a convenience sample of clinical diagnostic tests could be biased and should be avoided.
- Define and report standard procedures for collection of respiratory samples and RT-PCR testing.
- Restrict enrolment to patients with a duration of illness of 7 days or fewer to minimise misclassification of influenza status.
- Exclude patients vaccinated within 14 days before illness onset because of latent period between vaccination and serological response.
- Report source of vaccination data. Use medical records or registries to confirm vaccine receipt, dates (including previous season vaccination), and manufacturer whenever possible. Describe influenza vaccine manufacturers and products used in the study population.
- Include parameters for age group and calendar time in VE logistic regression models; studies done in multiple sites should adjust for enrolment location. Other potential confounders should be individually assessed to establish whether they change the unadjusted odds ratio by 10% or more, although this threshold is arbitrary and can be adjusted up or down. Covariates that exceed this threshold are potential confounders and should be included in the adjusted model.
- Report VE estimates by type, subtype, and lineage whenever sample size is sufficient. Report age-stratified VE estimates separately for paediatric and older adult age groups.
- Restrict VE analysis to periods of continuous local influenza circulation. One approach is to exclude controls with symptom onset before the week of the first influenza-positive case and those with symptom onset after the week when the last influenza case was identified.
- When previous season vaccination data are available, analyse the independent and combined effect of current and previous season vaccination with classification of vaccine exposure into four groups: vaccinated current season and previous season, vaccinated current season only, vaccinated previous season only, and unvaccinated in both current and previous season (referent group).

Contributors
EAB and HQM designed the study. EAB, JPK, MES, NSK, and HQM screened and abstracted publications. MDS, HQM, and EAB analysed data. EAB wrote the manuscript, with editorial contributions from MTO and HQM. All authors reviewed the manuscript for accuracy and scientific content.

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