The Efficacy of Influenza Vaccine for Healthy Children

A Meta-Analysis Evaluating Potential Sources of Variation in Efficacy Estimates Including Study Quality

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Background: Two systematic reviews evaluating influenza vaccine efficacy in healthy children have recently been published. Although quantitative summary estimates were similar, authors' conclusions were quite contrasting. We carried out another meta-analysis reevaluating study inclusion criteria and using metaregression techniques in addition to sensitivity and subgroups analyses to evaluate potential sources of heterogeneity of efficacy estimates, including methodologic quality of studies.

Methods: Only randomized clinical studies assessing the efficacy of influenza vaccine in healthy children/adolescents (age ≤18 years) for preventing naturally occurring influenza and/or acute otitis media cases were included. Summary estimates of effect were obtained using a random effects model. The methodologic quality of each study was assessed using 3 systems: Chalmers scale, Jadad scale and Schulz components (randomization, allocation concealment and double-blinding).

Results: The overall vaccination efficacy was 36% (95% confidence interval: 31–40%) against clinically diagnosed illnesses (evaluated by 19 randomized clinical studies for a total of 247,517 children); 67% (51–78%) against laboratory-confirmed cases (18 trials, n = 8574); and 51% (21–70%) against acute otitis media (11 trials, n = 11,349). Significant sources of between-study heterogeneity were participants' age and study quality both directly correlated with the efficacy. When the analysis was performed excluding USSR studies, the overall efficacy of the vaccine in preventing clinical cases substantially increased (from 36% to 61%).

Conclusions: These findings may indicate that the vaccine efficacy might be greater than the overall estimates. Although no safety and cost considerations are addressed in this analysis, the present findings support vaccination as a possible option for the prevention of influenza in healthy children and adolescents.

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The prevention of influenza epidemics has been centered on vaccination for several decades, and immunization is currently recommended worldwide for the elderly and individuals with specific chronic disorders.[1] Recently, the American Academy of Pediatrics and the U.S. Centers for Disease Control and Prevention Advisory Committee on Immunization Practices recommended routine influenza immunization for healthy children aged 6 to 23 months with the aim of reducing influenza incidence and mortality of elderly people in families with children.[2,3] However, this action needs to be supported by decision analysis processes, which require reliable data on the safety, efficacy and costs of influenza vaccination in the pediatric population.[4,5]

Two systematic reviews evaluating influenza vaccine efficacy in healthy children have recently been published.[6,7] Although quantitative summary estimates were similar, authors' conclusions were quite contrasting. One group focused on the "striking difference between efficacy and effectiveness," expressing skepticism on universal pediatric immunization as a public health policy and concluding that "no convincing evidence that vaccines can reduce mortality, admissions, serious complications, and community transmission of influenza was recorded."[6] The other group stated that "in terms of public health implications, even a 30% reduction in clinical influenza among children has important relevance," but conclusions on infants younger than 2 years were avoided because "available data were too scanty to allow meaningful analyses in separate age-groups."[7] The process toward a steadfast conclusion is also complicated by the fact that these meta-analyses differed on several relevant issues, the most important of which is the selection of studies for inclusion.[6,7] Finally, although significant heterogeneity between trial results was found in both meta-analyses, potential sources of heterogeneity were not fully investigated.

We carried out another meta-analysis on the efficacy of influenza vaccination for healthy children/adolescents, carefully reassessing studies' inclusion criteria and examining all the evidence available from previous researches. Furthermore, we used metaregression techniques, in addition to

sensitivity and subgroups analyses, to evaluate potential sources of heterogeneity of efficacy estimates, including methodological quality of studies.

METHODS

Bibliographic Search. Trials evaluating influenza vaccine efficacy were initially searched in MEDLINE using the following search strategy: (influenza OR flu) AND vaccin* AND (child* OR pediatric OR pediatric OR adolescent* OR young) as words in the title/abstract from 1966 up to May 2005. Additional searches in EMBASE and the Cochrane Library were carried out using "influenza vaccine" or "influenza vaccination" as search terms, and bibliographies of all relevant articles including reviews and meta-analyses were reviewed for further references. No restriction of language was applied.

Study Selection. A study was included in the present analysis if:

It had a randomized or quasi-randomized design and compared influenza vaccines with placebo, control vaccines or no intervention;
It assessed protection to naturally occurring influenza;
More than 70% of the participants were healthy individuals equal or less than 18 years old; and
It reported sufficient data to estimate vaccine efficacy for the prevention of at least one of the following outcomes: clinically confirmed cases of influenza, laboratory confirmed cases (LCC) of influenza, acute otitis media.

When more than one published report was generated using the same trial data, only the most recent published results were used.

Data Extraction and Quality Assessment. Each included article was independently assessed by 2 reviewers who were blind to authors, institution and journal. The following data were extracted: year, location, randomization method, outcomes definitions; type of control (placebo, other vaccinations or no intervention); type of vaccine (parenteral inactivated or live aerosol vaccine) along with information on vaccine and circulating strains; mean age of patients; and number of clinical cases of influenza, laboratory-diagnosed cases of influenza and cases of acute otitis media in both vaccine and control groups.

Studies were considered randomized when it was specifically stated in the text, although the method of randomization was not adequately described, whereas trials were defined as quasi-randomized when individuals were assigned to alternative forms of treatment using quasirandom methods of allocation such as alternation, date of birth or case record numbers.

To define clinically and LCC of influenza, to assess the compliance of the study vaccine with official recommendations and to evaluate the level of matching between the vaccine used and circulating strains, standard criteria described in a previous meta-analysis on healthy adults were used.[8]

The units of the meta-analysis were single comparisons of one treatment versus control in the influenza season after vaccine administration. Accordingly, when more than one treatment arm was included in the same study, the study was divided into subtrials and the placebo group was equally split between the subtrials. Subtrials were generated also when different years or populations were analyzed in the same study. For the sake of simplicity, studies conducted in the former Soviet Union have been defined in the text as "Russian studies," although some of them have been made in Kazakhstan.[9]

The methodologic quality of each study was assessed using 3 separate and widely used systems: 1) Chalmers scale, addressing several items and ranging from a minimum score of 0 up to 1[10]; 2) Jadad scale, evaluating 3 items pertaining to randomization, masking and dropouts/withdrawals and ranging from 0 to 5[11]; and 3) individual components known to affect estimation of intervention efficacy (Schulz components—randomization, allocation concealment and double-blinding[12]).

Data Analysis. Trials were grouped according to 3 outcomes (clinically confirmed cases of influenza, LCC and cases of acute otitis media [AOM]), and separate meta-analyses were conducted for each outcome. The risk ratios (RRs) with 95% confidence intervals (95% CIs) of vaccinated versus control children were computed for each trial, and summary estimates of effect were obtained using random effects model to account for the between-study variance.[13] The Mantel-Haenszel method (fixed effects model)[14] was also used to check the level of agreement with random effect conclusions. Vaccine efficacy was calculated as $100 \times (1 - \mathrm{RR})$.

Heterogeneity was quantified using I^2 statistic, which can be interpreted as the total variation across studies that is attributable to heterogeneity rather than chance.[15] The association between trial quality and other variables with estimated effects of influenza vaccination was examined by means of metaregression analyses with multiple covariates in addition to sensitivity and subgroups analyses.[16] To reduce potential overfitting and false-positive results, the number of variables included in both final and intermediate models (during modeling) was limited to 3 or 2 depending on the number of studies available for each outcome.[17] Final models were also checked for potential multicollinearity and interactions.

Potential publication bias was assessed using funnel plots (displaying RRs from individual studies versus their precision (1/standard error)[18]) and formally tested through Egger regression asymmetry test[19] and Begg adjusted rank correlation test.[20]

All meta-analyses including sensitivity and subgroups analyses were performed using RevMan software, version 4.2 (Cochrane Collaboration, Oxford, U.K., 2003), whereas metaregressions and tests investigating publication bias were carried out using STATA statistical software, version 8.2 (Stata Corp., College Station, TX, 2003).

RESULTS

Of the 1501 papers retrieved by our search (the complete list of which, with reasons for exclusion, is available from the corresponding author), 21 articles published between 1968 and 2003 met the selection criteria and were included in the meta-analysis.[9,21–40] Eight studies were split

into separate trials[21,26,29,30,32–34,39]; thus, a total of 32 substudies were available for the meta-analyses. The main characteristics and extracted data of each included study are available in the Appendix (online only). In summary, the trials were carried out during the period 1968 through 2000—17 in the United States, 12 in the former Soviet Union, and 3 in other countries (Italy, The Netherlands and United Kingdom). The age of the participants ranged from 6 months to 18 years. More than half evaluated the efficacy of parenteral inactivated vaccines (n = 17); the rest assessed live aerosol vaccines (n = 15). In most trials, the vaccines used complied with official recommendations (n = 24) and matched the circulating strains (n = 20). Clinically confirmed cases of influenza (influenza-like illness) was an outcome in 19 reports (for a total of 247,517 children); laboratory-confirmed cases (influenza cases) were recorded in 18 trials (n = 8574); and finally, 11 comparisons were available for the outcome AOM (n = 11,349).

The mean quality score of the 32 trials using the Chalmers scale was 0.37 (range, 0.13–0.72), whereas the average Jadad score was 2.0. The overall study quality significantly improves with the year of the trial, the most recent having the best score (Spearman rho = 0.67 for Chalmers scale, $P < 0.001$; rho = 0.40 for Jadad scale, $P = 0.024$). Selected items of the quality assessment systems used are presented in Table 1. In brief, with slight differences depending on the 3 approaches, few trials reported adequate methods of randomization (n = 7) or correct techniques of allocation concealment (n = 2) even among the most recent papers. Although 12 trials were originally classified as double-blind according to Schulz criteria, only 7 or 8 of them could be considered double-blind depending on the more stringent criteria of Jadad or Chalmers, respectively. Finally, only 5 studies adequately addressed the issue of withdrawals or dropouts after randomization.

TABLE 1. Frequency Counts and Percentages of 'Adequate' On Selected Quality Items in 32 Randomized or Quasi-Randomized Control Trials (contained in 21 published trials) of Influenza Vaccination in Healthy Children

Quality Items	No. Adequate (%)
Chalmers scale	
Selection description	10 (31)
Number and reasons for eligible patients not included in the study	0 (0)
Regimen definition	30 (94)
Blinding of randomization	2 (6)
Blinding of patients to therapy	17 (53)
Blinding of physicians/observers to therapy	8 (25)
Blinding of physicians/observers to ongoing results	0 (0)
Statistical estimate of sample size	2 (6)
Testing randomization	9 (28)
Testing blinding	0 (0)
Biologic equivalent	30 (94)
Dates of study	22 (69)
Results of prerandomization	3 (9)
Both test statistics and *P* value given	6 (19)
Confidence intervals given	15 (47)
Regression/correlation	10 (31)
Statistical analysis	13 (41)
Number and reasons for patients withdrawn after randomization	5 (16)
Withdrawals handled in several ways	2 (6)
Side effects discussion	20 (62)
Subgroups retrospective analysis	14 (44)
Jadad scale	
Randomization	7 (22)
Double-blinding	7 (22)
Withdrawals and dropout	5 (16)
Schulz components	
Randomization generation	7 (22)
Allocation concealment	2 (6)
Double-blinding	12 (37)

Children receiving influenza vaccination were significantly less likely to experience a clinically confirmed case of influenza in 14 of 19 trials (74%) and the overall vaccine efficacy for this outcome was 36% (95% CI: 31–40%) (Fig. 1). This estimate is very close to the 33% vaccine efficacy in preventing upper respiratory illness found in the most recent randomized clinical trial, which has not been included in this meta-analysis because data were available on the episodes of illness but not on the number of cases.[41]

Fourteen of 18 trials (78%) reported a significant protection of vaccination against laboratory-confirmed cases of influenza (Fig. 2). The meta-analysis of all studies using laboratory methods to detect the disease showed a 67% reduction of influenza cases with the vaccine (95% CI: 51–78%).

Cases of AOM occurred less frequently in the vaccinated group in 8 of 11 comparisons, although only 4 trials reported a significant reduction (Fig. 3). Overall, the efficacy of vaccination against AOM was 51% (95% CI: 21–71%); in contrast with the other 2 outcomes, Mantel-Haenszel estimate of efficacy substantially differed (17%; 95% CI: 5–29%).

The heterogeneity among studies was significant for all outcomes ($P < 0.001$). Potential sources of this heterogeneity include the study location, the age of participants, the type of vaccine and control used, the degree of antigenic match between vaccine and circulating strains and the methodologic quality of trials. For these variables, extensive sensitivity and subgroups analyses were performed for each of the outcomes considered (Table 2).

The vaccine efficacy against clinically confirmed cases varies substantially according to the study location: the estimate obtained pooling the 7 non-Russian trials is substantially higher compared with the estimate from the 12 Russian studies (61% versus 34%, respectively, with no overlap in confidence limits). The subgroup analyses conducted for the outcome clinically confirmed cases reveal no other relevant differences of effect by any of the other covariates, except for the double-blind design, which seems to increase the overall vaccine efficacy (RR = 0.48), although the confidence interval overlap of the overall estimates from trials with and without a double-blind design suggests a nonsignificant result.

A few studies from the former USSR examined the other 2 outcomes (LCC and AOM) and removal of these studies did not affect the size of the estimates. For children 2 years old or younger, the influenza vaccine was not significant in reducing both LCC and AOM; however, only few studies evaluated vaccine efficacy in this age range.

Trial methodological quality seems to exert a significant influence on vaccination effect against laboratory-con-

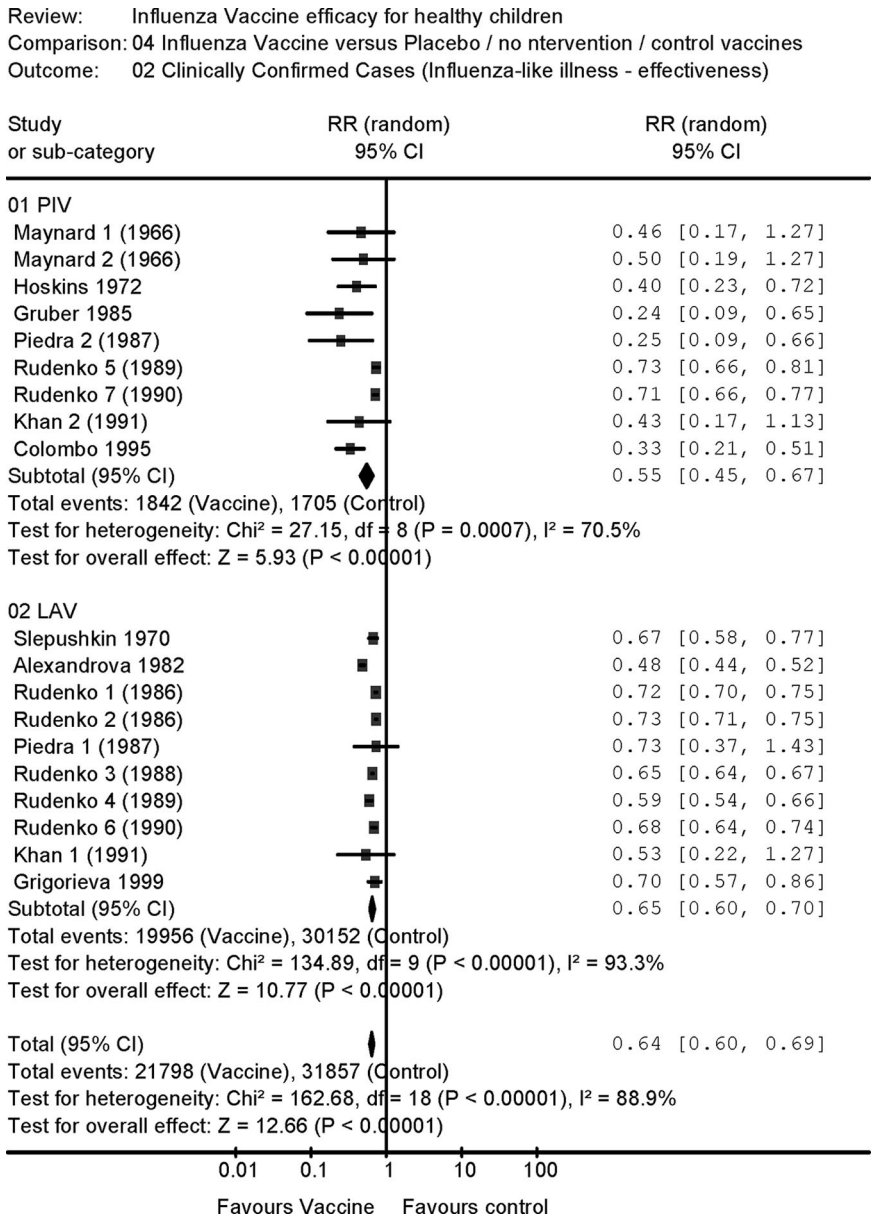

FIGURE 1. Meta-analysis of risk ratios by random effect model (DerSimonian and Laird method) of trials for the effect of vaccination on clinically confirmed cases of influenza in healthy children. The risk ratio pooled estimate for all trials is shown at the bottom of the graph.

firmed cases of influenza with vaccine efficacy being higher in studies achieving higher scores. However, trial quality as measured by the Chalmers scale seems to affect efficacy much more than quality measured by the Jadad scale, indicating that the influence of methodologic quality may depend on the quality assessment approach.

The influence of trial quality on the pooled estimates of studies evaluating the efficacy of influenza vaccine in preventing AOM does not seem substantial with the exception of the component "allocation concealment." It can be noted that for this outcome, live attenuated vaccines showed a statistically significant efficacy, whereas no benefit was found with parenteral inactivated vaccines.

The results of the univariate analysis were largely confirmed by multiple meta-regression (Table 3). For clinical cases of influenza, the only covariate that significantly affected the effect estimates was trial location, confirming a relevant increase in vaccine efficacy in non-Russian as compared with Russian studies. Vaccine efficacy against LCC significantly increased as Chalmers overall quality score and the mean age of participants increased as well as when allocation concealment was adequately addressed. The only factor impacting vaccine efficacy in preventing AOM was study quality, although solely intended as allocation concealment, whereas age did not show a statistically significant influence. The relationship between quality and effect was again in a positive direction: the 2 trials with appropriate allocation concealment showed a greater vaccination effect as compared with those studies that did not adequately addressed this component. None of the results of metaregres-

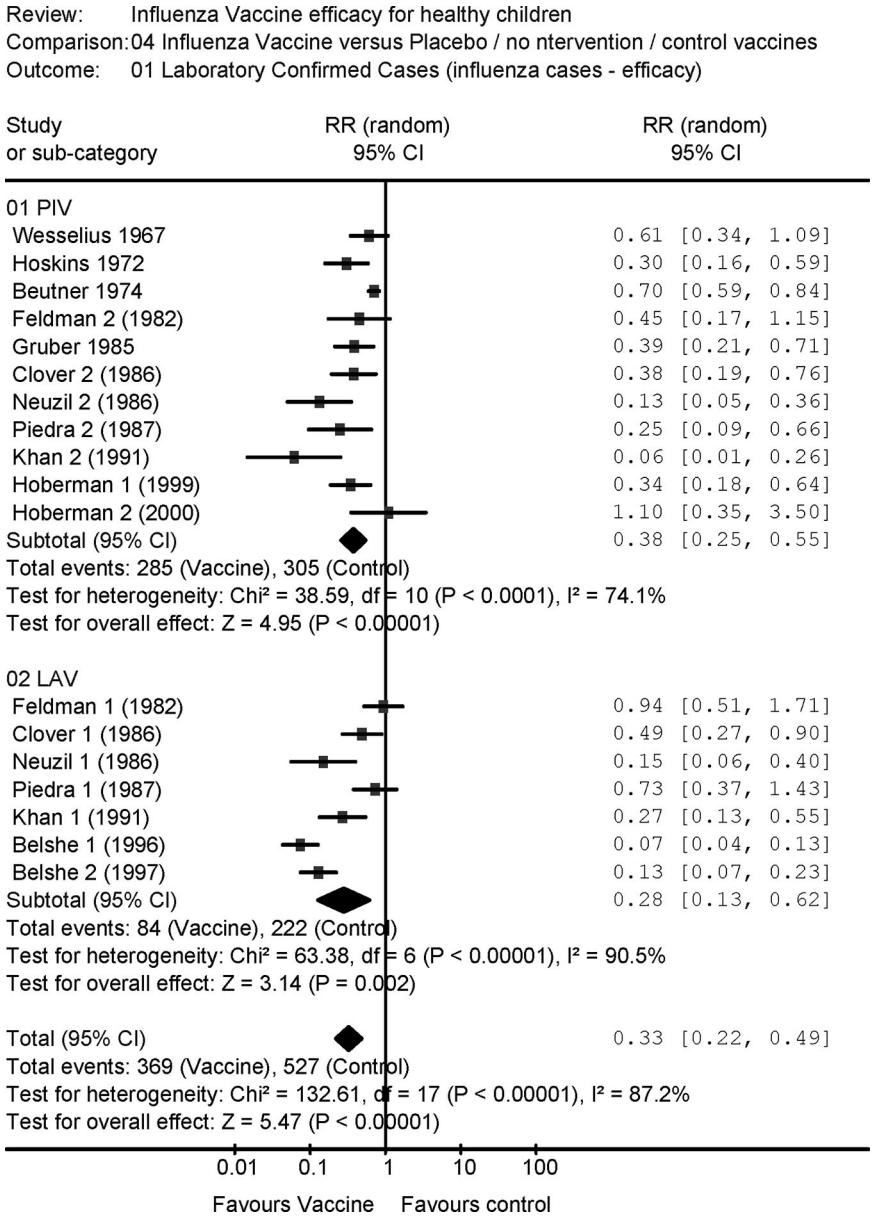

FIGURE 2. Meta-analysis of risk ratios by random effect model (DerSimonian and Laird method) of trials for the effect of vaccination on laboratory-confirmed cases of influenza in healthy children. The risk ratio pooled estimate for all trials is shown at the bottom of the graph.

sions substantially changed when the analysis was also adjusted for the year of study publication or study start.

Funnel plots displaying RRs of the individual studies versus the reciprocal of their standard error appear skewed to the left for trials evaluating vaccination efficacy in preventing clinical cases and AOM (Fig. 4A, C) but not for trials considering LCC (Fig. 4B). Neither Begg nor Egger tests were significant at the 0.1 level for studies assessing clinical cases, whereas only Egger weighted regression method detected a significant asymmetry for trials considering AOM ($P = 0.018$).

DISCUSSION

The efficacy of influenza vaccination for healthy children has recently been evaluated by 2 meta-analyses, which produced overall estimates of vaccine efficacy that were substantially concordant with regard to both clinically (ranging from 28% to 38%) and laboratory-confirmed cases of influenza (from 65% to 80%).[6,7] However, the authors gave different interpretations to their findings: Jefferson et al focused on the discordance between efficacy and effectiveness as a point of reflection for the need of routine immunization,[6] whereas Negri et al attributed a minor role to this issue, stating that "in terms of public health implications even a 30% reduction in clinical influenza among children has important relevance."[7] In addition, the first group concluded that "immunization of children aged 2 years or less is not supported by our findings,"[6] whereas the other group considered the available data "too scanty to allow meaningful analyses in separate age-groups."[7] The fact that the 2 meta-

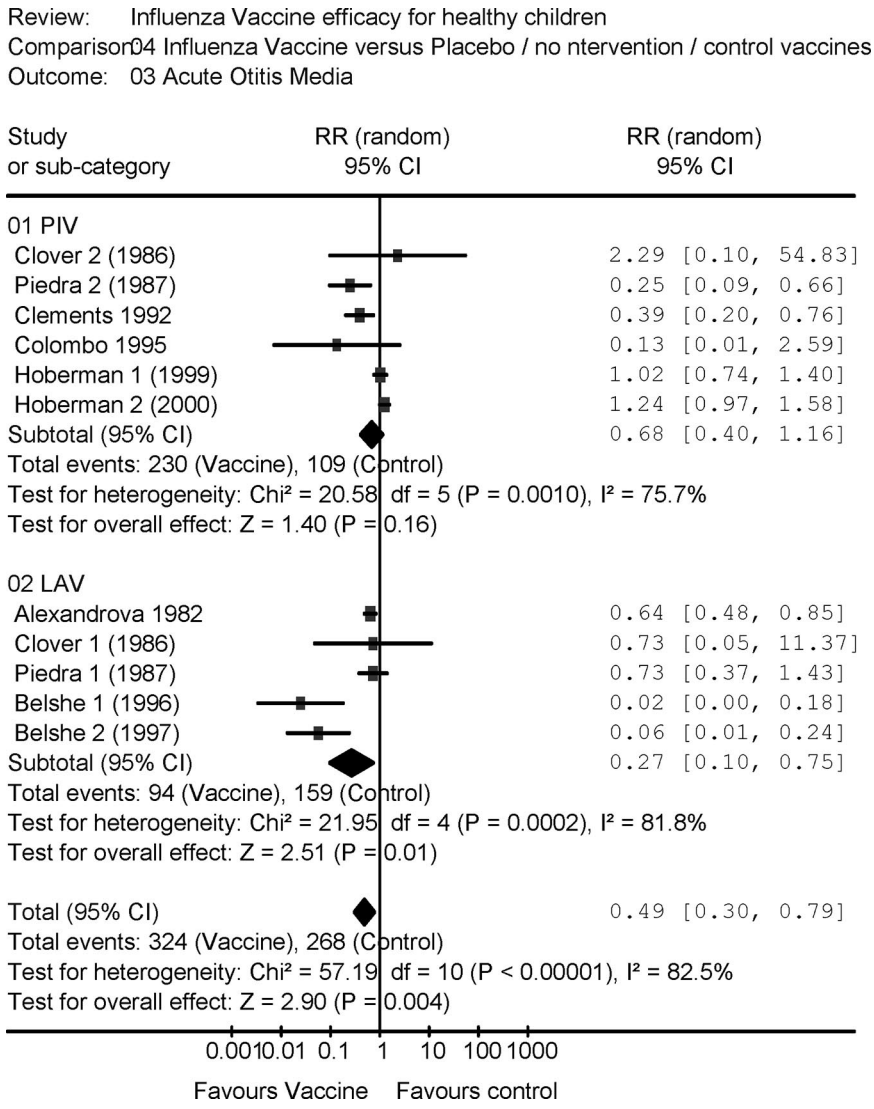

FIGURE 3. Meta-analysis of risk ratios by random effect model (DerSimonian and Laird method) of trials for the effect of vaccination on acute otitis media in healthy children. The risk ratio pooled estimate for all trials is shown at the bottom of the graph.

analyses differed with regard to relevant methodologic aspects (outcomes definition, placebo data extraction and criteria for study inclusion) further complicates the attainment of an opinion between these positions. As an example, Negri et al[7] limited their analysis to papers published in English after 1990 and included studies on children attending day care,[42,43] whereas Jefferson et al used no restriction for language or date but excluded daycare patients.[6] Because of this and other minor issues, the 2 meta-analyses had a relatively scarce overlap of data: only 9 of the 18 randomized controlled trials found by the 2 groups were included in both analyses.[9,28,29,33,34,36–39] Finally, although significant heterogeneity was found for most comparisons—even using stratified and subgroup analyses—the 2 meta-analyses did not investigate potential sources of heterogeneity including methodologic quality of trials, which has been repeatedly documented as a possible explanatory variable.[8,44,45]

The present systematic review offers the possibility of an in-depth evaluation of all these issues and has a larger sample size than the 2 previous meta-analyses. Indeed, this meta-analysis includes 6 randomized controlled trials that were not considered before[21,22,24,26,32,35] and all studies from the other meta-analyses with the exception of one paper without a stated randomization[46] and 2 studies without a clear indication of the health status of the participants.[42,43]

Our estimates of the overall efficacy of the vaccine were 36% for the prevention of clinical cases (35% for the live aerosol preparation, 45% for the parenteral inactivated one) and 67% for LCC (72% for the live attenuated vaccine, 62% for the inactivated one). This smaller effect of vaccination on clinically as compared with LCC was already documented in the 2 previous meta-analyses on healthy children[6,7] and healthy adults.[8,47] This finding should be attributed to the fact that clinically confirmed cases of influenza invariably include a number of misdiagnosed noninfluenza cases,[6–8] and a proportion of clinically diagnosed cases would not be prevented even by a totally efficacious vaccine. When the analysis was stratified excluding Russian studies, the overall

TABLE 2. The Influence of Trial Quality and Other Variables on Effect Size Estimates of Randomized Clinical Trials Evaluating the Efficacy of Vaccination in Preventing Influenza Cases (both clinically and laboratory-confirmed) and Acute Otitis Media in Healthy Children (sensitivity and subgroup analyses)

Variables	CCC		LCC		AOM	
	No. of Trials	RR (95% CI)*	No. of Trials	RR (95% CI)*	No. of Trials	RR (95% CI)*
All studies	19	0.64 (0.60–0.69)	18	0.33 (0.22–0.49)	11	0.49 (0.30–0.79)
Location						
Russian studies only	12	0.66 (0.62–0.71)	2‡	0.15 (0.04–0.61)	1	0.64 (0.48–0.85)
Non-Russian studies	7‡	0.39 (0.30–0.51)	16	0.35 (0.24–0.53)	10	0.44 (0.25–0.78)
Age†						
2 yr or younger	1	0.67 (0.58–0.77)	2	0.55 (0.18–1.69)	3	0.88 (0.54–1.42)
6 yr or younger	7	0.62 (0.56–0.69)	9	0.39 (0.19–0.80)	7	0.46 (0.26–0.83)
Older than 6 yr	15	0.65 (0.61–0.70)	9	0.33 (0.19–0.56)	2	Not estimable
Type of vaccine						
Parenteral inactivated vaccine	9	0.55 (0.45–0.67)	11	0.38 (0.25–0.55)	6	0.68 (0.40–1.16)
Live aerosol vaccine	10	0.65 (0.60–0.70)	7	0.28 (0.13–0.62)	5	0.27 (0.10–0.75)
Type of control group						
Placebo	14	0.64 (0.60–0.70)	17	0.33 (0.22–0.49)	9	0.53 (0.32–0.87)
No intervention	2	0.51 (0.23–1.10)	0	—	1	0.16 (0.01–3.12)
Other vaccination	3‡	0.43 (0.28–0.67)	1	0.30 (0.16–0.59)	1	0.39 (0.20–0.76)
Vaccine/circulating strains matching						
No	11	0.68 (0.64–0.73)	5	0.22 (0.13–0.37)	1	0.06 (0.01–0.24)
Yes	8	0.59 (0.51–0.69)	13	0.39 (0.25–0.60)	10	0.60 (0.39–0.92)
Trial quality (Chalmers scale)						
Low (<median value)	13	0.62 (0.57–0.68)	8	0.55 (0.41–0.73)	5‡	0.52 (0.36–0.75)
High (≥median value)	6‡	0.67 (0.61–0.73)	10	0.22 (0.13–0.37)	6	0.47 (0.22–1.03)
Trial quality (Jadad scale)						
Low (≤2)	17	0.65 (0.60–0.69)	10	0.40 (0.28–0.57)	3‡	0.44 (0.25–0.79)
High (>2)	2	0.49 (0.24–1.03)	8	0.27 (0.13–0.56)	8	0.54 (0.30–0.95)
Trial quality (Schulz components) randomization generation						
Nonadequate	16	0.63 (0.58–0.69)	14	0.36 (0.26–0.49)	5‡	0.47 (0.31–0.73)
Adequate	3	0.61 (0.45–0.81)	4	0.30 (0.08–1.12)	6	0.50 (0.27–0.93)
Allocation concealment						
Nonadequate	19	0.64 (0.60–0.69)	16	0.40 (0.30–0.54)	9	0.70 (0.49–1.01)
Adequate	0	—	2	0.10 (0.06–0.17)	2‡	0.04 (0.01–0.14)
Double-blinding						
Nonadequate	14	0.65 (0.60–0.69)	7	0.42 (0.28–0.64)	2	0.40 (0.21–0.77)
Adequate	5	0.48 (0.31–0.74)	11	0.29 (0.17–0.50)	9	0.53 (0.32–0.87)

*Risk ratio pooled estimate (random effects model, DerSimonian and Laird method).
†An approximation of ±1 yr was applied in some studies.[27,28,32,35,38]
‡The test for heterogeneity (between-study variance) was significant ($P < 0.05$) for all meta-analyses except those indicated.
CCC indicates clinically confirmed cases of influenza.

efficacy of the vaccine in preventing clinical cases substantially increased (from 36% to 61%), and this finding was confirmed by the metaregression results, which indicated that Russian studies report a lower efficacy than non-Russian ones. One possible explanation is related to the sample size of Russian studies, which are larger than non-Russian trials (average sample size 20,470 versus 268). Because it is known that many viruses other than influenza may confound the clinical diagnosis,[48] it is crucial that careful and standardized criteria are used to classify patients accurately. This condition is more likely to occur in limited settings rather than with very large samples in which control is inevitably lower. Alternatively, the observed higher efficacy of non-Russian studies might be the result of publication bias, rather than location, because non-Russian studies are smaller and it is known that smaller trials are less likely to be published than larger ones in case of nonsignificant results.[49] Indeed, the analysis of the funnel plot displaying individual trial RRs versus the reciprocal of their standard errors may suggest a publication bias because the graph was skewed to the left. However, Begg and Egger formal tests for publication bias were not significant for this outcome and, more importantly, the association between location and vaccination effect was still highly significant after controlling for study size (and non-English language; data not shown). Therefore, the effect of location on vaccine efficacy seems consistent.

Because the American Academy of Pediatrics recently recommended routine influenza immunization for all children aged 2 years or less, a precise quantification of vaccine effect in these age group would be of particular interest.[2] Unfortunately, like in previous analyses,[6,7] we were not able to find more than 3 studies for any of the outcomes. The only trial considering clinical cases showed a statistical significant protection conferred by vaccination, whereas the 2 studies assessing LCC of influenza (total number of individuals = 786)[39] and the 3 evaluating AOM (n = 853)[35,39] revealed no significant benefit from vaccination. However, the scarcity of data available suggests that any conclusion should be avoided until further trials are published.

A significant improvement in vaccine efficacy with increasing of age was found for LCC in metaregression analysis. This association was observed also for cases of

TABLE 3. Results of Multiple Metaregression Analyses Relating Trial Quality and Other Variables to Effect Size Estimates of Trials Evaluating the Efficacy of Vaccination in Preventing Cases of Influenza (clinically and laboratory-confirmed) and Acute Otitis Media in Healthy Children

	Regression Coefficient	*P*
Model 1: Clinically confirmed cases of influenza		
Variables included in the model*		
Location (Russians = 0; non-Russians = 1)	−0.519	0.001
Mean age of patients (in years, continuous)	+0.008	0.66
Chalmers overall quality score (continuous)	−0.001	0.94
Model 2: Laboratory-confirmed cases of influenza		
Variables included in the model*		
Mean age of patients (in years, continuous)	−0.061	0.019
Chalmers overall quality score (continuous)	−0.028	<0.001
Allocation concealment (nonadequate = 0; adequate = 1)	−0.743	0.019
Model 3: Cases of acute otitis media		
Variables included in the model*		
Mean age of patients (in years, continuous)	−0.065	0.18
Allocation concealment (nonadequate = 0; adequate = 1)	−2.819	<0.001

*See text for details on metaregression modeling.

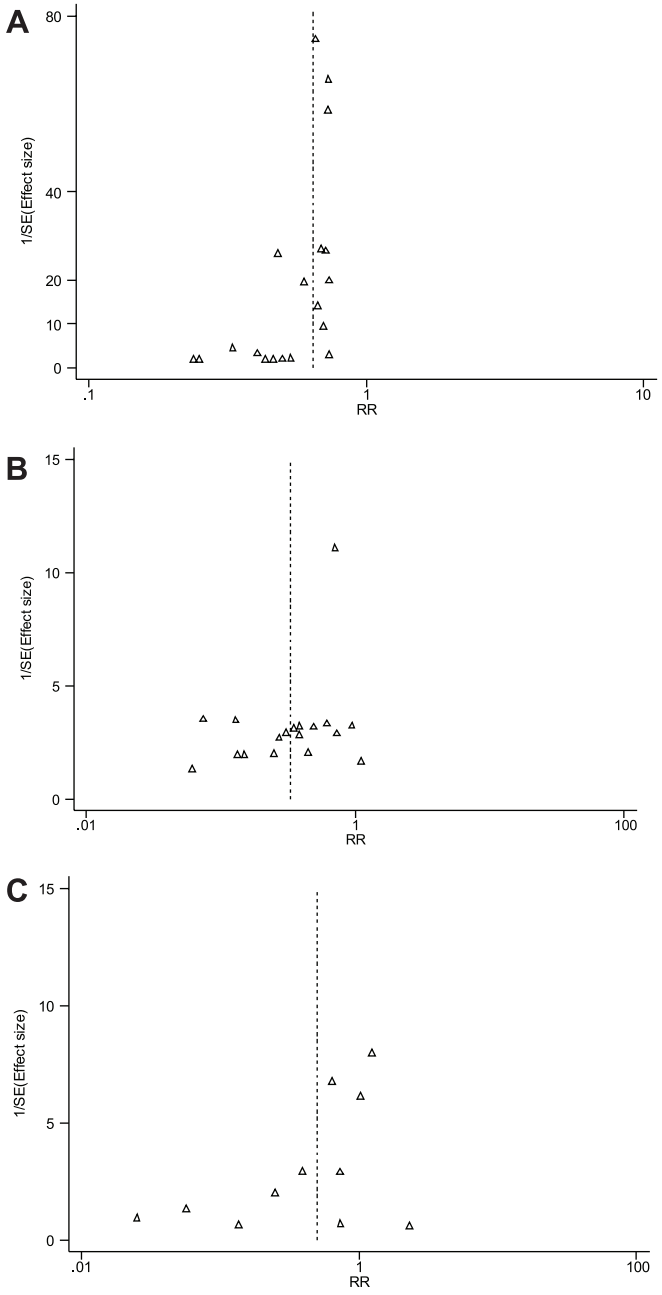

FIGURE 4. Funnel plot of the logarithm of risk ratios versus the reciprocal of their standard errors of trials evaluating the efficacy of influenza vaccination to prevent clinically confirmed cases (**A**), laboratory-confirmed cases (**B**), and acute otitis media (**C**) in healthy children. The vertical line indicates the pooled risk ratios.

AOM, although in this case, it did not achieve the statistical significance ($P = 0.19$). This finding, together with the previously mentioned lack of significance for children 2 years or younger, supports the hypothesis that the vaccination effect is greater in older than in younger children.[6,50] Because age also was found to affect immune responsiveness to vaccination in a meta-analysis on healthy adults,[8] but in an opposite direction, it may be speculated that influenza vaccine efficacy might peak in patients approximately 18 years old and decrease as age increases or decreases. However, because this hypothesis is not supported by the results regarding clinical cases, and it is known that metaregression in the presence of a relevant heterogeneity may produce false-positive results,[17] it should be interpreted with caution. In any case, it has to be taken into account that, even in case the vaccine is more efficacious in older than younger children, it does not necessarily imply that vaccination is ineffective for infants.

In contrast to Jefferson et al,[6] we found a significant reduction of cases of AOM in vaccinated children compared with controls with an overall efficacy of 51%. The different results may be explained by the fact that only 6 trials,[29,36,38,39] for a total of 2642 children, were analyzed in the previously mentioned review, whereas we pooled data from 11 trials including 11,350 subjects. It is worth noting, however, that the live attenuated preparation of the vaccine showed a significant benefit, whereas the parenteral inactivated vaccine did not. However, metaregression analysis did not show any clear difference between inactivated and live attenuated vaccines for all 3 outcomes in line with previous analyses.[6,7]

Previous meta-analyses have examined some of the potential sources of heterogeneity among studies, including setting, age of population and type of vaccine, using stratified and subgroups analyses.[6,7] The additional stratified and metaregression analyses that we performed to evaluate the

potential influence of these and other factors on vaccine efficacy estimates did not reveal a significant variation of effect—for any of the 3 outcomes—depending on the type of vaccine, the type of control group and the closeness of the antigenic match between vaccine and circulating strains.

Statistical heterogeneity may results from defects in methodologic quality, in which a low quality is usually related with an overestimation of intervention benefits.[44,45] A meta-analysis on influenza vaccine efficacy for healthy adults also documented the existence of a clear relationship between methodologic quality and vaccine efficacy estimates.[8] In the present review, only the efficacy of vaccination against laboratory-confirmed cases of influenza appeared substantially influenced by study quality, which in contrast was not related with vaccination effect in preventing clinical cases and was weakly associated with efficacy in reducing cases of AOM (only allocation concealment was significantly related to effect estimates). It is important to note, however, that the association observed in this study is in the opposite direction of that reported previously[8,44,45,51]: for both AOM and laboratory-diagnosed cases, the efficacy of influenza vaccination tended to be higher in higher-quality studies. This finding suggests that the efficacy of influenza vaccination in reducing LCC might be greater than the overall efficacy (67%), probably closer to the estimate obtained in the subgroup analysis pooling the 10 high-quality studies according to Chalmers score (78%).

A funnel plot asymmetry, as a result of the fact that smaller studies showed larger vaccination benefits,[18] was detected for clinically confirmed cases and AOM, although the statistical significance was reached only using Egger test on trials considering AOM. Potential explanations for this asymmetry include publication bias or study quality.[49] However, only publication bias could be a valid explanation, because for both outcomes, there were no significant differences in quality between smaller and larger studies (arbitrarily defined as those with n = >350; data not shown) and because low quality was not associated with greater vaccination effect. Importantly, the exclusion of smaller trials did not lead to a relevant variation of vaccine efficacy for AOM (RR = 0.52; 95% CI: 0.28–0.98). The effect estimates for clinical cases changed substantially (RR = 0.66; 95% CI: 0.61–0.71), but in this case, large trials, with one exception, were all Russian studies and, therefore, their lower efficacy could be the result of the reasons discussed—a less controlled setting for such a critical diagnosis—rather than true publication bias. Finally, it is interesting to note that, for the outcome LCC, the funnel plot appeared, if any, slightly skewed to the right. Because higher study quality does increase vaccine efficacy, this "reversed" asymmetry may be the result of the fact that small studies, that are of lower quality, underestimate vaccine efficacy.

Our review has some of the typical potential limitations of systematic reviews. Mainly, reasons of concern include the scarcity of data on young infants; the fact that methodologic quality of the published articles do not coincide with that of the trials, because reporting may be incomplete[44]; the high likelihood of false-positive results of metaregression analysis when heterogeneity among studies is relevant (however, we limited the number of covariates, used the random-effect approach and considered also the results of correspondent subgroup analyses)[17]; the low statistical power of tests for publication bias when a relatively low number of studies are examined[18]; and finally, the potential bias resulting from published studies that we might have missed in the bibliographic search.

In conclusion, this study indicates a relevant benefit of influenza vaccination for the prevention of clinically and laboratory-confirmed cases of influenza as well as acute otitis media in healthy children older than 2 years, whereas insufficient data was available to draw firm conclusions of children 2 years or younger. The efficacy of the vaccine was smaller for clinical illnesses as compared with laboratory-confirmed cases as already shown in previous meta-analyses on healthy children[6,7] and adults.[8,47] The only significant sources of heterogeneity between studies were the age of participants and study quality both directly correlated with vaccine efficacy. These and other findings of sensitivity analyses on studies performed outside the former USSR and high-quality trials may indicate that the efficacy of the vaccine could be greater than the overall pooled estimates. Although no safety and cost considerations are addressed in this analysis, with regard to efficacy, the present findings support vaccination as a possible option for the prevention of influenza in healthy children.

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