Systematic review of the non-specific effects of BCG, DTP and measles containing vaccines

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Erratum, 3 April 2014

A correction has been made to the text of the Conclusion in the last table in *Section 10 Summary of the evidence* (page 24). Also, some accidentally duplicated rows have been removed from Figures 3, 9 and 17.

Contents

1 Executive Summary

This report describes the results of a systematic review of epidemiological evidence concerning the non-specific effects of BCG vaccine, DTP vaccine and measles vaccine as routinely administered to infants and children. It focuses on all-cause mortality and, where data permit, examines the effects of various potential effect measure modifiers (gender of the child, age at vaccination, vitamin co-administration, order of vaccination). A detailed assessment of the risk of bias inherent in each study was also performed, and the available evidence subjected to a formal assessment of the quality of the evidence using the GRADE approach. In this executive summary we omit mention of studies assessed at being at such high risk of bias that they do not contribute useful information.

Five clinical trials and 9 observational studies provided comparisons of mortality rates among BCG-vaccinated and BCG non-vaccinated children in the neonatal period. The results indicated a beneficial effect of BCG on overall mortality in the first 6-12 months of life. Relevant follow-up in some of the trials was short, and all of the observational studies were regarded as being at risk of bias, so the confidence in the findings was rated as very low according to the GRADE criteria. No strong evidence of differential effects by gender or vitamin A was available, and there was a suggestion that BCG vaccination may be more beneficial the earlier it is given.

Ten observational studies (but no clinical trials) provided comparisons of DTP with no DTP. Oral polio vaccine was administered concomitantly with DTP in most included studies. The findings were inconsistent, with a majority of the studies indicating a detrimental effect of DTP, and two studies indicating a beneficial effect. All of the studies were regarded as being at risk of bias, so the confidence in the findings was rated as very low according to the GRADE criteria. One study reported a larger detrimental effect in girls, but overall there was not convincing evidence of a differential between girls and boys, or of differential effects by age at vaccination or vitamin A administration.

For measles vaccine, four randomized trials and 18 results from observational studies were included. There was consistent evidence of a beneficial effect of measles vaccine, although all observational studies were assessed as being at risk of bias and the GRADE rating was of low confidence. There was an apparent difference between the effect in girls and boys, with girls benefitting more from measles vaccination. We did not identify sufficient evidence to draw conclusions about effect modification by vitamin A, or about the age at which measles vaccination is most effective.

There was limited evidence on alternatives to the WHO-recommended ordering of vaccinations. Three observational studies provided a suggestion that simultaneous administration of BCG and DTP may be preferable to the recommended schedule of BCG before DTP; and there was suggestion that mortality risk may be higher when DTP is given with, or after, measles vaccine compared with when it is given before measles vaccine (from five, and three, observational studies, respectively). These results are consistent with hypotheses that DTP vaccine may have detrimental effects on mortality, although a majority of the evidence was generated by a group centred in Guinea-Bissau who have often written in defence of such a hypothesis.

2   Background

Over the past 30 or more years, an increasing number of vaccines have reached a greater proportion of the world's children, targeting some of the leading causes of morbidity and mortality, especially in children living in poor countries with high infant and child mortality rates. These vaccines, such as those against measles, diphtheria-pertussis-tetanus (DTP) and polio, have produced extraordinary declines in morbidity and mortality from the diseases targeted by the vaccines. In this context, a number of studies and related publications have suggested that some of the vaccines routinely administered to infants and children have non-specific effects on the immune system, and that these effects alter the risk of illness and death from conditions other than the specific infectious disease the vaccine is designed to prevent. These have come to be called non-specific effects of vaccines. Among hypotheses concerning these non-specific effects have been assertions that some vaccines (e.g. measles and Bacillus Calmette–Guérin (BCG) vaccines) are associated with lower subsequent risk of illness and death from other causes, while other vaccines (such as some DTP vaccines) are associated with higher subsequent risk of illness and death from other causes. It is further postulated that these effects may vary depending on factors including a child's gender and whether or not vitamin A supplements have been administered. Because hypotheses concerning possible non-specific effects of various infant immunizations arose after these vaccines had become part of the routine immunization schedule, randomized trials testing these hypotheses have been difficult or impossible to conduct on ethical grounds; as a result, with few exceptions, studies testing these hypotheses have been observational in nature.

This report describes the results of a systematic review of epidemiological evidence concerning the non-specific effects of vaccines routinely administered to infants and children. A separate review addresses immunological evidence. The present review was limited from the outset to studies of the effects of three vaccines: BCG vaccine, DTP vaccine and measles vaccine. Furthermore, it was decided to limit the review to studies, both published and unpublished, that permit an assessment of the effect, if any, of receipt of one of these vaccines on the subsequent risk of dying by five years of age. Children who had received medium or high titre measles vaccine were excluded. The protocol for the review specified a primary outcome of mortality from causes other than those associated with the disease the vaccine is intended to prevent (i.e. the non-specific effect of the vaccine on mortality) and a secondary outcome of mortality from all causes. This report focuses on the latter outcome of all-cause mortality, for which there is more evidence. Whenever the available data permitted, the effects of various potential effect measure modifiers (e.g. gender of the child, vitamin co-administration, order of vaccination) on the relationship between receipt of a given vaccine and the subsequent risk of dying were examined.

Because re-analyses and multiple analyses of data from some of the relevant studies have been published or are available, particular attention was given to avoiding the use of information for the same child for the same period of time more than once in the review. As described further below, a detailed assessment of the risk of bias inherent in each study was also performed, and the available evidence was subjected to a formal assessment of the quality of the evidence using the GRADE approach required for all WHO reviews.

The methods of the review are summarized in Section 11, and the numbers of articles identified and included are summarized diagrammatically in Section 12. Details of the included papers are provided in Annex A; data selected for presentation in the present report are explained in Annex B; and assessments of risk of bias are provided in Annex C. Findings for mortality from causes other than the disease the vaccine is intended to prevent are presented in Annex D, and a list of abbreviations is provided in Annex E. A series of appendices provides additional supplementary information.

3   Is administration of BCG vaccine in infancy associated with an effect on all-cause mortality?

Five clinical trials (two clearly randomized (1, 2) and three less clear in their allocation methods (3-5)), 12 cohort studies (6-19) and one case-control study (20) were identified that provided comparisons of mortality rates among BCG-vaccinated and BCG non-vaccinated children in the neonatal period and are presented in Figure 1. Four results from the cohort studies were considered to be at very high risk of bias in relation to the effect of BCG, and are presented separately at the bottom of the forest plot. We consider results that are assessed as being at very high risk to be uninformative, and such results do not contribute to our conclusions or GRADE assessments.

The clinical trials all pointed towards a beneficial effect of BCG on mortality. The randomized trials are essentially two periods of a single trial, involving new-borns recruited before and after it was restarted. We present 1-month mortality data from these, since the control group went on to receive BCG at a delayed time point (recommended age 6 weeks). The main phase of the trial found a halving of mortality risk among the BCG-vaccinated children, with a 95% confidence interval excluding 'no effect' (ES = 1). For the quasi-randomized trials (which allocated children to groups by alternation, or unclear methods), we could obtain only longer follow up (to 2 or 5 years of age). The quasi-randomized

trials were all performed in North America in the mid-20th century. They are the only studies in the review that were undertaken in high-income countries.

Excluding the results considered to be at very high risk of bias, the results of the nine non-randomized studies (all considered nevertheless to be at high risk of bias) also indicated a beneficial effect of BCG on overall mortality in the first 6-12 months of life. Four results had confidence intervals that excluded 'no effect', each demonstrating a beneficial effect of BCG. Estimated effects are in the region of a halving of mortality risk. Many of the analyses were undertaken by the Guinea-Bissau investigators, comprising four in which they conducted the original study [Guinea-Bissau 1984-1985, #851 (9); Guinea-Bissau 1989-1999, #839 (10); Guinea-Bissau 1990-1996, #9466 (8); Senegal 1996-1999, #9433 (18)] and two in which results of other investigators' data were re-analysed [India 1987-1989, #8996 (14); Malawi 1995-1997, #664 (16)].

The four studies with particularly high risks of bias produced highly variable results. Their results however point in the same direction as the other studies: of a beneficial effect of BCG on overall mortality.

As noted in the Background, the available studies typically provided data on all-cause mortality, and did not allow examination of the 'non-specific effects' of BCG vaccine on deaths from causes other than tuberculosis. However, deaths from tuberculosis are infrequent in infants and children in the first five years of life, so any effect of BCG vaccine on all-cause mortality is not likely to be attributable to any great extent to fewer deaths from tuberculosis (i.e. to a specific effect of BCG vaccine against tuberculosis).

3.1   Is there a difference in the effect between boys and girls?

Nine of the cohort studies provided comparisons of BCG with no BCG separately for boys and girls, including one randomized trial, six cohort studies without very high risk of bias and two cohort studies with very high risk of bias for the main effects in Figure 1. The findings are shown in Figure 2. They reflect the main observation above of a beneficial effect of BCG. To examine the potential for differential effects between boys and girls, we need to look at the (statistical) interaction between them. Figure 3 illustrates the difference in vaccine effect between boys and girls, which is equivalent to the comparison in boy-girl mortality ratios between BCG-vaccinated and BCG-unvaccinated children[1]. The studies do not provide any suggestion of a differential effect on mortality of BCG between boys and girls.

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[1] For one study [Senegal 1996-1999, #9433], boy-girl mortality ratios are used to compute interactions in Figure 3 rather than the data in Figure 2, since they allow for adjustment for age.

Figure 1. BCG and all-cause mortality.

BCG and all-cause mortality

Birth cohort	Article Gp	Pub'n year	Deaths/ Children	Subseq. DTP*	Subseq. MV*	Age at first dose	Observation period**	Adjustment	ES (95% CI)	Vaccine efficacy
1. Randomized and quasi-randomized trials										
Canada 1933-1945	Canada	1949	(53+63)/609	None	None	10 days	age 60 months	None	0.94 (0.67, 1.32)	6% (-32%, 33%)
Guinea-Bissau 2002-2008 (early)	Guinea-Bissau A	2012	(2+6)/105	None	None	2 days	age 1 month	Age	0.28 (0.06, 1.37)	72% (-37%, 94%)
Guinea-Bissau 2002-2008 (main)	Guinea-Bissau A	2011	(27+48)/2343	None	None	2 days	age 1 month	Age	0.55 (0.34, 0.89)	45% (11%, 66%)
USA c.1935	USA A	1948	(12+13)/3008	None	None	0-4 years	age 48 months	None	0.91 (0.41, 1.99)	9% (-99%, 59%)
USA c.1941	USA B	1961	(4+9)/451	None	None	7-10 days	age 60 months	None	0.42 (0.13, 1.35)	58% (-35%, 87%)
2. Case-control studies										
Benin 1983-1987	Benin	1991	(34+39)/294	Many	Many	NR	age 4-36 months	Age, gender, others	0.68 (0.38, 1.23)	32% (-23%, 62%)
3. Cohort studies										
Guinea-Bissau 1984-1985	Guinea-Bissau E	2004	NR/1657	Many	Some	NR (0-8 months)	age 8 months	Age, gender, DTP, others	0.63 (0.30, 1.33)	37% (-33%, 70%)
Guinea-Bissau 1989-1999	Guinea-Bissau I	2004	(2+14)/695	Many	Few	1-7 days	age 6 months	Age, gender, others	0.05 (0.01, 0.46)	95% (54%, 99%)
Guinea-Bissau 1990-1996	Guinea-Bissau D	2002	(92+97)/4418	Many	Some	Median 1 month	6 months follow-up	Age, DTP, others	0.56 (0.37, 0.84)	44% (16%, 63%)
India 1987-1989	India E	2012	(3+29)/3072	None	Some	Median 1.6 months	age 12 months	None	0.60 (0.18, 1.97)	40% (-97%, 82%)
India 1998-2002	India A	2005	208/10274	Many [cens]	Few	Median 19 days	age 6 months	Age	0.44 (0.29, 0.66)	56% (34%, 71%)
Malawi 1995-1997	Malawi	2006	NR/751	Many [cens]	Few	Median 16 days	age 8 months	Age, others	0.45 (0.16, 1.23)	55% (-23%, 84%)
Papua New Guinea 1989-1994	Papua New Guinea	2005	NR/3937	Many	Few	Median 1 month	age 1-6 months	Age, DTP, others	0.17 (0.09, 0.34)	83% (66%, 91%)
Senegal 1996-1999	Senegal D	Unpub.	(9+372)/4421	Many [cens]	Many [cens]	NR (by 12 months in 44%)	age 24 months	Age, others	0.98 (0.50, 1.90)	2% (-90%, 50%)
Excluded (Very high risk of bias)										
Bangladesh 1986-2001	Bangladesh A	2004	184/9704	Many (OS)	Many	0-2 months	age 0-60 months	Age	0.20 (0.07, 0.54)	80% (46%, 93%)
Burkina Faso 1985-1993	Burkina Faso	2004	(28+280)/9085	Many (SS)	Many [cens]	Mean 4.8 months	6 months follow-up	Age, others	0.50 (0.34, 0.75)	50% (25%, 66%)
Ghana 1998-2004	Ghana C	2010	NR/17967	Many	Many	NR (by 12 months in 57%)	age 60 months	Age, others	0.18 (0.17, 0.20)	82% (80%, 83%)
India 2006-2011	India G	2013	(45+285)/11390	Few	None	Mean 17 days	age 1.2 months	None	0.12 (0.09, 0.16)	88% (84%, 91%)

.2   .5   1   2   5
Vaccine beneficial   Vaccine harmful

ES = effect size (hazard ratio, rate ratio or risk ratio; odds ratio for the case-control study). NR = not reported.

Deaths/Children = (BCG deaths + Non-BCG deaths)/Total children or Total deaths/Total children

In the two cohort studies with 'None' as adjustment for confounding, we computed unadjusted rate ratios from rates presented in the paper.

Vaccine efficacy is computed as (1 – ES) × 100%. A non-negative number describes the proportion of deaths prevented by the vaccine. A negative number reflects a higher death rate among the vaccinated. For example, if vaccine efficacy = -100%, then an *additional* 100% of the deaths that would have occurred without vaccine would occur with the vaccine.

Guinea-Bissau 2002-2008 (early): early phase of the trial stopped prematurely because of faulty randomization procedure in one of the centres; Guinea-Bissau 2002-2008 (main): main trial report with larger sample size.

*Subseq. DTP: what proportion of children were likely to receive DTP during the period of observation. Subseq. MCV: what proportion of children were likely to receive measles vaccine during the period of observation. [cens] means this event was censored in the analysis].

SS = sometimes given simultaneously with DTP; OS = often given simultaneously with DTP

**This is the period of observation applicable to the result presented in the forest plot, aiming to capture the effect of BCG with minimal impact of subsequent vaccinations. The full study may have had a longer period of follow up.

Figure 2. BCG and all-cause mortality: results for boys and girls separately.
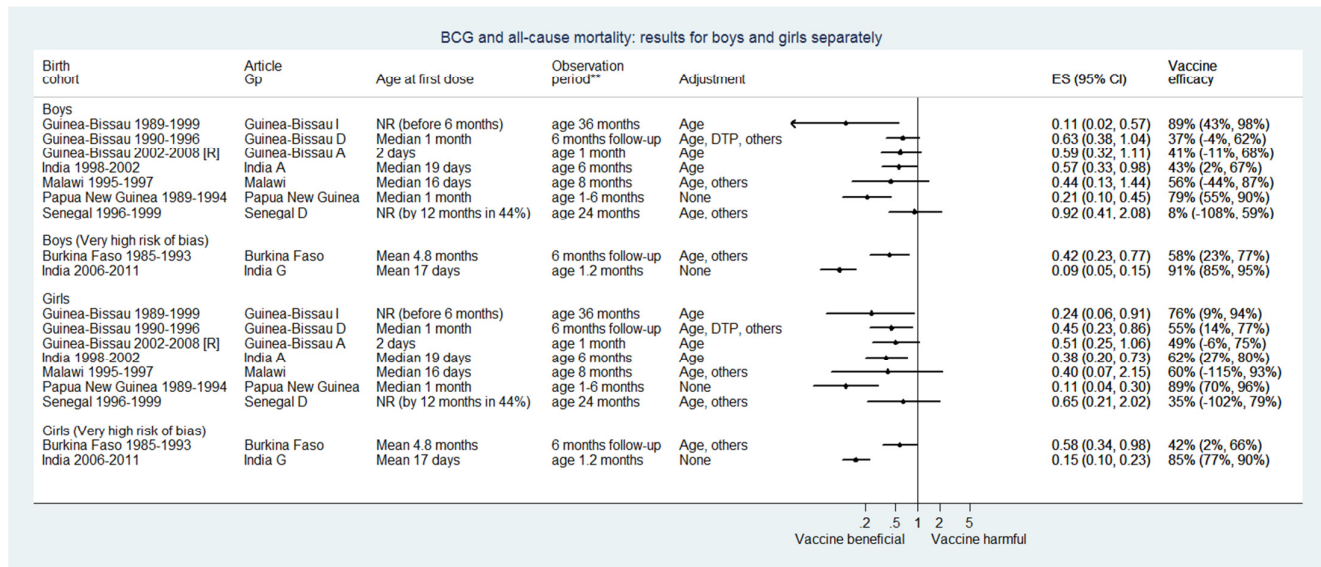
ES = effect size (hazard ratio, rate ratio or risk ratio)
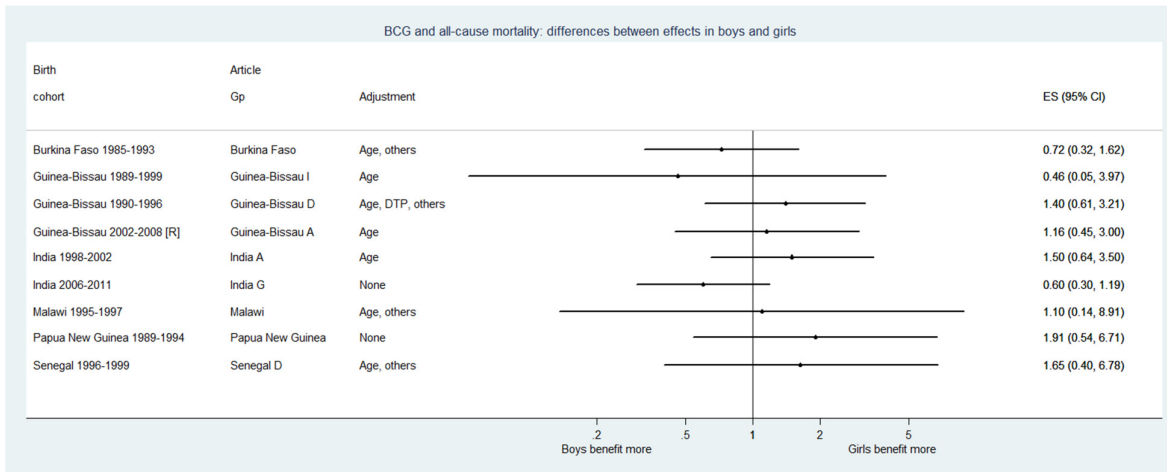R = Randomized trial (the main phase of the trial) (all other studies are cohort studies)
**This is the period of observation applicable to the result presented in the forest plot, aiming to capture the effect of BCG with minimal impact of subsequent vaccinations. The full study may have had a longer period of follow up.

Figure 3. BCG and all-cause mortality: differences between effects in boys and girls.

ES = effect size (ratio of hazard ratios, rate ratios or risk ratios)
R = Randomized trial (the main phase of the trial) (all other studies are cohort studies)

3.2   Is there a difference in the effect by age?

Age at vaccination

The average age at which BCG vaccination was administered varied across studies (see Figure 1), from very soon after birth to 4.8 months of age or later. However, there is not strong evidence of a pattern of association between observed effects on mortality and age of vaccination in Figure 1.

In two studies, effects were reported for children vaccinated at different ages. In both Bangladesh 1986-2001 (6) and Guinea-Bissau 1989-1999, #839 (10), the effect lessened as the age of vaccination increased. These results are illustrated at the top of Figure 4.

The two randomized comparisons examined BCG at birth versus delayed BCG (recommended at 6 weeks) among low birth weight infants. After 1 year, the early phase of the trial observed 5 deaths among 51 allocated to the at-birth arm and 11 deaths among 54 allocated to the delayed arm (risk ratio 0.48, 95% CI 0.18 to 1.29). After the same period of follow-up, the larger second phase observed 105 deaths among 1182 allocated to the at-birth arm and 124 deaths among 1161 allocated to the delayed arm (risk ratio 0.83, 95% CI 0.65 to 1.06). These results suggest a possible benefit of early BCG over delayed BCG.

Further research may help determine whether these effects are real or artefactual.

Age at follow-up

For Papua New Guinea 1989-1994 (17), results were available for multiple follow-up times. These results are illustrated in the middle portion of Figure 4. Interpretation of these is made challenging by the effects of subsequent vaccines and by the potential for selection biases to push results for later follow-up periods towards (and even beyond) the no effect line. Smaller effects are seen at later ages.

Other results by age

One study reports results by age of the child the first time they were seen ('first visit') so the findings likely reflect both age at vaccination and age at follow-up [Guinea-Bissau 1990-1996, #2726 (21)]. Again, a potential pattern of smaller effects at later ages is seen.

Figure 4. BCG and all-cause mortality: results by age.
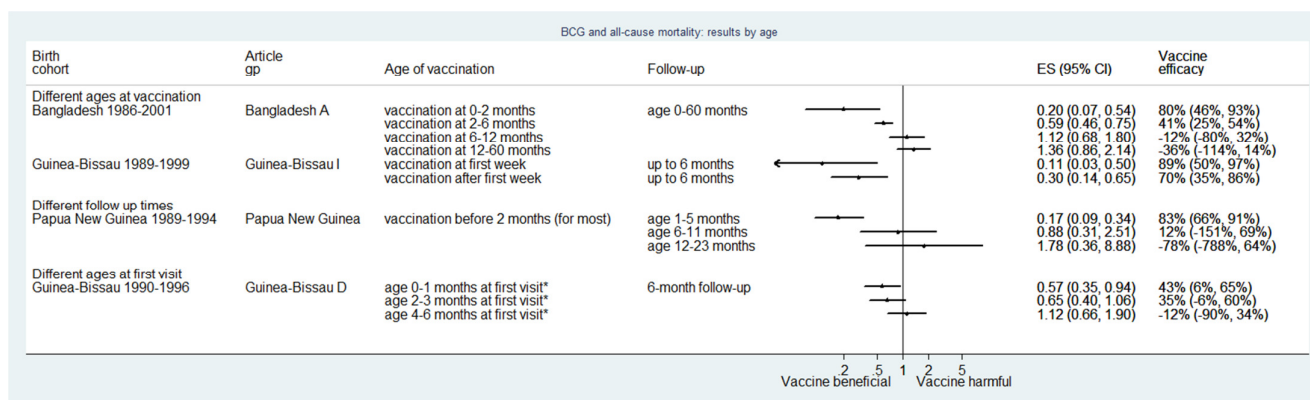
ES = effect size (hazard ratio, rate ratio or risk ratio)
All studies are cohort studies.
*Differences by age at first visit may reflect a combination of ages at vaccination and ages at follow up.

3.3   Is there a difference in the effect by vitamin A administration?

We sought results for interaction between BCG effect and (prior or concurrent) administration of vitamin A. To understand interaction we needed either (i) the effect of BCG separately among vitamin A recipients and vitamin A non-recipients or (ii) the effect of vitamin A separately among BCG recipients and BCG non-recipients. One cohort [India 1998-2002, #741 (13)] provided the former (presented in Figure 5) and one presented the latter [Guinea-Bissau 2002-2008, #339 (22)]. The resulting estimates of interaction are illustrated in Figure 6. There is insufficient evidence to determine any difference in effect of BCG according to vitamin A status.

Figure 5. BCG and all-cause mortality: results for vitamin A recipients and vitamin A non-recipients separately.

ES = effect size (hazard ratio, rate ratio or risk ratio; odds ratio for the case-control study)

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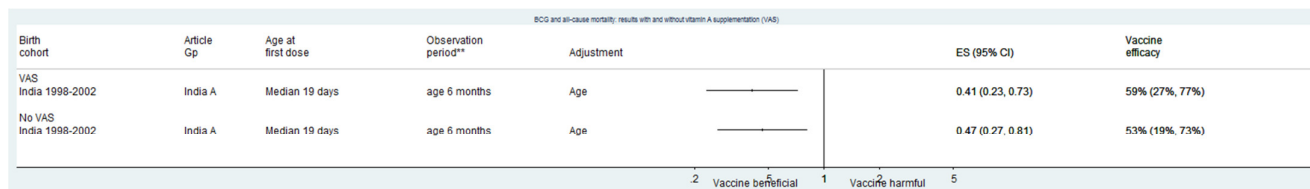
Figure 6. BCG and all-cause mortality: differences between effects between vitamin A recipients and vitamin A non-recipients.
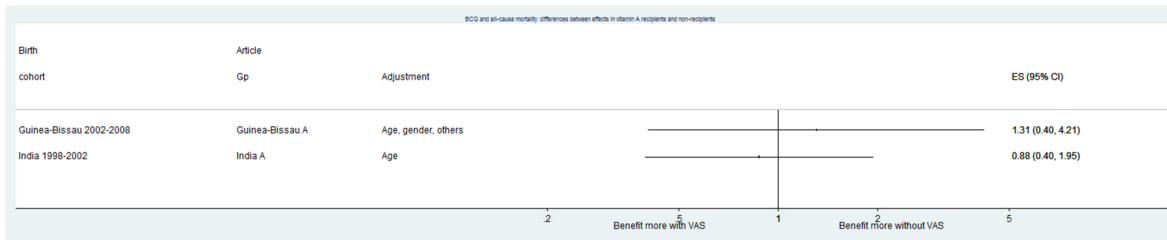
ES = effect size (ratio of hazard ratios, rate ratios or risk ratios)
Both studies are cohort studies.

3.4   Comments on study methodology and bias

All of the results from observational studies were judged to be at high risk of bias or very high risk of bias, so the findings above should be interpreted with caution. The main potential sources of bias in the observational studies were confounding (inherent differences in children vaccinated and children not vaccinated: no studies were considered to have overcome this); misclassification bias relating to determination of non-vaccination status; bias arising from selection of participants after vaccines were given (and hence after they could have impacted on mortality); co-interventions including administration of DTP vaccine; and misclassification bias relating to lack of information about vaccinations that were administered (including 'survival bias' arising from taking a retrospective approach to the analysis (23).

The very high risk of bias studies were seriously affected by co-administration of DTP [Bangladesh 1986-2001, #797 (6)], starting follow up long after BCG vaccination [Burkina Faso 1985-1993, #799 (7)], highly correlated co-interventions [Ghana 1998-2004, #9464 (19)], and strong confounding by age; [India 2006-2011, #9463 (15)]. Some of these studies had additional reasons for serious concern, but for which we were unable to make a judgement from the written reports.

We regard the estimates of interaction (for differences by gender and vitamin A) to be less affected by bias, since many of the biases affecting direct estimates of vaccine effects are likely to cancel out when these are contrasted between boys and girls or between vitamin A recipients and vitamin A non-recipients.

For full details of methodological features and assessments of risk of bias, see Annex C.

4   Is administration of DTP vaccine in infancy associated with an effect on all-cause mortality?

Fifteen cohort studies (7-9, 13-19, 24-28) and one case-control study (20) were identified that provided comparisons of DTP with no DTP. These results are presented in Figure 7. No randomized trials of DTP versus no DTP were identified. Oral polio vaccine (OPV) was administered concomitantly with DTP in most included studies; two studies did not report OPV co-administration [Burkina Faso 1985-1993, #799 (7); India 2006-2011, #9463 (15)]. Six results from the cohort studies were considered to be at very high risk of bias in relation to the effect of DTP, and are presented separately at the bottom of the forest plot.

Excluding the results considered to be at very high risk of bias, the results of the 10 studies (all considered nevertheless to be at high risk of bias) produced diverse results, ranging from a halving of mortality risk after DTP administration to a four-fold increase in mortality risk after DTP administration. The majority of studies indicated a deleterious effect of DTP on mortality. Three of these had 95% confidence intervals that excluded no effect. These were all undertaken in Guinea-Bissau [#25 (26); #9466 (8); #851 (9)]. Three of the other results were from the Guinea-Bissau investigators [Bangladesh 1986-2001 #9477 (24); Malawi 1995-1997, #664 (16); Senegal 1996-1999, #9433 (18)], two of which were re-analyses of studies undertaken by other teams [Bangladesh 1986-2001 #9477 (24); Malawi 1995-1997, #664 (16)]. Two of these suggested possible deleterious effects and one (a re-analysis of a Bangladesh study[2]) had a 95% confidence interval favouring a beneficial effect of DTP. The three studies from different investigator teams produced more equivocal

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[2] The result reported by the original investigators, which was considered to be at very high risk of bias for this research question due to high rates of co-administration of DTP with BCG, was an ES (mortality rate ratio) of 0.76 (95% CI 0.67 to 0.88).

Figure 7. DTP and all-cause mortality.

DTP and all-cause mortality

Birth cohort	Article Gp	Pub'n year	Deaths/ Children	Prior BCG*	Simult. OPV	Subseq. MV*	DTP doses	Age at first dose	Observation period**	Adjustment		ES (95% CI)	Vaccine efficacy
1. Case-control studies													
Benin 1983-1987	Benin	1991	(15+18)/132	NR	Yes	Many	1 dose	NR	age 4-36 months	Age, gender, others		2.20 (0.93, 5.22)	-120% (-422%, 7%)
2. Cohort studies													
Bangladesh 1986-2001	Bangladesh A	Unpub.	(26+51)/6410	All	Yes	Few [cens]	1 dose	Median 2.8 months	1.5-9 mo	Age, gender, others		0.52 (0.31, 0.87)	48% (13%, 69%)
Burkina Faso 1985-1993	Burkina Faso	2004	(33+28)/9085	All (SS)	NR	Many (SS)	1-3 doses	Mean 6.3 months	6 months follow-up	Age, others		1.00 (0.60, 1.67)	0% (-67%, 40%)
Guinea-Bissau 1984-1985	Guinea-Bissau E	2004	(47+20)/1657	Some	Yes	Few	1-3 doses	NR (3-8 months)	age 8 months	Age, gender, BCG, others		1.92 (1.04, 3.52)	-92% (-252%, -4%)
Guinea-Bissau 1990-1996	Guinea-Bissau D	2002	(61+128)/4418	Many	Yes	Some	1 dose	Median 3 months	6 months follow-up	Age, BCG, others		1.74 (1.10, 2.75)	-74% (-175%, -10%)
Guinea-Bissau 2002-2008	Guinea-Bissau A	2012	(20+14)/935	All	NR	Few [cens]	1 dose	NR (1.5-6 months)	age 6 months	Age, gender, others		4.33 (1.54, 12.19)	-333% (-1120%, -54%)
India 1998-2002	India A	2005	NR/1723	All	Yes	Few	1-3 doses	Median 2 months	age 6 months	Age		1.64 (0.87, 3.07)	-64% (-207%, 13%)
Malawi 1995-1997	Malawi	2006	NR/805	Many	Yes	Few	1 dose	Median 2.2 months	age 8 months	Age, others		3.19 (0.80, 12.79)	-219% (-1180%, 20%)
Papua New Guinea 1989-1994	Papua New Guinea	2005	NR/2788	All	Yes	Few	1 dose	Before 3 months for most	age 1-5 months	Age, others		0.48 (0.22, 1.09)	52% (-9%, 78%)
Senegal 1996-1999	Senegal D	Unpub.	(9+9)/319	All	Yes	Many [cens]	1 dose	NR (before 9 months)	age 24 months	Age, others		1.37 (0.54, 3.47)	-37% (-247%, 46%)
Excluded (Very high risk of bias)													
Ghana 1984-1991	Ghana A	2012	(25+4)/665	Many (OS)	Yes	Many (OS)	1-2 doses	NR	age 10-39 months	Age, others		2.39 (0.82, 6.99)	-139% (-599%, 18%)
Ghana 1998-2004	Ghana C	2010	NR/17967	NR	Yes	Many	1 dose	NR (by 12 months in 47%)	age 60 months	Age, others		0.15 (0.14, 0.16)	85% (84%, 86%)
Guinea-Bissau 1989-1999	Guinea-Bissau P	2002	(19+2)/533	Most	Yes	Few	1-3 doses	NR (from 1.2 months)	age 1.25-20 months	Age, gender, others		1.58 (0.36, 7.02)	-58% (-602%, 64%)
India 1987-1989	India E	2012	(9+3)/1723	All	Yes	Some	1-3 doses	Median 3.8 months	age 12 months	None		1.11 (0.30, 4.12)	-11% (-312%, 70%)
India 2008-2011	India G	2013	(136+44)/10274	Many	NR	Few	1-3 doses	Mean 2 months	age 8 months	None		0.28 (0.20, 0.40)	72% (60%, 80%)
Philippines 1988-1991	Philippines	2007	(79+6)/10231	All	Yes	Many [cens]	1-3 doses	NR (before 7 months)	age 30 months	Age, gender, others		0.87 (0.33, 2.29)	13% (-129%, 67%)

.2   .5   1   2   5

Vaccine beneficial   Vaccine harmful

ES = effect size (hazard ratio, rate ratio or risk ratio; odds ratio for the case-control study)
Deaths/Children = (DTP deaths + Non-DTP deaths)/Total children or Total deaths/Total children
All studies are cohort studies.
In the two studies with 'None' as adjustment for confounding, we computed unadjusted rate ratios from rates presented in the paper.
Vaccine efficacy is computed as (1 – ES) × 100%. A non-negative number describes the proportion of deaths prevented by the vaccine. A negative number reflects a higher death rate among the vaccinated. For example, if vaccine efficacy = -100%, then an *additional* 100% of the deaths that would have occurred without vaccine would occur with the vaccine.
*Prior BCG: whether children studied had received BCG. Subseq. MCV: what proportion of children were likely to receive measles vaccine during the period of observation [*cens* means this event was censored in the analysis].
SS = sometimes given simultaneously with DTP; OS = often given simultaneously with DTP.
**This is the period of observation applicable to the result presented in the forest plot, aiming to capture the effect of DTP with minimal impact of subsequent measles vaccination. The full study may have had a longer period of follow up.
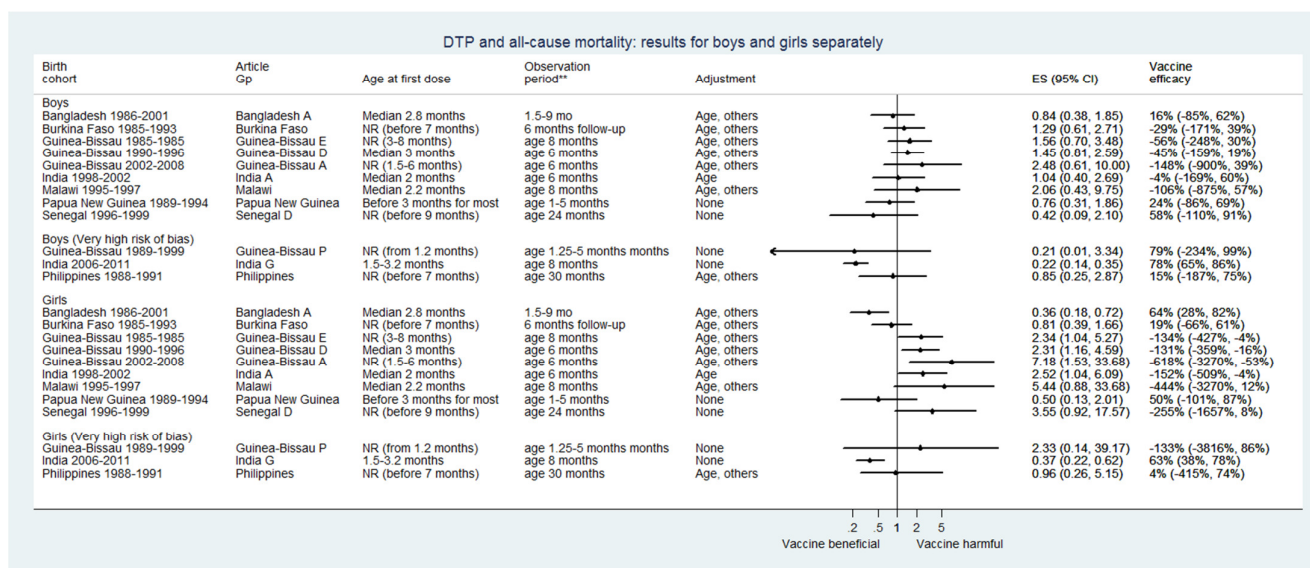
results, with one suggesting a beneficial effect of DTP [Papua New Guinea 1989-1994, #784 (17)], one providing rate ratios in the region of 1 [Burkina Faso 1985-1993, #799 (7)] and two suggesting deleterious effects [Benin 1983-1987, #9372 (20), India 1998-2002, #741 (13)].

The six studies with very high risks of bias produced highly variable results, and do not contribute to our conclusions or GRADE assessments.

4.1 Is there a difference in the effect between boys and girls?

Twelve of the 15 cohort studies provided comparisons of DTP with no DTP separately for boys and girls, including three that were regarded as being at very high risk of bias. The findings are shown in Figure 8. They broadly reflect the main findings above, but suggest that effects may be more deleterious or variable in girls. To examine the potential for differential effects between boys and girls, we need to look at the (statistical) interaction between them. Figure 9 illustrates the difference in vaccine effect between boys and girls, which is equivalent to the comparison in boy-girl mortality ratios between DTP-vaccinated and DTP-unvaccinated children[3]. One of the studies found evidence of a difference [Senegal 1996-1999, #9433 (18)], with 95% confidence intervals indicating that boys benefit more (or equivalently, boys suffer less; this analysis does not tell us about whether DTP vaccine is beneficial or deleterious). For none of the other studies was there similarly strong evidence of a difference in either direction. Four studies in Guinea-Bissau, and three others by these investigators (including the Senegal study), found a tendency for DTP to have a more beneficial effect in boys than in girls. Two of the remaining four studies observed more beneficial effects in girls, with one pointing in neither direction and one observing more beneficial effects in boys; all four were inconclusive.

Figure 8. DTP and all-cause mortality: results for boys and girls separately.

DTP and all-cause mortality: results for boys and girls separately

Birth cohort	Article Gp	Age at first dose	Observation period**	Adjustment	ES (95% CI)	Vaccine efficacy
Boys						
Bangladesh 1986-2001	Bangladesh A	Median 2.8 months	1.5-9 mo	Age, others	0.84 (0.38, 1.85)	16% (-85%, 62%)
Burkina Faso 1985-1993	Burkina Faso	NR (before 7 months)	6 months follow-up	Age, others	1.29 (0.61, 2.71)	-29% (-171%, 39%)
Guinea-Bissau 1985-1985	Guinea-Bissau E	NR (3-8 months)	age 8 months	Age, others	1.56 (0.70, 3.48)	-56% (-248%, 30%)
Guinea-Bissau 1990-1996	Guinea-Bissau D	Median 3 months	age 6 months	Age, others	1.45 (0.81, 2.59)	-45% (-159%, 19%)
Guinea-Bissau 2002-2008	Guinea-Bissau A	NR (1.5-6 months)	age 6 months	Age, others	2.48 (0.61, 10.00)	-148% (-900%, 39%)
India 1998-2002	India A	Median 2 months	age 6 months	Age	1.04 (0.40, 2.69)	-4% (-169%, 60%)
Malawi 1995-1997	Malawi	Median 2.2 months	age 8 months	Age, others	2.06 (0.43, 9.75)	-106% (-875%, 57%)
Papua New Guinea 1989-1994	Papua New Guinea	Before 3 months for most	age 1-5 months	None	0.76 (0.31, 1.86)	24% (-86%, 69%)
Senegal 1996-1999	Senegal D	NR (before 9 months)	age 24 months	None	0.42 (0.09, 2.10)	58% (-110%, 91%)
Boys (Very high risk of bias)						
Guinea-Bissau 1989-1999	Guinea-Bissau P	NR (from 1.2 months)	age 1.25-5 months months	None	0.21 (0.01, 3.34)	79% (-234%, 99%)
India 2006-2011	India G	1.5-3.2 months	age 8 months	None	0.22 (0.14, 0.35)	78% (65%, 86%)
Philippines 1988-1991	Philippines	NR (before 7 months)	age 30 months	Age, others	0.85 (0.25, 2.87)	15% (-187%, 75%)
Girls						
Bangladesh 1986-2001	Bangladesh A	Median 2.8 months	1.5-9 mo	Age, others	0.36 (0.18, 0.72)	64% (28%, 82%)
Burkina Faso 1985-1993	Burkina Faso	NR (before 7 months)	6 months follow-up	Age, others	0.81 (0.39, 1.66)	19% (-66%, 61%)
Guinea-Bissau 1985-1985	Guinea-Bissau E	NR (3-8 months)	age 8 months	Age, others	2.34 (1.04, 5.27)	-134% (-427%, -4%)
Guinea-Bissau 1990-1996	Guinea-Bissau D	Median 3 months	age 6 months	Age, others	2.31 (1.16, 4.59)	-131% (-359%, -16%)
Guinea-Bissau 2002-2008	Guinea-Bissau A	NR (1.5-6 months)	age 6 months	Age, others	7.18 (1.53, 33.68)	-618% (-3270%, -53%)
India 1998-2002	India A	Median 2 months	age 6 months	Age	2.52 (1.04, 6.09)	-152% (-509%, -4%)
Malawi 1995-1997	Malawi	Median 2.2 months	age 8 months	Age, others	5.44 (0.88, 33.68)	-444% (-3270%, 12%)
Papua New Guinea 1989-1994	Papua New Guinea	Before 3 months for most	age 1-5 months	None	0.50 (0.13, 2.01)	50% (-101%, 87%)
Senegal 1996-1999	Senegal D	NR (before 9 months)	age 24 months	None	3.55 (0.92, 17.57)	-255% (-1657%, 8%)
Girls (Very high risk of bias)						
Guinea-Bissau 1989-1999	Guinea-Bissau P	NR (from 1.2 months)	age 1.25-5 months months	None	2.33 (0.14, 39.17)	-133% (-3816%, 86%)
India 2006-2011	India G	1.5-3.2 months	age 8 months	None	0.37 (0.22, 0.62)	63% (38%, 78%)
Philippines 1988-1991	Philippines	NR (before 7 months)	age 30 months	Age, others	0.96 (0.26, 5.15)	4% (-415%, 74%)

.2 .5 1 2 5
Vaccine beneficial   Vaccine harmful

ES = effect size (hazard ratio, rate ratio or risk ratio; odds ratio for the case-control study)
All studies are cohort studies.
**The is the period of observation applicable to the result presented in the forest plot, aiming to capture the effect of DTP with minimal impact of subsequent measles vaccination. The full study may have had a longer period of follow up.

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[3] For two studies [Guinea-Bissau 1989-1999, #2622; Senegal 1996-1999; #9433], boy-girl mortality ratios are used to compute interactions rather than the data in Figure 8, since they allow adjustment for age.

Figure 9. DTP and all-cause mortality: differences between effects in boys and girls.
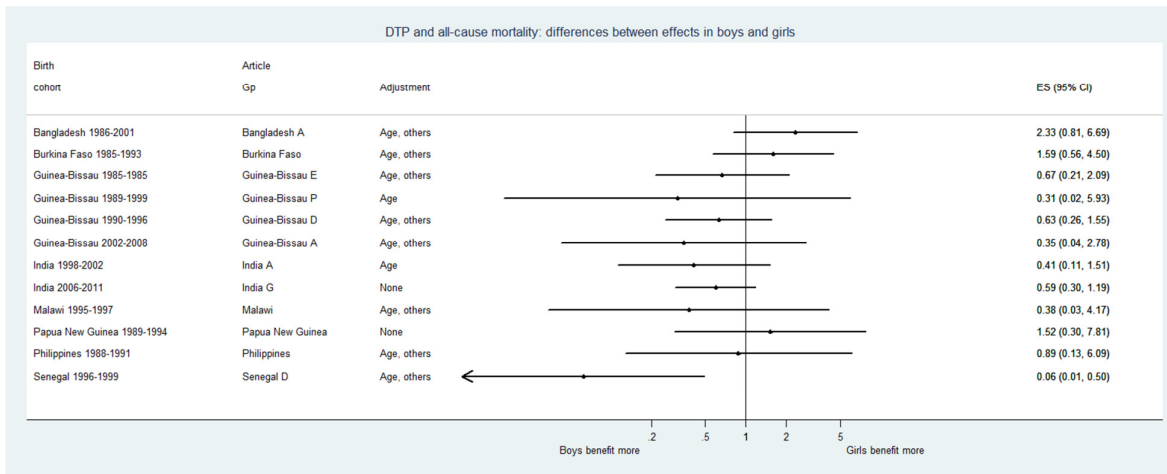
ES = effect size (ratio of hazard ratios, rate ratios or risk ratios)

All studies are cohort studies.

For Guinea-Bissau 1989-1999 and Senegal 1996-1999, interaction estimates are computed from male-female mortality ratios for among DTP recipients and DTP non-recipients; for all other studies they are computed from results in Figure 8.

**The is the period of observation applicable to the result presented in the forest plot, aiming to capture the effect of DTP with minimal impact of subsequent measles vaccination. The full study may have had a longer period of follow up.

4.2 Is there a difference in the effect by age?

Age at vaccination

As can be seen in Figure 7, age at vaccination was variable both within and across studies, and detailed information was not available in many studies. No studies directly reported results for different ages at DTP vaccination. Meaningful examination of differences in effect of DTP according to age at administration was therefore not possible. Further research may be warranted on this question.

Age at follow-up

Four studies report different ages of follow-up [Bangladesh 1986-2001, #797 (6); Ghana 1984-1991, #3294 (25); Guinea-Bissau 1989-1999, #2622 (27); Papua New Guinea 1989-1994, #784 (17)]. These results are illustrated at the top of Figure 10. No consistent pattern is apparent.

Other results by age

Two studies report results by age of the child the time they were seen ('first visit' or 'visit') so the findings either reflect both age at vaccination and age at follow-up [Guinea-Bissau 1990-1996, #2726 (21)] or may be only loosely correlated with age at vaccination [Guinea-Bissau 1984-1985, #851 (9)]. Again no pattern is apparent.

Figure 10. DTP and all-cause mortality: results by age.
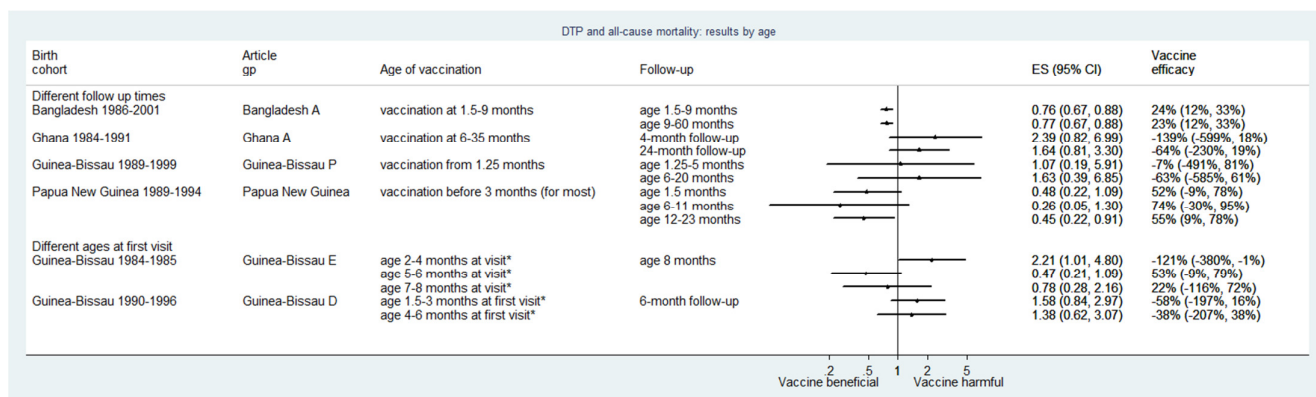
ES = effect size (hazard ratio, rate ratio or risk ratio)

All studies are cohort studies.

*When we know only the age of the children at their visit, the differences likely reflect a combination of age at vaccination and age at follow-up. For Guinea-Bissau 1984-1985, 178 children were seen more than once.

## 4.3 Is there a difference in the effect by vitamin A administration?

We sought results for interaction between DTP effect and (prior or concurrent) administration of vitamin A. Although results relating to the impact of vitamin A on mortality are prevalent in the literature, to understand interaction we needed either (i) the effect of DTP separately among vitamin A recipients and vitamin A non-recipients or (ii) the effect of vitamin A separately among DTP recipients and DTP non-recipients. Only one cohort study [India 1998-2002, #741 (13)] provided this information. This was based on a randomized trial of vitamin A supplementation at birth. The results are illustrated in Figure 11 and Figure 12. There is insufficient evidence concerning any difference in effect of DTP according to vitamin A status.

Figure 11. DTP and all-cause mortality: results for vitamin A recipients and vitamin A non-recipients separately.
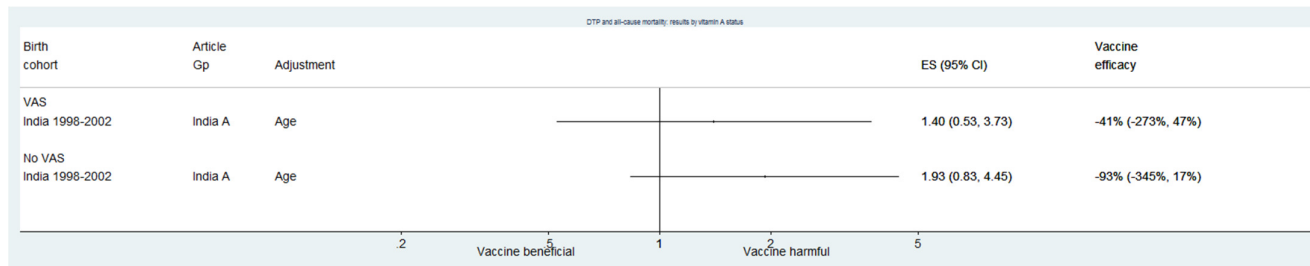
ES = effect size (hazard ratio, rate ratio or risk ratio)
All studies are cohort studies.

Figure 12. DTP and all-cause mortality: differences between effects between vitamin A recipients and vitamin A non-recipients.
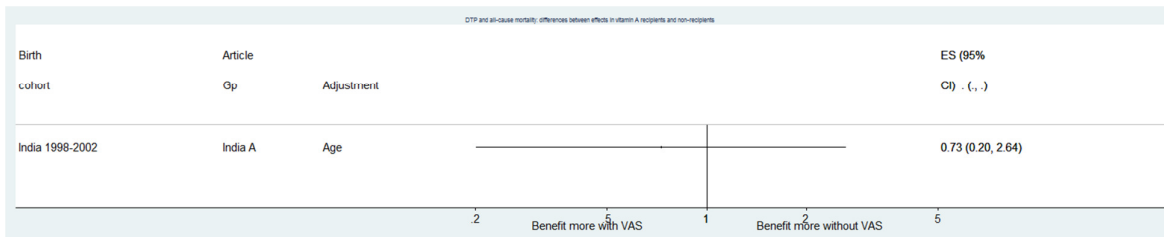
ES = effect size (ratio of hazard ratios, rate ratios or risk ratios)
All studies are cohort studies.

4.4 Comments on study methodology and bias

All of the results from observational studies were judged to be at high risk of bias or very high risk of bias, so all the findings above should be interpreted with caution. Full methodological details and assessments of risk of bias are included in Annex C. We re-emphasize here that DTP was almost always given with OPV, and the findings should be interpreted in that context.

There were 10 observational studies with slightly lesser methodological concerns. All of these were regarded as at risk of bias due to confounding (inherent differences in vaccinated and unvaccinated children). Although most results were adjusted for some confounding factors, only one study addressed a measure from each of our four pre-specified domains of confounding (health of the child, socio-economic status, age and gender), and this was achieved in part by matching children in a case-control design [Benin 1983-1987, #9372 (20)]. Only one further study adjusted for the first three domains [Burkina-Faso 1985-1993, #799 (7)]. Three studies adjusted for a measure of health status of the child [Benin 1983-1987, #9372 (20); Burkina-Faso 1985-1993, #799 (7); Guinea-Bissau 2002-2008, #25 (26)]. Six studies included a measure of socio-economic status [Bangladesh 1986-2001, #9477 (24); Benin 1983-1987, #9372 (20); Burkina-Faso 1985-1993, #799 (7); Guinea-Bissau 1990-1996, #9466 (8); Guinea-Bissau 1984-1985, #851 (9); Senegal 1996-1999, #9433 (18)], mostly using a measure of geographic location.

Four studies were considered to be at risk of bias because children were recruited after vaccines had been given (and hence after the vaccine could have impacted on mortality). In the Burkina-Faso study, children had to survive until the first visit (up to 7 months of age) to be included in the 'landmark approach' analysis, which we selected in preference to the 'retrospective approach' analysis [Burkina-Faso 1985-1993, #799 (7)]. However, we considered this to be at risk of bias

because any child who died before the first visit was unable to contribute to the analysis, as well as misclassification bias (since some children who were vaccinated between visits did not have their status updated). The latter would lead to bias towards the null, which is the reason it is selected in preference to the 'retrospective approach', but the former was considered to lead to a more serious risk of bias, having the potential to switch the direction of effect. In three Guinea-Bissau studies, children were included only if they were seen at a date subsequent to most DTP vaccinations, again raising the risk of bias [Guinea-Bissau 2002-2008, #25 (26); Guinea-Bissau 1990-1996, #9466 (8); Guinea-Bissau 1984-1985, #851 (9)].

Potential for misclassification bias was identified in six further results which made assumptions about non-vaccination of children in the absence of concrete information [Burkina-Faso 1985-1993, #799 (7); Guinea-Bissau 1990-1996, #9466 (8); Guinea-Bissau 1984-1985, #851 (9); India 1998-2002, #741 (13); Malawi 1995-1997, #664 (16); Senegal 1996-1999, #9433 (18)]. One of these also used vaccination information that was updated retrospectively, although the interval between visits to children was short (at two weeks) so this would be unlikely to be very problematic [India 1998-2002, #741 (13)]. In most of the studies, we also had concerns about the possibility of co-interventions (post-vaccination differences between vaccinated and non-vaccinated children), particularly in relation to subsequent measles vaccination. This would be more serious for studies with longer follow-up. Although some studies censored subsequent measles vaccination, we considered this to introduce a different risk of bias because measles vaccination is potentially related to both DTP vaccination and to mortality risk (often known as 'informative censoring').
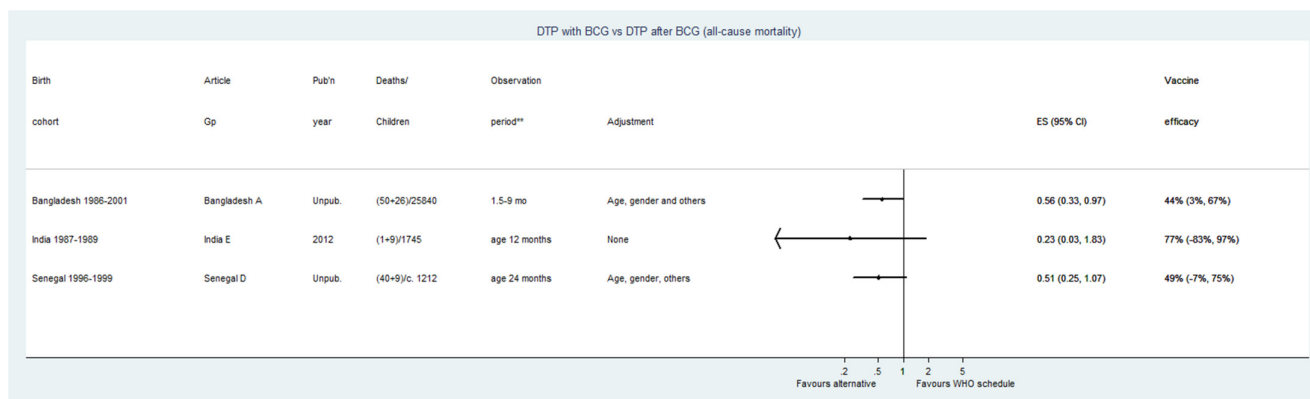
The six 'very high' risk of bias studies were seriously affected by co-administration of BCG [Ghana 1984-1991, #3294 (25)], high correlation between BCG, DTP and measles vaccines [Ghana 1998-2004, #9464 (19)], starting follow-up after DTP vaccination [Ghana 1984-1991, #3294 (25); Guinea-Bissau 1989-1999, #2622 (27); Philippines 1988-1991, #555 (28)], restricting the sample according to measles vaccination [Guinea-Bissau 1989-1999, #2622 (27)], and strong confounding by age [India 1987-1989, #8996 (14); India 2006-2011, #9463 (15)]. Some of these studies had additional reasons for concern (including a possibility that vaccination could be seriously misclassified for children who had died), but for which we were unable to make a firm judgement from the written reports.

We regard the estimates of interaction (for differences by gender and vitamin A) to be less affected by bias, since many of the biases affecting direct estimates of vaccine effects are likely to cancel out when these are contrasted between boys and girls or between vitamin A recipients and vitamin A non-recipients.

5   Does co-administration of BCG and DTP affect all-cause mortality?

Three studies provided results for the comparison of DTP given *simultaneously* with BCG against the current WHO recommendation of DTP after BCG [Bangladesh 1986-2001, #9477 (24); India 1987-1989, #8996 (14); Senegal 1996-1999, #9433 (18)] and these are presented in Figure 13. All results were reported by the Guinea-Bissau investigators; two of them were adjusted for age differences as well as other potential differences between children receiving the different schedules. The three results would suggest that simultaneous administration may be associated with lower mortality; one of them had a 95% confidence interval that excluded 'no difference'.

Figure 13. Sequence of DTP and BCG and all-cause mortality: simultaneous administration of DTP and BCG compared with BCG before DTP.

Birth cohort	Article Gp	Pub'n year	Deaths/ Children	Observation period**	Adjustment		ES (95% CI)	Vaccine efficacy
Bangladesh 1986-2001	Bangladesh A	Unpub.	(50+26)/25840	1.5-9 mo	Age, gender and others		0.56 (0.33, 0.97)	44% (3%, 67%)
India 1987-1989	India E	2012	(1+9)/1745	age 12 months	None		0.23 (0.03, 1.83)	77% (-83%, 97%)
Senegal 1996-1999	Senegal D	Unpub.	(40+9)/c. 1212	age 24 months	Age, gender, others		0.51 (0.25, 1.07)	49% (-7%, 75%)

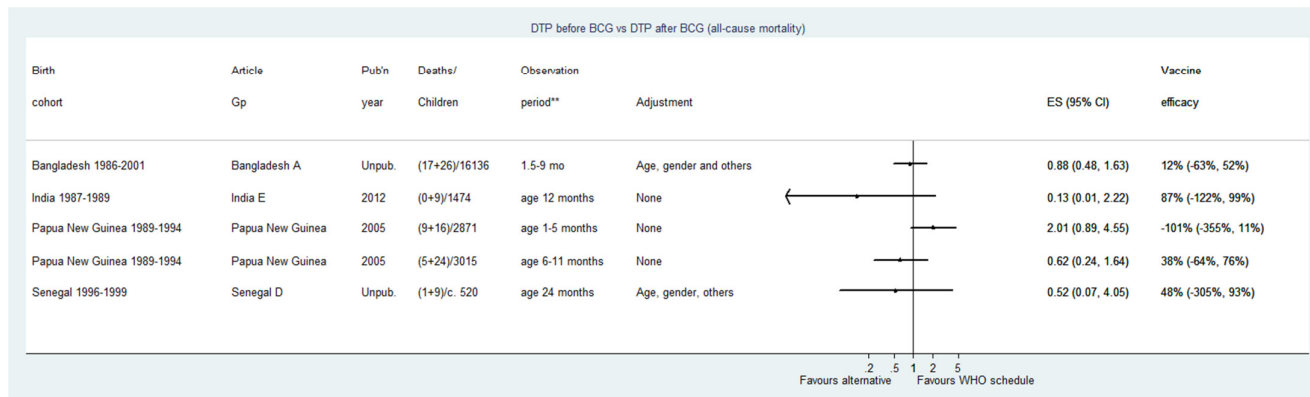ES = effect size (hazard ratio, rate ratio or risk ratio)
Deaths/Children = (Deaths simultaneous + Deaths WHO recommended)/Total children or Total deaths/Total children
All studies are cohort studies.

6 Does order of BCG and DTP affect all-cause mortality?

Three studies reported results for the comparison of DTP given *before* BCG against the current WHO recommendation of DTP after BCG [Bangladesh 1986-2001, #9477 (24); India 1987-1989, #8996 (14); Senegal 1996-1999, #9433 (18)]. A fourth study reported on DTP vaccine given *before or with* BCG versus the current WHO recommendation [Papua New Guinea 1989-1994, #784 (17)]. We present these results, including two different periods of observation for Papua New Guinea, in Figure 14. No clear differences are apparent.

Figure 14. Sequence of DTP and BCG and all-cause mortality: administration of DTP before BCG compared with BCG before DTP.

DTP before BCG vs DTP after BCG (all-cause mortality)

Birth cohort	Article Gp	Pub'n year	Deaths/ Children	Observation period**	Adjustment	ES (95% CI)	Vaccine efficacy
Bangladesh 1986-2001	Bangladesh A	Unpub.	(17+26)/16136	1.5-9 mo	Age, gender and others	0.88 (0.48, 1.63)	12% (-63%, 52%)
India 1987-1989	India E	2012	(0+9)/1474	age 12 months	None	0.13 (0.01, 2.22)	87% (-122%, 99%)
Papua New Guinea 1989-1994	Papua New Guinea	2005	(9+16)/2871	age 1-5 months	None	2.01 (0.89, 4.55)	-101% (-355%, 11%)
Papua New Guinea 1989-1994	Papua New Guinea	2005	(5+24)/3015	age 6-11 months	None	0.62 (0.24, 1.64)	38% (-64%, 76%)
Senegal 1996-1999	Senegal D	Unpub.	(1+9)/c. 520	age 24 months	Age, gender, others	0.52 (0.07, 4.05)	48% (-305%, 93%)

.2 .5 1 2 5
Favours alternative   Favours WHO schedule

ES = effect size (hazard ratio, rate ratio or risk ratio)
Deaths/Children = (Deaths reverse order + Deaths WHO recommended)/Total children or Total deaths/Total children
All studies are cohort studies.

7 Is administration of measles containing vaccine in infancy associated with an effect on all-cause mortality?

Four randomized trials (29-32), 22 cohort studies (6, 14-19, 21, 25, 33-44) and two case-control studies (20, 45) were identified that provided comparisons of mortality among children who had or had not received measles vaccine. These results are presented in Figure 15. Six results from the cohort studies were considered to be at very high risk of bias in relation to the effect of measles vaccine, and are presented separately at the bottom of the forest plot.
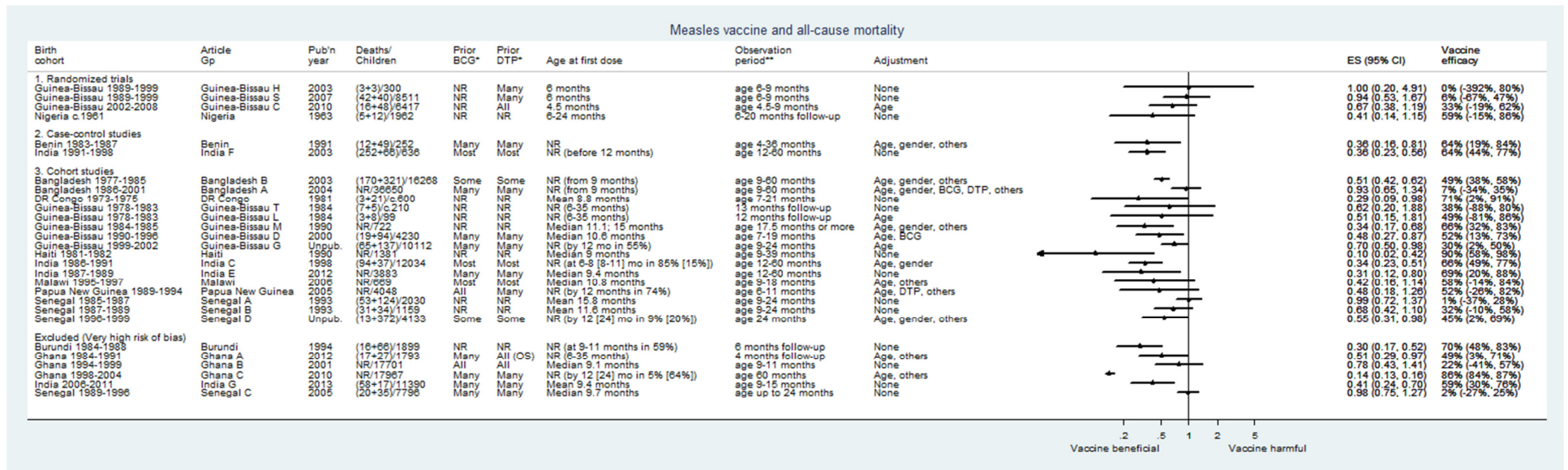
From the randomized trials in Guinea-Bissau, we present results for mortality up to 9 months, at which point all three administered measles vaccine in the control group. Due to the short follow-up, the numbers of deaths were low and the findings inconclusive. Directions of effect in these trials, as well as the fourth trial in Nigeria, pointed towards a beneficial effect of measles vaccine.

Excluding the results considered to be at very high risk of bias, the results of the 18 non-randomized studies (all considered nevertheless to be at high risk of bias) consistently observed effects indicating a beneficial effect of measles vaccine on mortality. For 11 of these, the 95% confidence interval excluded no effect. Estimated effects are in the region of a halving of mortality risk. Again, most of the analyses were undertaken by the Guinea-Bissau investigators.

The six studies with very high risks of bias produced variable results. Their results however point in the same direction as all the other studies: of a beneficial effect of measles vaccine on overall mortality.

As noted in the Background, we present here the data on all-cause mortality, rather than an examination of the 'non-specific effects' of measles vaccine on deaths from causes other than measles. In populations with very high coverage of measles vaccine, deaths from measles should be infrequent. In Annex D we present the results we extracted where measles deaths had been removed or censored. They suggest that if these effects are real then they are not fully explained by deaths that were established as due to measles.

Figure 15. Measles vaccine and all-cause mortality.

Measles vaccine and all-cause mortality

Birth cohort	Article Gp	Pub'n year	Deaths/Children	Prior BCG*	Prior DTP*	Age at first dose	Observation period**	Adjustment	ES (95% CI)	Vaccine efficacy
1. Randomized trials										
Guinea-Bissau 1989-1999	Guinea-Bissau H	2003	(3+3)/300	NR	Many	6 months	age 6-9 months	None	1.00 (0.20, 4.91)	0% (-392%, 80%)
Guinea-Bissau 1989-1999	Guinea-Bissau S	2007	(42+40)/8511	NR	Many	6 months	age 6-9 months	None	0.94 (0.53, 1.67)	6% (-67%, 47%)
Guinea-Bissau 2002-2008	Guinea-Bissau C	2010	(16+48)/6417	NR	All	4.5 months	age 4.5-9 months	Age	0.67 (0.38, 1.19)	33% (-19%, 62%)
Nigeria c.1961	Nigeria	1963	(5+12)/1962	NR	NR	6-24 months	6-20 months follow-up	None	0.41 (0.14, 1.15)	59% (-15%, 86%)
2. Case-control studies										
Benin 1983-1987	Benin	1991	(12+49)/252	Many	Many	NR	age 4-36 months	Age, gender, others	0.36 (0.16, 0.81)	64% (19%, 84%)
India 1991-1998	India F	2003	(252+66)/636	Most	Most	NR (before 12 months)	age 12-60 months	None	0.36 (0.23, 0.56)	64% (44%, 77%)
3. Cohort studies										
Bangladesh 1977-1985	Bangladesh B	2003	(170+321)/16268	Some	Some	NR (from 9 months)	age 9-60 months	Age, gender, others	0.51 (0.42, 0.62)	49% (38%, 58%)
Bangladesh 1986-2001	Bangladesh A	2004	NR/36650	Many	Many	NR (from 9 months)	age 9-60 months	Age, gender, BCG, DTP, others	0.93 (0.65, 1.34)	7% (-34%, 35%)
DR Congo 1973-1975	DR Congo	1981	(3+21)/c.600	NR	NR	Mean 8.8 months	age 7-21 months	None	0.29 (0.09, 0.98)	71% (2%, 91%)
Guinea-Bissau 1978-1983	Guinea-Bissau T	1984	(7+5)/c.210	NR	NR	NR (6-35 months)	13 months follow-up	None	0.62 (0.20, 1.88)	38% (-88%, 80%)
Guinea-Bissau 1978-1983	Guinea-Bissau L	1984	(3+8)/99	NR	NR	NR (6-35 months)	12 months follow-up	Age	0.51 (0.15, 1.81)	49% (-81%, 86%)
Guinea-Bissau 1984-1985	Guinea-Bissau M	1990	NR/722	NR	NR	Median 11.1; 15 months	age 17.5 months or more	Age, gender, others	0.34 (0.17, 0.68)	66% (32%, 83%)
Guinea-Bissau 1990-1996	Guinea-Bissau D	2000	(19+94)/4230	Many	Many	Median 10.6 months	age 7-19 months	Age, BCG	0.48 (0.27, 0.87)	52% (13%, 73%)
Guinea-Bissau 1999-2002	Guinea-Bissau G	Unpub.	(65+137)/10112	Many	Many	NR (by 12 mo in 55%)	age 9-24 months	Age	0.70 (0.50, 0.98)	30% (2%, 50%)
Haiti 1981-1982	Haiti	1990	NR/1381	NR	NR	Median 9 months	age 9-39 months	None	0.10 (0.02, 0.42)	90% (58%, 98%)
India 1986-1991	India C	1998	(94+37)/12034	Most	Most	NR (at 6-8 [8-11] mo in 85% [15%])	age 12-60 months	Age, gender	0.34 (0.23, 0.51)	66% (49%, 77%)
India 1987-1989	India E	2012	NR/3883	Many	Many	Median 9.4 months	age 12-60 months	None	0.31 (0.12, 0.80)	69% (20%, 88%)
Malawi 1995-1997	Malawi	2006	NR/669	Most	Most	Median 10.8 months	age 9-18 months	Age, others	0.42 (0.16, 1.14)	58% (-14%, 84%)
Papua New Guinea 1989-1994	Papua New Guinea	2005	NR/4048	All	Many	NR (by 12 months in 74%)	age 6-11 months	Age, DTP, others	0.48 (0.18, 1.26)	52% (-26%, 82%)
Senegal 1985-1987	Senegal A	1993	(53+124)/2030	NR	NR	Mean 15.8 months	age 9-24 months	None	0.99 (0.72, 1.37)	1% (-37%, 28%)
Senegal 1987-1989	Senegal B	1993	(31+34)/1159	NR	NR	Mean 11.6 months	age 9-24 months	None	0.68 (0.42, 1.10)	32% (-10%, 58%)
Senegal 1996-1999	Senegal D	Unpub.	(13+372)/4133	Some	Some	NR (by 12 [24] mo in 9% [20%])	age 24 months	Age, gender, others	0.55 (0.31, 0.98)	45% (2%, 69%)
Excluded (Very high risk of bias)										
Burundi 1984-1988	Burundi	1994	(16+66)/1899	NR	NR	NR (at 9-11 months in 59%)	6 months follow-up	None	0.30 (0.17, 0.52)	70% (48%, 83%)
Ghana 1984-1991	Ghana A	2012	(17+27)/1793	Many	All (OS)	NR (6-35 months)	4 months follow-up	Age, others	0.51 (0.29, 0.97)	49% (3%, 71%)
Ghana 1994-1999	Ghana B	2001	NR/17701	All	All	Median 9.1 months	age 9-11 months	None	0.78 (0.43, 1.41)	22% (-41%, 57%)
Ghana 1998-2004	Ghana C	2010	NR/17967	Many	Many	NR (by 12 [24] mo in 5% [64%])	age 60 months	Age, others	0.14 (0.13, 0.16)	86% (84%, 87%)
India 2006-2011	India G	2013	(58+17)/11390	Many	Many	Mean 9.4 months	age 9-15 months	None	0.41 (0.24, 0.70)	59% (30%, 76%)
Senegal 1989-1996	Senegal C	2005	(20+35)/7796	Many	Many	Median 9.7 months	age up to 24 months	None	0.98 (0.75, 1.27)	2% (-27%, 25%)

Forest plot axis: .2   .5   1   2   5
Vaccine beneficial | Vaccine harmful

ES = effect size (hazard ratio, rate ratio or risk ratio; odds ratio for the case-control studies)

Deaths/Children = (Measles vaccine deaths + Non-measles vaccine deaths)/Total children or Total deaths/Total children

In most observational studies with 'None' as adjustment for confounding, we computed unadjusted rate ratios from rates presented in the paper.

Vaccine efficacy is computed as (1 – ES) × 100%. A non-negative number describes the proportion of deaths prevented by the vaccine. A negative number reflects a higher death rate among the vaccinated. For example, if vaccine efficacy = -100%, then an *additional* 100% of the deaths that would have occurred without vaccine would occur with the vaccine.

*Prior BCG: whether children studied had received BCG. Prior DTP: whether children studied had received DTP.

OS = often given simultaneously with DTP.

**This is the period of observation applicable to the result presented in the forest plot. The full study may have had a longer period of follow up.

7.1   Is there a difference in the effect between boys and girls?

Nine of the cohort studies and two randomized trials provided comparisons of measles vaccine with no measles vaccine separately for boys and girls; three of the cohort studies were considered to be at very high risk of bias. The findings are shown in Figure 16. Effects of the vaccine in girls appear to be more beneficial than in boys. Figure 17 illustrates the difference in vaccine effect between boys and girls, which is equivalent to the comparison in boy-girl mortality ratios between measles-vaccinated and measles-unvaccinated children[4]. Three of the studies found statistical evidence of a difference, indicating that girls benefit more. The other studies did not find convincing evidence in either direction.

Figure 16. Measles vaccine and all-cause mortality: results for boys and girls separately.
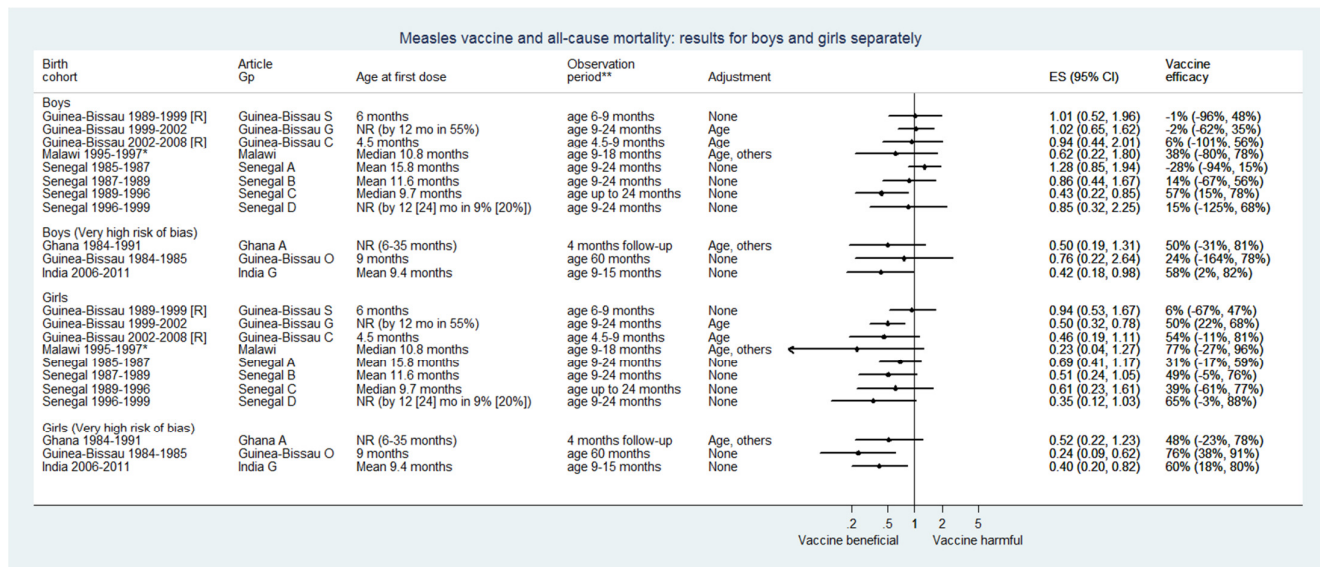
ES = effect size (hazard ratio, rate ratio or risk ratio)
R = Randomized trial (all other studies are cohort studies)
*Results from an analysis with vaccination status updated retrospectively. In an analysis with vaccination status updated prospectively (the preferred landmark approach) there were no deaths among the girls, so interaction cannot be computed along with a confidence interval. Among the boys, the MRR was 0.66 (95% CI 0.22, 2.03)
**The is the period of observation applicable to the result presented in the forest plot. The full study may have had a longer period of follow up.

Figure 17. Measles vaccine and all-cause mortality: differences between effects in boys and girls.
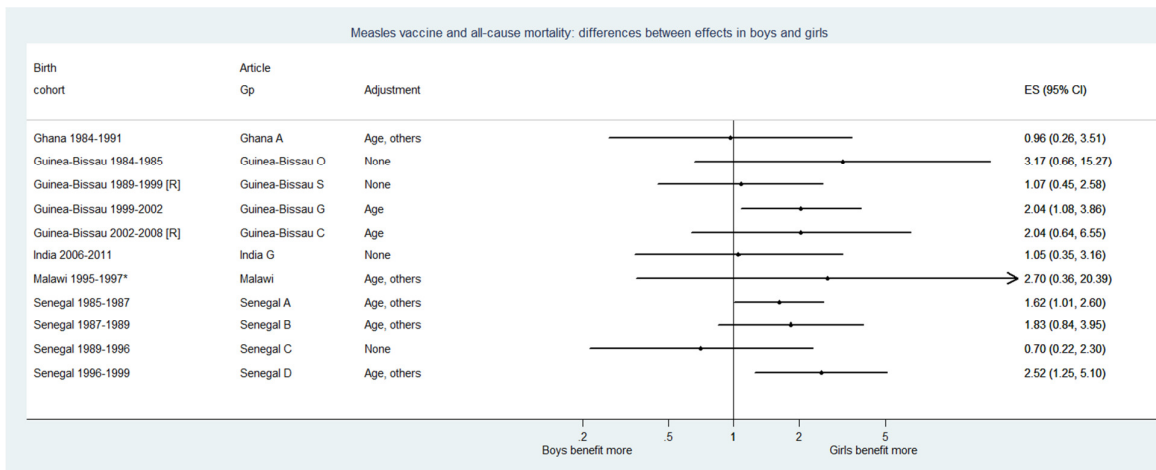
ES = effect size (hazard ratio, rate ratio or risk ratio)
R = Randomized trial (all other studies are cohort studies)
*Results from analysis with vaccination status updated retrospectively.

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[4] For three studies [Senegal 1985-1987 and 1987-1989, #6904; Senegal 1996-1999, #9433], boy-girl mortality ratios are used to compute interactions rather than the data in Figure 16, since they allow for adjustment for age.

7.2 Is there a difference in the effect by age?

Age at vaccination

As can be seen in Figure 15, age at vaccination was not always reported. Where vaccination ages were available, they ranged from 4.5 months in one of the randomized trials to a median of 15.8 months in one of the cohort studies. Vaccination typically took place shortly after children were 9 months old. Meaningful examination of differences in effect of measles vaccination according to age at administration was not possible from these results. One case-control study reported effects separately for children vaccinated before 12 months and after 12 months, shown at the top of Figure 18.

The three randomized trials involving early measles vaccination (at 4.5 or 6 months) included vaccination for all children at 9 months, so a comparison of early versus later vaccination is not offered by these trials. Another included article provided some relevant information, although the results were not included formally in the review because our eligibility criteria did not include direct comparisons of ages at vaccination: in the Guinea-Bissau 1978-1983 cohort (46), a comparison of mortality after vaccination at age 4-8 months with vaccination at age 9-11 months suggested a more beneficial effect in the earlier period. Further research may be warranted on the question of age at measles vaccination.

Age at follow-up

Figure 18 presents results for different follow-up periods in nine studies. The Figure also includes results for a study that provided effect estimates for children entering the study at different ages. No consistent patterns are discernible across these findings.

Figure 18. Measles vaccine and all-cause mortality: results by age.
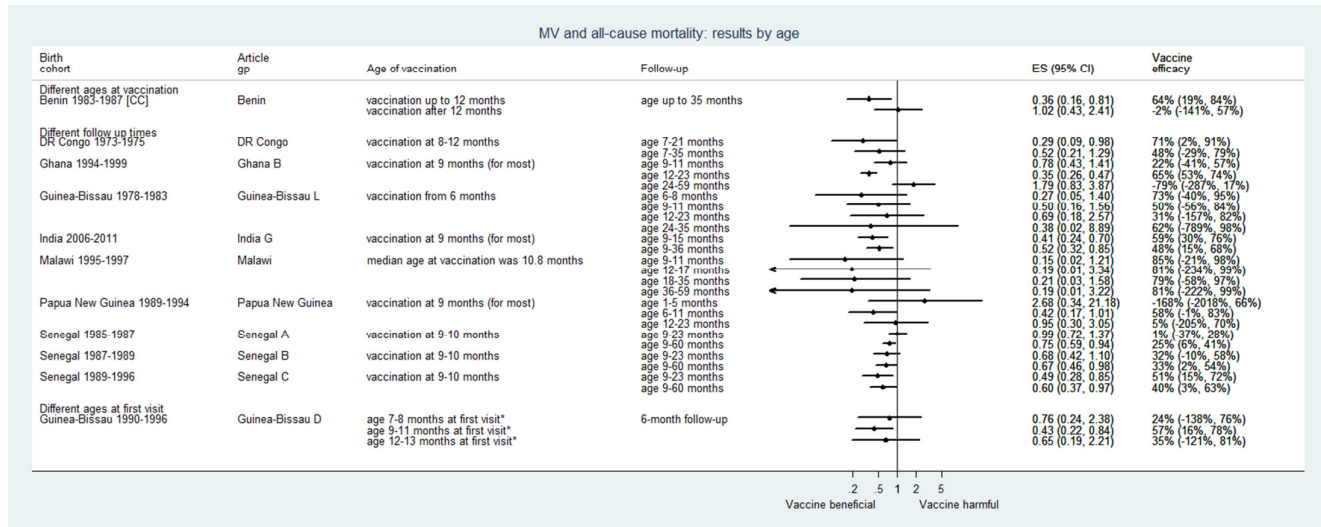
ES = effect size (hazard ratio, rate ratio or risk ratio)
CC = Case-control study (all others studies are cohort studies).
*When we know only the age of the children at their first visit, the differences likely reflect a combination of age at vaccination and age at follow-up.

7.3 Is there a difference in the effect by vitamin A administration?

We identified three studies providing results for interaction between measles vaccination and (prior or concurrent) administration of vitamin A. The vaccine effects are presented separately for vitamin A recipients and non-recipients in Figure 19, and the differences between them are given in Figure 20. There is no consistent message across these studies concerning a difference in effect of measles vaccine according to vitamin A status.

Figure 19. Measles vaccine and all-cause mortality: results for vitamin A recipients and vitamin A non-recipients separately.

Birth cohort	Article Gp	Adjustment		ES (95% CI)	Vaccine efficacy
VAS					
Guinea-Bissau 1999-2002	Guinea-Bissau G	None		0.81 (0.11, 6.04)	19% (-504%, 89%)
Guinea-Bissau 1989-1999 [R]	Guinea-Bissau H	None		0.14 (0.01, 2.65)	86% (-165%, 99%)
Guinea-Bissau 2002-2008	Guinea-Bissau A	None		1.13 (0.54, 2.38)	-13% (-138%, 46%)
No VAS					
Guinea-Bissau 1999-2002	Guinea-Bissau G	None		4.32 (0.87, 21.37)	-332% (-2037%, 13%)
Guinea-Bissau 2002-2008	Guinea-Bissau A	None		0.08 (0.00, 1.33)	92% (-33%, 100%)
Guinea-Bissau 1989-1999 [R]	Guinea-Bissau H	None		7.19 (0.38, 136.87)	-619% (-13581%, 62%)
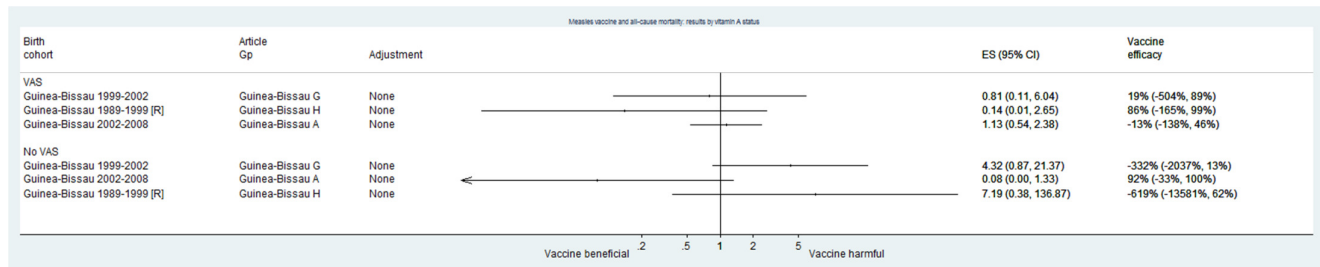
ES = effect size (hazard ratio, rate ratio or risk ratio)
R = Randomized trial (all other studies are cohort studies)

Figure 20. Measles vaccine and all-cause mortality: differences between effects between vitamin A recipients and vitamin A non-recipients.

Birth cohort	Article Gp	Adjustment	ES (95% CI)
Guinea-Bissau 1989-1999 [R]	Guinea-Bissau H	None	0.02 (0.00, 1.25)
Guinea-Bissau 1999-2002	Guinea-Bissau G	None	0.19 (0.01, 2.44)
Guinea-Dissau 2002-2000	Guinea-Dissau A	None	14.13 (0.77, 250.42)
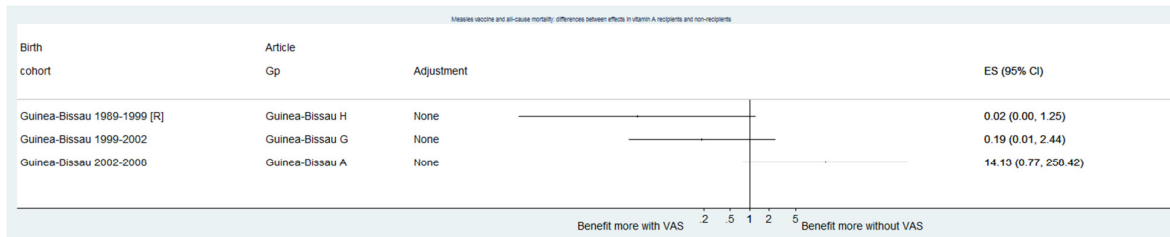
ES = effect size (hazard ratio, rate ratio or risk ratio)
R = Randomized trial (all other studies are cohort studies)

7.4   Comments on study methodology and bias

All of the results from observational studies were judged to be at high risk of bias or very high risk of bias, so all the findings above should be interpreted with caution. As for the other vaccines, the main potential sources of bias were confounding (again an important issue for all studies); bias arising from selection of participants some time after vaccines had been administered; misclassification bias relating to ascertainment of vaccination status; and differences between groups with regard to DTP administration. For instance, among the 18 observational studies that were at slightly lesser risk of bias, four were restricted to children observed at a time point after measles vaccinations had taken place, raising the possibility of selection bias [Guinea-Bissau 1990-1996, #2726 (21); Guinea-Bissau 1999-2002, #9441 (37); Haiti 1981-1982, 7013 (41); India 1986-1991, #6720 (42)]. Three results made assumptions about non-vaccinated children [Guinea-Bissau 1990-1996, #2726 (21); India 1987-1989, #8996 (14); Malawi 1995-1997, #664 (16)]. Information about potential co-interventions between administration of measles vaccine and the end of the follow-up period was seldom available.

The very high risk of bias studies were seriously affected by recruiting children long after their vaccinations [Burundi 1984-1988, #6889 (34)]; strong confounding by DTP and/or BCG administration [Ghana 1984-1991, #3294 (25); Ghana 1998-2004, #9464 (19); Senegal 1989-1996, #740 (44)] or by age [India 2006-2011, #9463] and various problems caused by visits to children being annual [Ghana 1994-1999, #7190]. Some of these studies had additional reasons for serious concern, but for which we were unable to make a judgement from the written reports.

We regard the estimates of interaction (for differences by gender and vitamin A) to be less affected by bias, since many of the biases affecting direct estimates of vaccine effects are likely to cancel out when these are contrasted between boys and girls or between vitamin A recipients and vitamin A non-recipients.

For full details of methodological features and assessments of risk of bias, see Annex C.

8   Does co-administration of DTP and measles vaccine affect all-cause mortality?

Five studies provided results for the comparison of DTP given *simultaneously* with measles vaccine against the current WHO recommendation of measles vaccine after DTP and these are presented in Figure 21 [combined analysis of Guinea-Bissau 1990-1996 and 1999-2002, #2218 (47); Guinea-Bissau 1999-2002, #9442 (48); India 1987-1989, #8996 (14); Malawi, #664 (16); Senegal 1996-1999, #9433 (18)]. All results were reported by the Guinea-Bissau investigators; three of them

were adjusted for age differences and two of these also for other potential differences between children receiving the different schedules. The five results would suggest that simultaneous administration may be associated with higher mortality.

Figure 21. Sequence of DTP and measles vaccine and all-cause mortality: simultaneous administration of DTP and measles vaccine compared with DTP before measles vaccine.
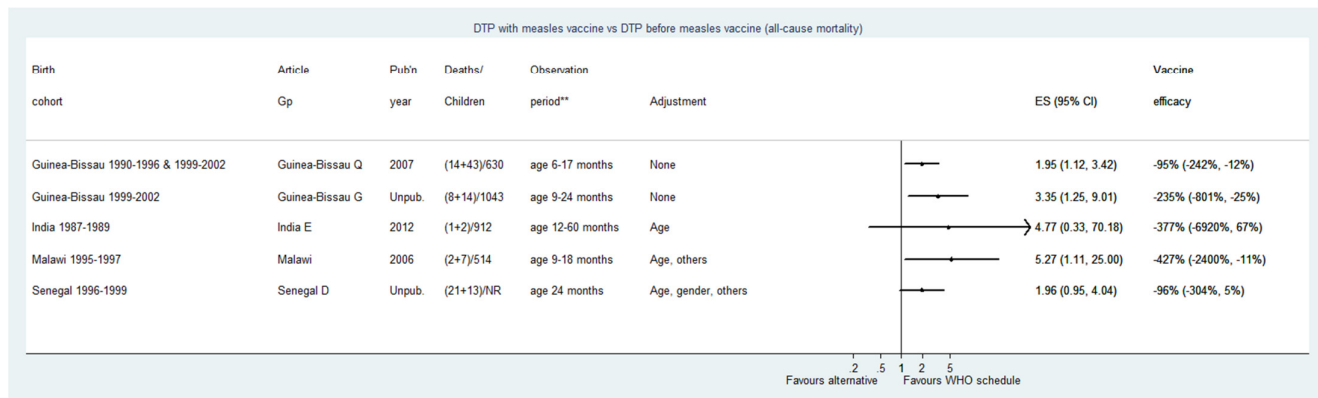
ES = effect size (hazard ratio, rate ratio or risk ratio)
Deaths/Children = (Deaths simultaneous + Deaths WHO recommended)/Total children or Total deaths/Total children
All studies are cohort studies.

9   Does order of DTP and measles vaccine affect all-cause mortality?

Three studies reported results for the comparison of DTP given *after* measles vaccine against the current WHO recommendation of DTP before measles vaccine [combined analysis of Guinea-Bissau 1990-1996 and 1999-2002, #2218 (47); India 1987-1989, #8996 (14); Senegal 1996-1999, #9433 (18)]. We present these results in Figure 22. The three results would suggest that giving DTP after measles vaccine may be associated with higher mortality.

Figure 22. Sequence of DTP and measles vaccine and all-cause mortality: administration of DTP after measles vaccine compared with DTP before measles vaccine.
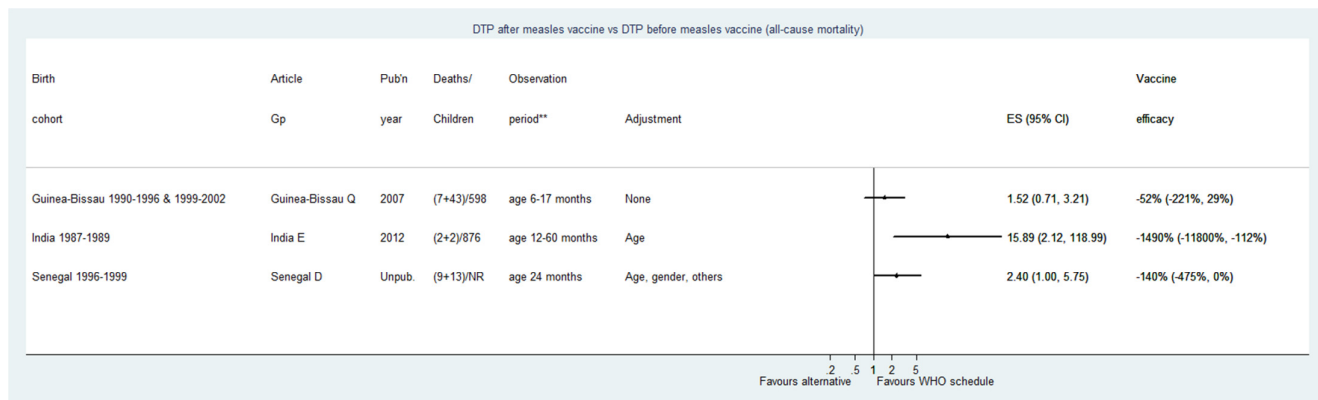
ES = effect size (hazard ratio, rate ratio or risk ratio)
Deaths/Children = (Deaths reverse order + Deaths WHO recommended)/Total children or Total deaths/Total children
All studies are cohort studies.

10 Summary of the evidence

Here we present GRADE assessments, including summary conclusions, for the seven comparisons addressed in the report. Because in each case a large majority of the included evidence is from non-randomized studies, the starting point for each assessment is a score of 2 (equivalent to the interpretation 'Our confidence in the estimate of the effect on the health outcome is limited'). The score can be decreased or increased according to specific factors. In no instance did we regard it appropriate to increase the score, and in most instances we had less confidence so assigned a score of 1 (equivalent to the interpretation 'We have very little confidence in the estimate of the effect on the health outcome').

Does administration of BCG vaccine in infancy affect all-cause mortality?				
			Rating	Adjustment to rating
Quality Assessment	No of studies/starting score		14 studies (5 trials, 9 observational)[1]	2
	Factors decreasing confidence	Limitation in study design	Serious[2]	(0)
		Inconsistency	None serious	0
		Indirectness	Serious[3]	−1
		Imprecision	None serious	0
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Large effect[5]	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			1
Summary of findings	**Statement on quality of evidence**			We have very little confidence in the evidence about the effect of BCG vaccine on all-cause mortality.
	Conclusion			BCG vaccine may reduce risk of all-cause mortality.

[1]Excluding studies assessed to be at very high risk of bias.
[2]Addressed by starting score of 2.
[3]Large proportion of studies from one region in West Africa; short follow-up used in randomized trials.

[4]Many confidence intervals were compatible with higher, unchanged and lower risk of mortality.   [5]Typical rate ratio in the region of 50% reduction.

Does administration of DTP* vaccine in infancy affect all-cause mortality?				
			Rating	Adjustment to rating
Quality Assessment	No of studies/starting score		10 observational studies[1]	2
	Factors decreasing confidence	Limitation in study design	Serious[2]	(0)
		Inconsistency	Very serious[3]	−1
		Indirectness	Serious[4]	(0)
		Imprecision	Serious[5]	(0)
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Not demonstrated	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			1
Summary of findings	**Statement on quality of evidence**			We have very little confidence in the evidence about the effect of DTP vaccine on all-cause mortality.
	Conclusion			Insufficient evidence to draw a conclusion about the effect of DTP vaccine on all-cause mortality.

*DTP was nearly always administered with OPV.
[1]Excluding studies assessed to be at very high risk of bias.
[2]Addressed by starting score of 2.
[3]Inconsistent directions of effect.

[4]Large proportion of studies from one region in West Africa.
[5]Many confidence intervals were compatible with higher, unchanged and lower risk of mortality.

Does <u>simultaneous</u> administration of DTP and BCG vaccine in infancy affect all-cause mortality (compared with BCG followed by DTP)?

			Rating	Adjustment to rating
Quality Assessment	No of studies/starting score		3 observational studies	2
	Factors decreasing confidence	Limitation in study design	Serious[1]	(0)
		Inconsistency	None serious	0
		Indirectness	Serious[2]	(0)
		Imprecision	Serious[3]	−1
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Large effect[4]	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			1
Summary of findings	**Statement on quality of evidence**			We have very little confidence in the evidence about the effect of simultaneous administration of DTP and BCG vaccines on all-cause mortality.
	Conclusion			Simultaneous administration of DTP and BCG vaccines may reduce risk of all-cause mortality compared with BCG followed by DTP.

[1]Addressed by starting score of 2.
[2]Two of the three studies from one region in West Africa.
[3]Only three studies (135 deaths).
[4]Typical rate ratio in the region of 50% reduction.

Does administration of DTP <u>before</u> BCG vaccine in infancy affect all-cause mortality (compared with BCG followed by DTP)?

			Rating	Adjustment to rating
Quality Assessment	No of studies/starting score		4 observational studies[1]	2
	Factors decreasing confidence	Limitation in study design	Serious[2]	(0)
		Inconsistency	None apparent	0
		Indirectness	Not apparent	0
		Imprecision	Serious[3]	−1
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Not demonstrated	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			1
Summary of findings	**Statement on quality of evidence**			We have very little confidence in the evidence about the effect of ordering of DTP and BCG vaccines on all-cause mortality.
	Conclusion			Insufficient evidence to draw a conclusion about the effect of order of administration of BCG and DTP vaccines on all-cause mortality.

[1]Including one study with DTP vaccine given *before or with* BCG, for which we presented two time periods in the forest plot.
[2]Addressed by starting score of 2.
[3]Only five studies (116 deaths); confidence intervals were compatible with higher, unchanged and lower risk of mortality.

Does administration of measles vaccine in infancy affect all-cause mortality?				
			Rating	Adjustment to rating
Quality Assessment		No of studies/starting score	22 studies (4 trials, 18 observational)[1]	2
	Factors decreasing confidence	Limitation in study design	Serious[2]	(0)
		Inconsistency	None serious	0
		Indirectness	None serious[3]	(0)
		Imprecision	None serious	0
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Large effect[4]	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			2
Summary of findings			**Statement on quality of evidence**	Our confidence in the effect of measles vaccine on all-cause mortality is limited.
			Conclusion	Measles vaccine may reduce risk of all-cause mortality (an effect that appears stronger in girls than boys).

[1]Excluding studies assessed to be at very high risk of bias.
[2]Addressed by starting score of 2.
[3]We have some concerns, however, about the large proportion of studies from one region in West Africa, and about the short follow-up available in randomized trials.
[4]Typical rate ratio in the region of 50% reduction.

Does <u>simultaneous</u> administration of DTP and measles vaccine in infancy affect all-cause mortality (compared with DTP followed by measles vaccine)?				
			Rating	Adjustment to rating
Quality Assessment		No of studies/starting score	5 observational studies	2
	Factors decreasing confidence	Limitation in study design	Serious[1]	(0)
		Inconsistency	None serious	0
		Indirectness	Serious[2]	(0)
		Imprecision	Serious[3]	−1
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Large effect[4]	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			1
Summary of findings			**Statement on quality of evidence**	We have very little confidence in the evidence about the effect of simultaneous administration of DTP and measles vaccines on all-cause mortality.
			Conclusion	Simultaneous administration of DTP and measles vaccines may be associated with higher risk of all-cause mortality compared with DTP followed by measles vaccine.

[1]Addressed by starting score of 2.
[2]Three of the five studies are from one region in West Africa.
[3]Only five studies (125 deaths).
[4]Typical rate ratio compatible with more than two-fold higher mortality rate.

Does administration of measles <u>before</u> DTP vaccine in infancy affect all-cause mortality (compared with DTP followed by measles vaccine)?				
			Rating	Adjustment to rating
Quality Assessment	No of studies/starting score		3 observational studies	2
	Factors decreasing confidence	Limitation in study design	Serious[1]	(0)
		Inconsistency	None serious	0
		Indirectness	Serious[2]	(0)
		Imprecision	Serious[3]	−1
		Publication bias	Not apparent	0
	Factors increasing confidence	Strength of association	Large effect[4]	N/A
		Dose-response	Not demonstrated	N/A
		Mitigated bias and confounding	Not demonstrated	N/A
	Final numerical score of quality of evidence			1
Summary of findings	Statement on quality of evidence			We have very little confidence in the evidence about the effect of ordering of DTP and measles vaccines on all-cause mortality.
	Conclusion			Reversing the usual order of administration of DTP and measles vaccines may be associated with higher risk of all-cause mortality compared with DTP followed by measles vaccine.

[1]Addressed by starting score of 2.
[2]Two of the three studies are from one region in West Africa.
[3]Only three studies (76 deaths).

[4]Typical rate ratio compatible with more than two-fold higher mortality rate..

10.1 Closing remarks on risk of bias

Some of the biases described in our assessment were related to the decisions we made in selecting data to best address the policy questions set forth for this review; however in no case was there a relevant result with a lower risk of bias. Different biases were considered likely to operate in different directions. Baseline confounding, if ignored, would tend to lead to bias towards a beneficial effect of the vaccine, because children with a worse prognosis generally tended to be vaccinated later or not vaccinated at all (sometimes described as 'frailty bias'). Some selection biases were expected to operate in the opposite direction: if children are recruited some time after vaccination then early deaths among unvaccinated children – that might have been prevented had they been vaccinated – are not counted and the bias works against the vaccine and can switch the direction of effect. Misclassification of vaccinated children as unvaccinated would lead to bias towards the null (no effect), as occurs when a 'landmark' approach is taken to the analysis (23). Previous receipt, co-administration and subsequent administration of other vaccines (e.g. DTP or measles vaccine when examining BCG) would lead to biases that depend on the effects of these vaccines and combinations, which we cannot infer in the context of this review. Therefore we do not predict the direction of bias for individual studies or for the accumulated body of evidence. A further potential source of bias, which is very difficult to assess, is the selective reporting (and non-reporting) of results, both through mechanisms that lead papers to be written and published, and through decisions about what results to present in papers. There is not a single approach to design and analysis of studies in this research area, leaving open the possibility that investigators may have tried multiple ways to select and analyse the data, thereby putting the accessible literature as a whole at risk of bias.

11 Methods

The final version of our *a priori* protocol is available in Appendix 1. Due in part to the complexity of the material, a number of modifications and additional steps were made, and these are described below.

Key differences between the planned and implemented methods are as follows:

- **Outcomes of interest**: we focus in the report on all-cause mortality, for several reasons. First, there were considerably more data for this outcome, particularly for BCG and DTP (fewer than 20% of included articles contribute data on non-targeted mortality). Second, we had important concerns about the ability to determine cause of death, particularly among very young children. Third, incidence of the diseases being targeted would be low in many of the populations studied, so most deaths would be from other causes. Finally, there is a technical concern about how the deaths from the targeted infections should be addressed in the analysis. For example, a standard analysis of death counts from the causes other than the infection the vaccine is designed to prevent would not be appropriate, since it would combine the deaths from the targeted infection with the children staying alive. Censoring follow-up time is one option, but the exact implications of this for risk of bias are unclear. See Annex D for details of the included studies providing data on non-targeted mortality.
- **Vaccines of interest**: since polio vaccine is usually administered with DTP, we could not separate the effects of these vaccines. Our results for DTP should therefore be interpreted as results for the combination of vaccines.
- **Organization of articles and data**: we had to devise processes to manage the overlap of children in multiple articles reporting on different subsets of children from the same area.
- **Selection of results**: we had to devise processes to select one key result from each birth cohort of children, from among multiple results within an article and from among multiple articles covering the same birth cohort.
- **Risk of bias assessment**: we had made it clear in the protocol that development work would take place on the approach to assessing risk of bias in non-randomized studies, and we summarize our method below. We changed the labels we use to describe our judgements (from 'critical' and 'serious' risk of bias to 'very high' and 'high' risk of bias).
- **Statistical synthesis**: the Working Group requested that meta-analyses not be done, so none of the statistical syntheses are included in the report.

11.1 Study eligibility

As per protocol, we considered for inclusion studies with the following designs: (i) randomized controlled trials; (ii) quasi-randomized controlled trials; (iii) cohort studies (prospective, historical and ambi-directional); and (vi) case-control studies. We excluded animal or laboratory studies, and studies with the following designs: (i) ecological studies, (ii) uncontrolled studies (i.e. case reports, case series studies and studies in which all children received the same vaccine(s)), (iii) studies including only individuals with the outcome of interest in the analyses ["case only" studies], and (iv) self-controlled case series studies. Studies containing data related to the vaccination of children up to 5 years with BCG, DTP or measles-containing vaccine were eligible for inclusion if they compared one of the vaccines with no vaccination (BCG, DTP or measles vaccine) or with simultaneous administration of another vaccine. Since DTP is usually administered with polio vaccine, the effects cannot be separated and they are considered here together. Comparisons of different sequences of vaccine administration were also included.

To facilitate an assessment of the risk of bias in each study, we included (i) primary research papers (published or unpublished), (ii) re-analyses of primary studies with full articles describing methodology; (iii) follow-up commentaries and letters about studies written by the authors of the original article; and we excluded (i) results available only in reviews and meta-analyses and (ii) commentaries or letters about studies not performed by their authors.

11.2 Study selection

The search strategies are provided in Appendix 2. Search results were uploaded to a web-based system (DistillerSR®, www.systematic-review.com). The 5,600 identified titles and abstracts were inspected independently by two reviewers from among four (Michelle Beam, Emi Han, Emma Smith, Paul Zhang), coordinated by one co-principal investigator (Arthur Reingold). The full-text versions of 846 articles were obtained (six articles could not be located), and were again independently inspected by two of the same four reviewers (MB, EH, ES, PZ). Any discrepancies were resolved by the co-principal investigator (Arthur Reingold).

After the completion of the full-text screening, 238 potentially relevant references were cross-checked for eligibility by two reviewers from the Enhance Reviews team (Artemisia Kakourou, Maria Christou). Any disagreements between the two reviewers were discussed with the co-principal investigator Karla Soares-Weiser who took the final decision on inclusion. Justifications for excluding articles from the review were documented.

A flow chart of all screened articles is presented in Section 12, the table of characteristics of the included articles is presented in Annex 1, and a list of references of excluded articles (and articles that could not be located) is provided in Appendix 3.

11.3 Data collection

As per protocol, the data collection took place at article level. In the main round of data extraction, information on study characteristics extracted for each article separately were: study design, demographic and participant characteristics, methods of collecting outcome data and vaccination status, sequence of vaccine, age of vaccination, follow up, co-administration of vaccines or vitamin A, and main outcome data or results. The data extraction forms are provided in Appendix 4.

A second stage of data collection was undertaken by a statistician, with a focus on extraction of all-cause mortality outcome data. The main results collected in the first stage were checked, and supplemented with other findings, such as for different age groups, time points, genders or vitamin A statuses. Where effects could be computed from available data, we extracted the data and computed the effect sizes (see below). The approach at this point was still liberal: we extracted any comparison in which (i) child-time after vaccination of BCG, DTP or measles vaccine was compared with child-time after the vaccine had not been administered; (ii) different vaccination sequences had been compared (e.g., DTP3 before vs. after measles vaccine). The resulting compilation of results (included in Appendix 5) includes a large degree of overlap in samples, repetition, strong biases and extreme comparisons (e.g. children who received all three versus children who received none).

Adjusted and unadjusted effect estimates were collected where available; the estimate providing the most reliable evidence would usually be that including adjustment for one or more potential confounders. We collected all available effect measures stratified by gender (or computed them where the needed information was reported). We also collected all effect measures stratified by receipt or not of Vitamin A supplementation (or computed them if the needed information was available).

Effect metrics

The hierarchy for selection or computation of effect metrics was as follows.

   a) Cohort studies:
      1) Hazard ratio (HR) in preference to
      2) Mortality rate ratio (MRR) in preference to
      3) Mortality ratio (MR) in preference to
      4) Odds ratio (OR).
   b) Case-control studies: odds ratios.

Computations

Adjusted effect estimates could be computed manually in a few cases where the comparison of interest was to be made between two exposures groups that had been compared to a common reference category (and reference category was not of interest to us). For example, to compare DTP (after BCG) vs. No DTP (BCG only) we might extract the comparison of each to an unvaccinated reference group. In such cases, the effect was computed as a ratio between the two effects reported in the article, and the standard error was computed based on the methods described by Greenland and Longnecker (49).

In some instances we combined results across subgroups to obtain an adjusted estimate of main effect. For example, combining mortality ratios within boys and within girls, or combining mortality ratios estimated within different age groups. Such combination was performed using fixed-effect meta-analysis methods on the log scale (50).

Most of the effect estimates manually computed were unadjusted. These could be calculated where the article reported the required information per group: deaths and person-time for MRRs; deaths and group size for MRs; cell frequencies for ORs. None of the articles included provided the needed information to compute a HR. We followed standard formulae to compute rate ratios, risk ratios and odds ratios and their standard errors on the log scale (50, 51). If there were no deaths on one group, we added 0.5 to the number of deaths in both groups and added 1 to the denominator (either total children or total children-years).

11.4 Organization of articles and data

Grouping related articles

After each included article had been fully data extracted, information about country, location within a country, design, vaccine comparisons, years of enrolment, and years of birth dates were tabulated. Articles were informally grouped to assist in the identification of results from the same children and hence to avoid double-counting of participants. One of the challenges we encountered with this process was that half of the included articles were from a single country (Guinea-Bissau) and many of the birth dates from different articles overlapped. We therefore plotted the different birth dates against the year of publication for all articles from countries on which more than one study was reported (Bangladesh, Ghana, Guinea-Bissau, India, and Senegal) and attempted to identify details of a given study that would make a particular group of articles unique. As an example, seven articles from Guinea-Bissau published since 2006 (or unpublished) in birth dates from 1999 to 2010 were divided into two groups: *Guinea-Bissau A* reported on a randomized trial conducted in low-birth weight infants in the Bandim area on which the main objective was the impact of vitamin A supplementation; however, these children also received the recommended vaccines and data on BCG and measles vaccine were reported; *Guinea-Bissau C* reported on a randomized trial conducted to evaluate the impact of two vs. one dose of measles vaccine in the same area. These groups of articles are used to organize the articles in Annex A. They also appear on the forest plots in the earlier part of this report to aid location of further information relating to the results plotted.

Birth cohorts

After the process of organizing articles into related groups had been completed, it became clear that it was still not possible to discard considerable overlaps across some of these groupings. For example, although *Guinea-Bissau A* and *Guinea-Bissau C* clearly related to different studies, it is likely that part of the population receiving BCG at birth (*Guinea-Bissau A*) later on participated in the measles vaccine trial (*Guinea-Bissau C*). Therefore, in order to prevent double-counting of participants in the analyses, we further organized the article groups into 'birth cohorts' based on geographical area and time period. In some instances there was more than one independent data set within a birth cohort. Information about overlap of studies was obtained from study authors (specifically, for studies in Guinea-Bissau and Senegal).

Details of final groupings of studies for analyses are provided in Annex B.

11.5 Selection of results

Selection of one result

Having determined the (broadly) non-overlapping birth cohorts, we needed to select one result from each cohort to avoid double counting of children. We devised the following algorithm (which evolved iteratively) to achieve this:

1. Select comparison with vaccination sequence according to the WHO recommendations (e.g., BCG, DTP1-3, MCV). We depict 'BCG before DTP' as 'BCP<DTP'
2. Select estimates from randomized trials.
3. Select estimates adjusted for age and other vaccines.
4. Estimates of primary interest
    – BCG
        A. BCG at birth vs. no BCG in preference to
        B. BCG vs. no BCG
    – DTP
        A. BCG<DTP (any number of doses) vs. BCG in preference to
        B. BCG<DTP (1 or 1-2 doses) vs. BCG in preference to
        C. BCG<DTP (2 or more doses) vs. BCG in preference to
        D. DTP (any number of doses) vs. no DTP in preference to
        E. DTP (1 or 1-2 doses) vs. no DTP in preference to
        F. DTP (2 or more doses) vs. no DTP
    – MCV
        A. BCG<DTP<MCV vs. BCG<DTP in preference to
        B. BCG<MCV vs. BCG in preference to
        C. DTP<MCV vs. DTP in preference to
        D. MCV vs. no MCV

5. Select comparison with least co-administration of other vaccines, particularly when vs. unvaccinated children
6. Select comparison involving children from the same area (e.g. #7108)
7. Select estimate obtained using landmark (rather than retrospective) approach
8. Select estimate obtained from general population children rather than subgroups (e.g., hospitalized children)
9. Select comparison including the most comprehensive adjustment for potential confounders.
10. Select result for the shortest period of follow-up
11. Select result with the largest sample size
12. Select comparison with vaccination strategies according to the WHO recommendations (e.g., BCG at birth, MCV vaccine at 9 months)
13. Select estimate using the methodological approach claimed to be superior or more correct (e.g. #9014)
14. Select result from more recent article

We discarded studies in which all children in one of the comparison groups had two of the vaccines administered simultaneously. The sources of data used in the forest plots are described in the first column of the tables in Annex C.

11.6   Risk of bias assessment

As per protocol, we used the Cochrane Collaboration's tool for assessing risk of bias in randomized trials (52). For non-randomized studies, we used a version of a tool under development by the team that developed the randomized trials tool (led by Jonathan Sterne and Julian Higgins at the University of Bristol), informed by methodological consideration specific to this research area (23, 53).

Assessment of risk of bias in non-randomized studies was highly iterative. This was partly because the tool itself was evolving in its development, partly because iteration is inherent in the evaluation of non-randomized studies (with the need to return to papers to consider potential problems, biases, confounders and co-interventions identified in other studies) and partly to respond to refinements to the research questions posed by this particular review. Details of data extracted to assess risk of biases in the included articles, and the judgements reached, are provided in Annex C.

11.6.1   Preliminary considerations for risk of bias in non-randomized studies

We pre-specified potential confounders in four domains:

- Age of child
- Gender of child
- Child's health (including nutritional status and birth weight)
- Socioeconomic status (including poverty, education, health insurance, urban/rural, hygiene conditions)

We pre-specified potentially important co-interventions:

- Malaria interventions
- De-worming
- Micronutrient supplements
- Breast feeding
- Hygiene programmes
- Other vaccines (Hepatitis B, yellow fever. Polio vaccine not considered as a co-intervention for assessing risk of bias, instead being considered an integral part of the DTP vaccination exposure.)

11.6.2   Process of assessing risk of bias

The assessment was informed by thinking about a hypothetical 'target trial' that makes the same comparison as the result being evaluated. Specific information about potential confounders and co-interventions was collected where available. Risks of bias were assessed in seven domains, facilitated by considering pertinent questions about the conduct of the study and analysis. Within each domain, risk of bias was to be rated as 'low' (meaning comparable to a well-performed randomized trial); 'moderate' (meaning sound for an observational study); 'high' (meaning there are some important problems); or 'very high' (meaning the study is too problematic to provide useful evidence). A short explanatory note outlining the reason for any 'high' or 'very high' risk-of-bias judgement is provided. The overall risk-of-bias judgement was specified as the lowest among the domain-level judgements.

At least two reviewers evaluated the risk of bias for study (Katherine Chaplin, Julian Higgins, Hannah Christensen, Natasha Martin), and face-to-face consensus discussions were held for each result.

1. Bias due to confounding (including frailty bias)

Issues for consideration: (i) Did the authors conduct an appropriate analysis that controlled for all the critically important confounding domains?; (ii) If yes, were all of the confounding domains measured validly and reliably by the variables adjusted for in this study?; (iii) Did the authors avoid adjusting for post-intervention variables?

A very high risk of bias would arise if the vaccine groups being compared had very little overlap in distributions of the confounders, particularly if no adjustment was made for this, which was sometime the case for age.

2. Bias in selection of participants into the study (including inception bias)

Issues for consideration: (i) Are all eligible children included in the analyses (and was selection unrelated to intervention or outcome)?; (ii) Do start of follow-up and start of intervention coincide?

A very high risk of bias would arise if follow-up started somewhat after vaccines were administered in such a way that vaccines had potential to affect mortality rates before the start of follow-up.

3. Bias in measurement of interventions (including survival bias)

Issues for consideration: (i) Were the methods of assessment of vaccination status comparable for participants with different outcomes?; (ii) Was the approach to analysis 'landmark' or 'retrospective'?; (iii) If a retrospective approach was used, is it unlikely that substantial numbers of dead children have been assigned to the wrong vaccination status?

A high risk of bias would arise if vaccination status was assumed rather than measured. A (possibly very) high risk of bias might arise if time of vaccination was assigned using information collected at a later time point, since the information may be missing differentially in dead compared with living children. The seriousness of this depends on the interval between attempts to collect vaccination data.

4. Bias due to departures from intended interventions (performance bias)

Issues for consideration: Were the critical co-interventions balanced across intervention groups?

A very high risk of bias would arise if there were high rates of co-administration (or subsequent administration) of the vaccines of interest. If *all* children received a co-administered vaccine (other than polio vaccine with DTP) then the study would be excluded from the analysis altogether.

5. Bias in measurement of outcome (detection bias)

Issues for consideration: (i) Were outcome assessors unaware of the intervention received by study participants?; (ii) Were the methods of outcome assessment comparable across intervention groups?

Because all-cause mortality is an objective measure, we did not consider there to be important problems in this domain.

6. Bias due to missing outcome data (attrition bias)

Issues for consideration: Are outcome data reasonably complete?

We did not consider there to be important problems in this domain.

7. Bias in selection of the reported result (reporting bias)

Issues for consideration: Is the reported effect estimate unlikely to be prone to selective reporting (on the basis of the results) from among multiple analyses?

This is a particularly challenging domain to assess in the absence of *a priori* analysis plans, and for all studies we defaulted to an assessment of 'moderate risk of bias'.

11.7   Analyses

11.7.1   Main effects

All results are presented in forest plots as effect estimates along with 95% confidence intervals, following hierarchies for choice of effect size and selection of data described above. In these plots, a null effect of one would suggest that the vaccine had no effect on infant mortality and arrows at the base of the plot indicate directions of effect. Effects are presented separately for the three vaccines (evaluated in the context of existing WHO policy). Studies assessed as being at very high risk of bias are presented separately at the bottom of the plots, and do not contribute to the main findings or GRADE tables. We also address various sequences of vaccines. In the investigation of sequences, our main goal was to retrieve data points where the sequences recommended by WHO had been compared with alternatives (specifically simultaneous administration of BCG and DTP, DTP before BCG, simultaneous administration of DTP and measles vaccine, DTP after measles vaccine).
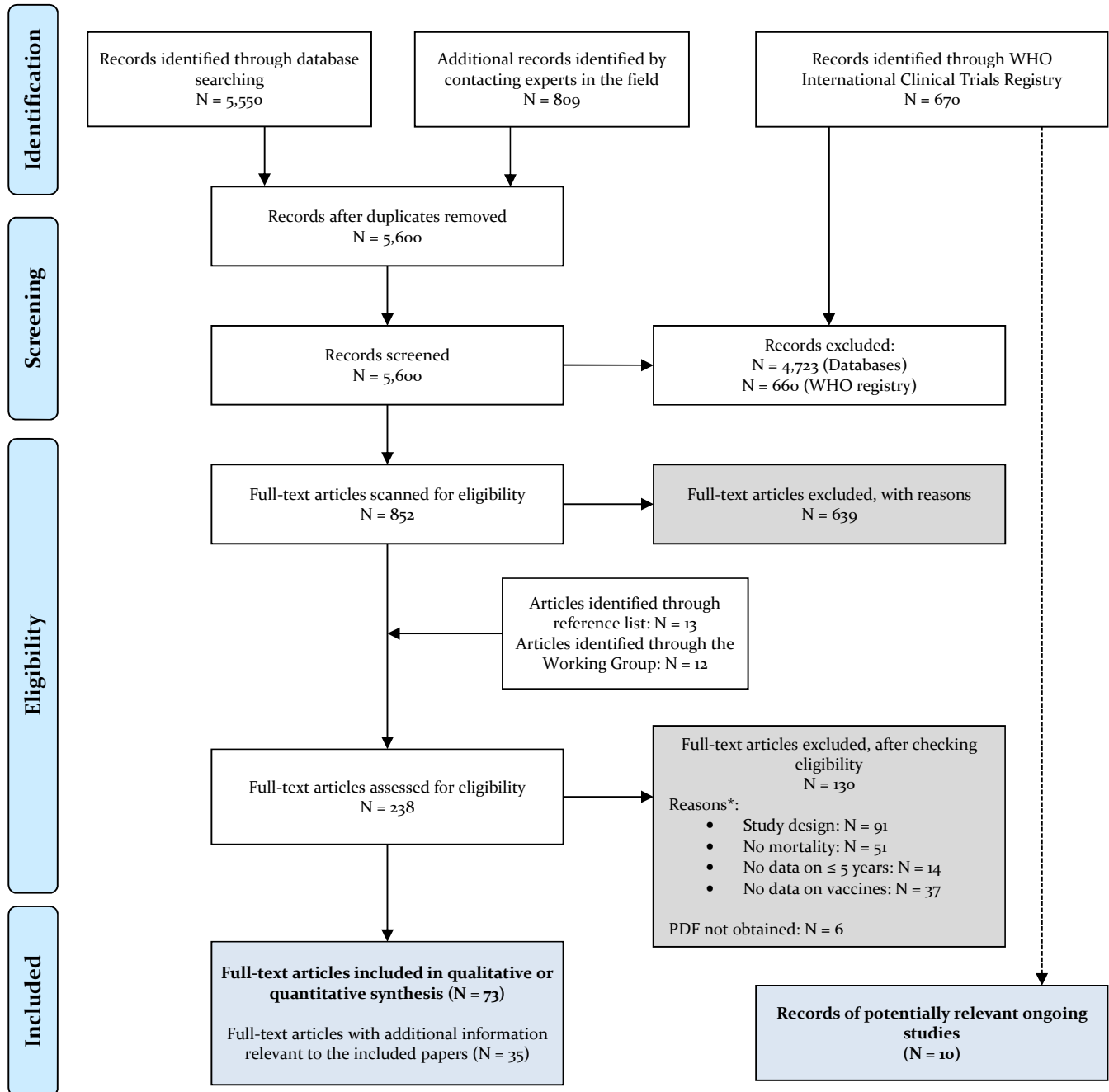
11.7.2 Gender

Results were displayed using separate forest plots for each vaccine with data points presented in two different ways. First, the estimates for each vaccine were grouped in the forest plot by gender to provide an overview of the vaccine effect for boys and girls across birth cohorts. In these plots, a null effect of one would suggest that the vaccine had no effect on infant mortality. Second, we computed the differences between the vaccine effects for boys and girls in each birth cohort, and presented these differential effects for to facilitate assessment of whether one subgroup experienced a higher decrease on mortality than the other. In these plots, a null effect of one would suggest that there was no interaction, i.e. that the vaccine effect on overall mortality was identical for both genders. The sources of data used in the forest plots are described in Appendix 6.

11.7.3 Vitamin A

We also collected all effect measures stratified by receipt or not of vitamin A supplementation (or computed them if the needed information was available). Analyses followed the strategy describe above for gender. The sources of data used in the forest plots are described in Appendix 6.

12 Flow diagram for articles identified in the review

Identification

Records identified through database searching
N = 5,550

Additional records identified by contacting experts in the field
N = 809

Records identified through WHO International Clinical Trials Registry
N = 670

Records after duplicates removed
N = 5,600

Screening

Records screened
N = 5,600

Records excluded:
N = 4,723 (Databases)
N = 660 (WHO registry)

Eligibility

Full-text articles scanned for eligibility
N = 852

Full-text articles excluded, with reasons
N = 639

Articles identified through reference list: N = 13
Articles identified through the Working Group: N = 12

Full-text articles assessed for eligibility
N = 238

Full-text articles excluded, after checking eligibility
N = 130

Reasons*:
- Study design: N = 91
- No mortality: N = 51
- No data on ≤ 5 years: N = 14
- No data on vaccines: N = 37

PDF not obtained: N = 6

Included

Full-text articles included in qualitative or quantitative synthesis (N = 73)

Full-text articles with additional information relevant to the included papers (N = 35)

Records of potentially relevant ongoing studies (N = 10)

13 Contributors and acknowledgements

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