The WHO review of the possible non-specific effects of diphtheria-tetanus-pertussis vaccine

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Abstract

Background: WHO recently reviewed the possible non-specific effects of diphtheria-tetanus-pertussis (DTP) vaccine. The results were considered inconsistent though most studies suggested deleterious effects. We examined whether inconsistencies in results reflected differences in effect of DTP or differences in the methodology used in different studies.

Methods: If children remain unvaccinated because they are frail or if children (including dead ones) with no information on vaccination status are classified as ‘unvaccinated’, the mortality rate becomes unnaturally high among ‘unvaccinated’ controls. To measure this bias, we defined the “bias index” as the mortality rate ratio (MRR) between unvaccinated and vaccinated children.

Results: Five studies had frail or poorly defined control groups and survival bias, the bias index being 2.0-8.0; in these studies DTP was associated with a MRR of 0.39 (0.18-0.83). Eight studies determined ‘unvaccinated’ by vaccination card and the bias index was 0.5-1.7; in these studies DTP was associated with a MRR of 2.00 (1.50-2.67).

Conclusion: Hence, the observed inconsistencies in results were due to methodological differences between studies. Bias does not see to explain why DTP is associated with higher mortality.

Key words: diphtheria-tetanus-pertussis vaccine, DTP, frailty bias, non-specific effects of vaccine, survival bias
Introduction

WHO’s Strategic Advisory Group of Expert (SAGE) on Immunization recently recommended further research into the non-specific effects of vaccines (1). A growing number of studies have suggested heterologous or non-specific effects (NSE) of vaccines, i.e. vaccines may affect susceptibility to infections not targeted by the vaccine (2-7). The SAGE recommendation was based on a thorough review of the possible NSEs of BCG, whole-cell diphtheria-tetanus-pertussis (DTP) and measles vaccine (MV) on mortality of children less than 5 years of age (6). The review concluded that though most evidence came from observational studies with a high risk of bias, and the results should be interpreted with caution, BCG and MV were associated with nearly a halving of mortality, an effect which if true could not be explained by prevention of tuberculosis or measles infection. For DTP the majority of studies suggested a deleterious effect but the literature was considered inconsistent (6). In the present paper, we aim to elucidate the controversies surrounding the potential detrimental effects of DTP.

The first study showing that DTP might be associated with increased child mortality was published in the BMJ in 2000 (8). Subsequently, WHO’s Global Advisory Committee on Vaccine Safety (GACVS) sponsored a series of reanalyses of existing data sets to examine possible sex-differential and NSEs of DTP (9-13). In 2004, after a review by the Task Force for Child Survival, the GAVCS concluded that the issue of a deleterious effect of DTP was not supported by the evidence and decided to set the matter aside (14). However, it was subsequently acknowledged that many of the WHO-sponsored studies
had used incorrect methodology and introduced survival bias into the analysis (15-17).

In these studies, the date of DTP vaccination had been updated retrospectively at the next household visit. If the child died before the next household visit, updating of vaccination status did not happen because the family had usually discarded the vaccination card of a dead child (Figure 1, scenario 1). Hence, children who died, but had been vaccinated, would be misclassified as “unvaccinated” for lack of other information. In other words, these procedures allocate risk-free survival time to the vaccinated group and misclassify some dead vaccinated children as unvaccinated. The comparison of mortality for DTP-vaccinated children versus DTP-unvaccinated children will therefore be biased towards a survival benefit for DTP-vaccinated children. In contrast to this ‘retrospective updating approach’, we have favoured “landmark” analyses which use only prospective follow-up from the date vaccination status was assessed (16,17).

Following the methodological controversies, GAVCS recommended in 2008 to keep a watch for potential non-specific effects of vaccines, including possible deleterious effects of DTP (18).

In 2013-2014 SAGE organised a new comprehensive review of the potential NSEs of BCG, DTP and MV on mortality of children less than 5 years of age (6). The present paper deals only with the review of DTP. The SAGE review included 16 estimates for DTP-vaccinated children compared with DTP-unvaccinated children.

**Methods**
SAGE review of DTP studies: Assessment of risk of bias and selection of studies. The 16 studies included in the SAGE review are listed in Supplementary Table 1 and explained in more technical detail in Supplementary Table 2. There were two studies from Ghana (Ghana I,II), three from India (India I-III), four from Guinea-Bissau (Guinea-Bissau I-IV) and the remaining seven were from different countries.

The SAGE review put a major emphasis on assessing bias in the studies included in the review (6). The criteria for assessment are discussed in the Supplementary Material. Ten studies were considered to have “high risk of bias”. Six studies (marked in grey in Supplementary Table 1) had “very high risk of bias” and were not considered in the main analysis of the effect of DTP-vaccinated versus DTP-unvaccinated.

The assessment did not take the direction of bias into consideration nor did it examine whether the potential biases had the predicted effect (see below). It has little meaning to exclude a study due to “very high risk of bias” if it shows a negative effect of DTP and all known biases should lead to a positive estimate for DTP. We have therefore initially maintained all 16 studies in the analysis and explored how the biases may affect the direction of the estimated effect of DTP.

Bias increasing the benefit of vaccination. Frailty bias and survival bias are the most important biases exaggerating the benefit of vaccination.
Frailty bias: If it is the healthy children with the most educated and compliant mothers, living close to a health facility, who are brought first for vaccination, this would imply that the vaccinated children have inherent lower risk of dying (6); in other words, mortality is “unnaturally” high in the “unvaccinated” group and estimated benefits of vaccination may be exaggerated.

We reviewed the 16 studies in SAGE review for indication of whether healthy children were vaccinated first (Table 1).

Survival bias: Most of the existing DTP studies have been conducted in health and demographic surveillance system sites, where information on vaccination status and vital status is collected at home visits with certain intervals (Figure 1). Retrospective updating of vaccine status for survivors but not for children who died lead to survival bias; due to lack of information during follow-up dead children will be classified as unvaccinated even though they might have been vaccinated (Figure 1, scenario 1). Misclassification of dead children may also occur when children are classified as “unvaccinated” because no vaccination card was seen; some children may have been DTP-vaccinated but this is not known to the investigators because the child died before the information could be retrieved (Figure 1, scenario 2). Many DTP studies included in the SAGE review assumed that they had all relevant information and excluded no
children (16); hence, dead children with no recorded information about DTP were automatically classified as “DTP-unvaccinated”.

If the children have not been seen as unvaccinated in the age group being examined, imprecision in the day-of-birth and the day-of-death may easily lead to misclassified deaths in the unvaccinated group. In demographic surveillance systems, dates may not be known very precisely, and the day may just be classified as “15” and even the month may be uncertain. With this lack of precision, a child who biologically dies at 35 days of age, before DTP-vaccination is given at 6 weeks of age, may end up being classified as having died at 45 days as a DTP-unvaccinated child. We have called these inadequacies for a “poorly defined control group”; i.e. the group of “unvaccinated” has not been defined actively by determining that the children were “unvaccinated” and alive at the relevant age.

Bias reducing the benefit of vaccination. Other than publication bias (6), three biases have been suggested to lead to “unnatural” high mortality in the vaccinated groups (6,26).

Frailty bias: In contrast to the speculation above that healthy children are vaccinated first, it has also been suggested that sick children might come to health clinics and get preferential vaccination, creating a bias which would reduce the apparent benefits of
vaccination (26). As discussed below the data does not make that possibility very likely (Table 1).

Delayed entry: Starting observation time sometime after the actual vaccination occurred (as would happen when data is collected in a demographic surveillance system with regular data collection rounds and a “landmark approach”) could mean that frail children had already died in the unvaccinated group, leading to higher measured mortality in the DTP group (6). It has been shown specifically in one study that the delay in starting follow-up had no impact on the estimate (23) and several studies have started follow-up at the day of vaccination (22,24) and found similar strong negative effects so this bias is unlikely to be important.

Selective censoring of children vaccinated during follow-up: Censoring for measles vaccination during follow-up could mean that the healthiest DTP-vaccinated children had gone on to be measles vaccinated and frail children were left to die in the DTP group (6). The studies which have tested whether censoring for MV matters for the estimated DTP effect have found no effect or the opposite effect (30,31) and many studies have not censored for MV and still found a strong negative effect of DTP (8,11,22,28) so this bias is unlikely to be important.

Bias index. Frailty bias and different forms of survival bias (Figure 1) will lead to an unnaturally high mortality rate in the unvaccinated group (15-17). To measure this bias, we have defined a “bias index” as the mortality rate ratio (MRR) comparing children classified as completely unvaccinated versus children classified as having received at
least one vaccine. We calculated the bias index for the 14 of the 16 SAGE studies in which the necessary information was available (Figure 2, Supplementary Table 1).

**Direction of bias.** The direction of bias is just as important as the inclusion or exclusion of studies because of bias. For the sake of determining whether DTP is associated with increased mortality, based on a majority of studies showing mortality estimates above 1 when comparing DTP-vaccinated children versus DTP-unvaccinated children, the important question is whether a study has biases which would lead to *exaggeration* the harm from DTP. Such studies should be excluded or at least very carefully considered. In contrast, among studies, which find an estimate above 1, it should not be a reason to exclude studies that have biases which leads to *underestimation* of the harm from DTP.

**Results**

**Frailty bias.** Available data on links between nutritional status and vaccination are summarized in Table 1. Three studies reported that sick children were not vaccinated. Because WHO has previously recommended to vaccinate sick children, one study speculated that sick children might have been more likely to get vaccinated, but presented no data (26). Six studies reported nutritional status at time of vaccination or nutritional status and subsequent time to vaccination; all studies except one suggested that healthier children were vaccinated first. Hence, most studies suggest that frail children are less likely getting vaccinated, and we should expect that the DTP-unvaccinated children inherently have higher mortality than the DTP-vaccinated children.
DTP studies and the bias index. The 14 studies for which a bias index could be calculated are presented in Figure 2. Eight studies had prospective follow-up and no survival bias (8,22,23,24,26,27,28,29) (Supplementary Table 1); in seven of these a bias index could be calculated, being between 0.49 and 1.74. Two further studies had limited survival bias (11,25). In Senegal (25) the “unvaccinated” children could not be actively identified from the way the data had been collected. However, vaccination coverage was very low (25) so a few misclassified children in the unvaccinated group had little effect on the bias index which was only 1.48 (1.23-1.77). In India-II (11) it was not described that “unvaccinated” was documented; however, the children were visited every 2 weeks so most vaccinations would have been registered and the bias index was 1.64 (0.87-3.07). Inclusion or exclusion of these two studies made little difference to the overall results.

Of the remaining six studies, one had mainly given BCG and DTP simultaneously (21) and therefore did not evaluate the effect of DTP after BCG. The last five studies had a poorly defined control group and a bias index between 2.0 and 8.0 (Figure 2, Supplementary Table 1) (9,12,13,19,20).

Discordant studies

The different focus in the SAGE and our assessment of bias give rise to six discordant study classifications. The SAGE review included three studies, which we believe should
not have been included due to massive frailty and survival bias, and excluded three
studies which should have been included, because they had limited bias or the risk
estimate was above 1 and the direction of the bias was away from harm (towards 1).
First, the SAGE-review’s main analysis of DTP included three studies from Papua New
Guinea (PNG), Bangladesh and Burkina Faso, which all had high bias indexes. The PNG
had a 1-5 month mortality rate of 233 per 1000 person-years in unvaccinated children
compared to only 31 in vaccinated children (9). A rate of 233/1000 is unrealistic in a
community with a neonatal mortality rate of 18 per 1000, and a general post-neonatal
mortality rate of only 48 per 1000 person-years. The rate is also higher than in 1920s
before vaccine and antibiotics were introduced (33). A main reason may have been that
sick children were not vaccinated (9) and that the study had various forms of survival
bias. The Bangladesh study (30) is based on community registers and it was assumed
that all information was available since no child was excluded for lack of information;
thus, status as “unvaccinated” was not actively verified. The study had problems with
registration of early vaccination events; children who subsequently moved had
significantly lower vaccination coverage. Seventy per cents of the children started with
BCG+DTP1-first. Controlling for the relevant background factors, children who received
DTP1 after BCG as currently recommended by WHO had a MRR of 1.78 (1.03-3.06)
compared with children who had received BCG and DTP1 simultaneously (30). The SAGE
reviewers used the DTP1 group from the BCG-first arm to measure the effect of DTP
after BCG. However, if early events are not registered some DTP1-vaccinated children
who died may not have been registered and have ended up as misclassified deaths in
the BCG-group to produce a MRR of 0.52 (0.31-0.87) for DTP1 after BCG (Supplementary Table 1). Since the DTP1-after-BCG group had significantly higher mortality than children who had received BCG and DTP1 simultaneously, it seems strange to use this study to suggest that DTP after BCG has a good effect on child survival (30). The Burkina Faso study (12) assumed that children not seen were unvaccinated which would lead to survival bias. An undocumented proportion of the children had received DTP with BCG since the median age of BCG vaccination was 4.8 months, or received measles vaccine (MV) during follow-up, and both combinations would reduce any harm from DTP. Thus, the design of these three studies, which suggested benefit or no harm of DTP, implied a risk of bias that may have made DTP appear more beneficial than it was.

Second, the SAGE-review’s main analysis excluded three studies, Ghana-II, Guinea-Bissau IV, and India-III, with bias indexes of 0.67-1.74 (Supplementary Table 1) for having a “very high risk of bias” (6). Ghana-II (27) was classified as very high risk of bias because of delayed entry after DTP vaccination and because a high proportion had co-administration of BCG or MV; both of these vaccines reduce the apparent harm from DTP reported by the study so it should not be a reason to exclude the study. According to the SAGE review Guinea-Bissau-IV (28) had delayed entry after DTP and children who received MV were excluded (6). The study measured the effect of pre-war vaccination status during a civil war where there was little vaccination (28); to measure the effect of DTP, the children who had already received MV were evidently not included in the analysis of DTP. Hence, the reasons for excluding the study are unclear. India-III (29) was
excluded as “very high risk of bias” because of an unadjusted comparison of older DTP-vaccinated children with younger DTP-unvaccinated children and co-administration of MV. Both factors would reduce the apparent harm from DTP reported by the study and are not a reason to exclude. Furthermore, the paper did actually present another age and weight-adjusted estimate of 1.36 (0.63-2.92) which censored at 9 months to eliminate the effects of co-administration of MV. Thus, the three studies, which suggested harm of DTP, were excluded for reasons that had little or no effect on the estimate, or would reduce the apparent harm from DTP.

DTP and child survival

SAGE review. Based on the MRRs for DTP-vaccinated versus DTP-unvaccinated children presented in Supplementary Table 1 (last column), the executive summary of the SAGE review concluded that findings for DTP were inconsistent, with a majority of the studies indicating a detrimental effect of DTP and two studies (Bangladesh, PNG) indicating a beneficial effect (6). In the 10 studies in the SAGE analysis the MRR for DTP-vaccinated versus DTP-unvaccinated children was 1.38 (0.92-2.08).

In our assessment. In the five studies with major survival and frailty bias (bias index above 2) (Figure 2) there was no or a beneficial effect of DTP, the meta-estimate being 0.39 (0.18-0.83) (random effect model) (Supplementary Table 1).
In contrast, in the eight studies in which “unvaccinated” was assessed by vaccination card or health centre records, the MRR for DTP-vaccinated versus DTP-unvaccinated children was 2.00 (1.50-2.67) (Figure 3). Including the two studies with minimal survival bias did not change the result, the MRR being 1.89 (1.49-2.43) (11,25). It made no difference if the three studies judged by the SAGE reviewers to have ‘very high risk of bias’ were excluded (MRR=1.91 (1.46-2.50)).

Mortality after other vaccines in the same population. Given that the healthiest children are most likely to be vaccinated first (Table 1) no bias should be able to produce the counterintuitive trend of two-fold higher mortality for DTP (Figure 3). Had there been an unrecognised bias producing higher mortality for vaccinated children, this bias would presumably also have resulted in higher mortality for BCG and measles vaccinated children. As seen in Figure 4, in the studies, which estimated the effect of several vaccines, DTP was consistently associated with higher mortality whereas BCG and measles vaccine were associated with lower mortality; for studies estimating the effect of BCG the reduction was 44% (32-54%) and for MV 54% (40-65%).

Discussion

In 2004, GACVS reviewed the studies on DTP and concluded that a deleterious effect of DTP on child survival was not supported by the evidence (14). That conclusion was due to the inclusion of studies with survival bias (15,16,34); furthermore, most studies had given BCG and DTP simultaneous and not BCG at birth and DTP six weeks later as
recommended by WHO. In the recent SAGE review the studies with simultaneous BCG and DTP vaccinations have mostly been excluded since it was acknowledged that BCG and DTP simultaneously may have quite different effects from BCG followed by DTP (25,29). Furthermore, many of the studies with survival bias were excluded as it was recognised that they had very high risk of bias.

The SAGE review did not dismiss a possible deleterious effect of DTP. However, evoking the high risk of bias in all studies and inconsistent results, SAGE concluded that the available data neither excluded nor confirmed the possibility of beneficial or deleterious non-specific effects of DTP on all-cause mortality (6). However, the data presented in Figure 2 (Supplementary Table 1) suggests that the estimated effect of DTP-vaccination versus no DTP-vaccination is only beneficial when the bias index is very high because the mortality rate in the unvaccinated group is unnaturally high. If children for whom no information on vaccination is available are assumed to be “unvaccinated” one will automatically get a very good estimate for DTP-vaccinated compared with DTP-unvaccinated. On the other hand, when the status as “unvaccinated” has been documented, DTP was associated with a marked deleterious effect.

The SAGE review has apparently overlooked the extent and implications of survival and frailty bias. In spite of previous reviews of the methodologies and analyses of NSEs of vaccine (15-17, 34, 35) suggesting that we should focus less on identifying presence of
bias – which is inevitable in observational studies – and more on assessing the likely
impact on the results (17), the SAGE review included several studies with survival bias.
An illustration of the importance of survival bias stems from our initial DTP study (8). In
this study we used the “landmark approach” and only included prospective follow-up,
the bias index was 1.35 (0.97-1.89) and the estimate for DTP vaccinated versus DTP-
unvaccinated was 1.84 (1.10-3.10) (8). However, if we updated information on
vaccinations retrospectively in the same data set, thereby introduced survival bias and a
higher mortality rate in the unvaccinated group (Supplementary Table 1), the bias index
became 2.96 (2.15-4.08) and now DTP had a major beneficial effect on child survival
(MRR=0.62 (0.41-0.92)) (32).

There is no absolute value for when the bias index is unnaturally high but this example
(32) and the data presented in Figure 2 suggest that when the bias index is above 2
there is reason to be sceptical. By including studies with a “poor definition of the control
group” and a consequent high bias index, the SAGE review has produced inconsistent
results for DTP (6).

The studies in Supplementary Tables 1 and 2 without survival bias were not all ideal. In
several studies children who initially were DTP-unvaccinated may have received DTP
during follow-up; that bias would produce a conservative estimate and not exaggerate
the effect of DTP. Since the negative effect of DTP has been ascribed to an effect specific
to Guinea-Bissau (14), it should be noted that three quarters of the studies in Supplementary Table 1 were from other countries.

The interest in NSEs of vaccine was originally provoked by trials of high-titre measles vaccines (HTMV) conducted in the 1980s which found that early administration of HTMV at 4 months of age was effective against measles infection, but surprisingly associated with two-fold higher female mortality than standard MV delivered from 9 months of age (no difference for males) (7). WHO withdrew HTMV in 1992 (7). Both types of measles vaccine were fully protective against measles infection, so this was a non-specific effect. HTMV was administered at 4-5 months of age and most children received DTP or inactivated polio vaccine (IPV) after HTMV. When we reanalysed the HTMV studies from that perspective, the increased female mortality in the HTMV group was limited to girls who had received DTP/IPV after HTMV but there was no increase in female mortality when HTMV was the most recent vaccination (7). Hence, it was having DTP as the most recent vaccine rather than HTMV per se, which had caused excess female mortality.

**Implications**

In 2000, we first reported 84% (10-210%) higher mortality associated with DTP vaccination (8). Now many studies later, the combined estimate for studies with a comparable methodology is a two-fold increase in mortality. Not a single study without frailty bias and with prospective follow-up has shown DTP to be associated with a beneficial effect on child survival.
There are numerous implications of recognising that DTP may not be the best vaccine for child survival. We need to study both the underlying mechanisms and possible ways of preventing these negative effects. Given imprecision of date-of-death in most low-income country studies it has not been possible to determine whether there is a specific timing to the excess deaths after DTP. The increased mortality rate may also relate to general changes in immune profile which affect susceptibility whenever the child is exposed to other infections (4,5); in that scenario a clear timing pattern of deaths would not be expected. A negative effect of DTP could be minimised by following a live-vaccine-last policy and giving a live vaccine shortly after the last DTP (2); for example we have shown that an early MV shortly after DTP3 reduced overall mortality (36).

Following the same principle DTP should not be given with or after MV as this is always associated with increased mortality (2), as also recognised in the SAGE review (6). Several studies have suggested that co-administration of BCG and DTP reduces the negative effect of DTP for girls (23,25,29). Hence, there are reasons to explore whether there are other ways of reducing the DTP associated excess mortality. Furthermore, a live pertussis vaccine is being developed and apparently has beneficial non-specific effects in animal models (37).

The global health community has used the coverage for DTP3 as the main performance indicator for the global immunization programme (38). This has led to increases in the
DTP coverage but much less emphasis has been placed on the timeliness and coverage of BCG and MV, the vaccines associated with lower mortality. This may need to change.

SAGE recommended that the Immunization and Vaccine related Implementation Research Advisory Committee (IVIR-AC) should prioritise research questions on the non-specific effects of vaccines to inform policy. IVIR-AC has decided to guide the development of standard protocols and implementation of high quality prospective studies (39), but has also recently asserted that ‘the impact of DTP on all-cause mortality could not be determined’ (40). This seems to be sliding away from the unpleasant conclusion in the SAGE review that the majority of studies suggested a deleterious effect of DTP (6). Future randomised trials could examine the unbiased effect of booster DTP or of co-administering BCG and DTP. To the extent further observational studies are conducted it is important to prevent frailty and survival bias and to recognize that commonly considered biases cannot produce a negative effect of DTP.
Contributions: The first draft was written by PA; all authors contributed to the final version of the paper. PA will act as guarantor of the study.

Conflict of interest: nothing to declare

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Independence: The funding agencies had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

Data sharing: no additional data as the study is based on published studies
Table 1. Frailty or nutritional status in studies of DTP-unvaccinated and DTP-vaccinated children

<table>
<thead>
<tr>
<th>Study</th>
<th>Assessment</th>
<th>Comments and conclusions</th>
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<tbody>
<tr>
<td><strong>Vaccination of sick children</strong></td>
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<tr>
<td>PNG (9)</td>
<td>“sick children who are at greatest risk are brought to a clinic by their mothers in the hope of getting treatment, but these children never get vaccinated because of contraindications”</td>
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<tr>
<td>Guinea-Bissau I (22)</td>
<td>“Some children were considered too sick to be vaccinated”; unvaccinated children had lower nutritional status (see below)</td>
<td></td>
</tr>
<tr>
<td>Guinea-Bissau-III (23)</td>
<td>Children are coming to the health centre for treatment or for vaccination</td>
<td></td>
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<tr>
<td>Benin (26)</td>
<td>From case-control study: “Owing to a greater morbidity, cases (i.e. children who died) may be more likely to consult for curative care and consequently more likely to receive a single vaccination”. No data presented to support this possibility.</td>
<td>Estimates were adjusted for nutritional status</td>
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<tr>
<td><strong>Nutritional status at time of vaccination or nutritional status and time to vaccination</strong></td>
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<tr>
<td>DTP unvaccinated</td>
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<tr>
<td>India-II (11)</td>
<td>Low-birth weight children had lower vaccination coverage for BCG (RR=0.93 (0.90-0.97)) and DTP (RR=0.97 (0.94-1.00)).</td>
<td></td>
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<tr>
<td>Philippines (21)</td>
<td>Low-birth weight children had higher incidence of DTP vaccination (RR=1.05 (1.02-1.10))</td>
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<tr>
<td>Guinea-Bissau-I (22)</td>
<td>WAZ – 0.66 (-0.87 to - 0.44) (N=197)</td>
<td>The unvaccinated children, who were often unvaccinated because they were sick, had lower nutritional status than the vaccinated children (p=0.149).</td>
</tr>
<tr>
<td>Guinea-Bissau-III (23)</td>
<td>Poor nutritional status delayed vaccination: The DTP unvaccinated children had significantly lower birth weight, weight gain from birth to two month, mean weight, WAZ, length, HAZ, MUAC, head-</td>
<td>With adjustment for MUAC the estimate for DTP increased from 2.04 (0.77-5.41) to 4.33 (1.54-12.2)</td>
</tr>
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Circumference, abdominal circumference than the DTP vaccinated children.

<table>
<thead>
<tr>
<th>Country</th>
<th>Description</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Malawi (24)</td>
<td>Children with better WAZ and WHZ at 1 months of age received DTP1 significantly earlier</td>
<td>The healthiest children were vaccinated first</td>
</tr>
<tr>
<td>India-III (29)</td>
<td>The incidence of vaccination with BCG and DTP was 4-9% higher for each increase in weight-for-age z-score. Vaccinations were also related to birth weight.</td>
<td>The healthiest children were vaccinated first</td>
</tr>
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Notes: MUAC=mid-upper-arm-circumference; WAZ=weight-for-age-z-score; WHZ=weight-for-height-z-score

There studies (Burkina Faso, Guinea-Bissau-II, Ghana-II) reported surveys on nutritional status and vaccination coverage but it could not be assessed whether nutritional status was cause or effect. Bangladesh had too many missing information on arm-circumference to be used (13); India-I data on nutritional status was not available (19); Ghana-I had no information on nutritional status (20); Senegal did not have information on nutritional status (25); Guinea-Bissau IV (28) reported no data on nutritional status except by indicating that adjustment for arm-circumference had no effect on the inversion of the female-male mortality ratio after DTP and MV respectively.
The three children followed in a fictive demographic surveillance system illustrate two different ways of creating survival bias. In both situations child 1 is in the vaccinated group.

**Scenario 1 – Retrospective updating (comparing child 1 and 2):** Child 1 whose vaccination card is seen at 2nd visit has vaccination status updated retrospectively, i.e. from the date of vaccination. Child 2 dies between two visits and it is not known whether it was vaccinated or not and the child is therefore classified as “unvaccinated”. Hence, child 1 gets risk-free survival time from date of vaccination to surveillance visit because had it died it would not have had vaccinated time allocated.

**Scenario 2 – Poorly defined control group (comparing child 1 and 3):** Child 3 is never seen and dies before first visit. In the comparison child 3 is classified as “unvaccinated” due to lack of other information. Again, child 1 gets some risk-free survival time because had it died it would not have had time as “vaccinated” allocated.
Figure 1. Follow-up scenarios, which create “survival bias” in demographic surveillance system studies
Figure 2. Scatter plot of the mortality hazard ratio (HR) for DTP-vaccinated versus DTP-unvaccinated and for the bias index (mortality HR for unvaccinated versus vaccinated (any vaccine) children) (Supplementary Table 1). The GB-II study is represented with the originally published results (8) and with results when survival bias was introduced in the analysis (32).

Abbreviations and references: GB-III=Guinea-Bissau-III (23); GB-II=Guinea-Bissau-II (8); GB-I=Guinea-Bissau-I (22); GB-II with surv bias=Guinea-Bissau-II with survival bias (32); GB-IV=Guinea-Bissau-IV (28); BF=Burkina Faso (12); Bangla=Bangladesh (13,30); PNG=Papua New Guinea (9); Senegal (25); India-II (11); India-I (19); India-III (29); Ghana-II (27); Ghana-I (20); Malawi (24)
<table>
<thead>
<tr>
<th>Country</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>India-III</td>
<td>1.11 (0.30, 4.12)</td>
</tr>
<tr>
<td>Guinea-Bissau-IV</td>
<td>1.58 (0.36, 7.02)</td>
</tr>
<tr>
<td>Guinea-Bissau-II</td>
<td>1.74 (1.10, 2.75)</td>
</tr>
<tr>
<td>Guinea-Bissau-I</td>
<td>1.92 (1.04, 3.52)</td>
</tr>
<tr>
<td>Benin</td>
<td>2.20 (0.93, 5.22)</td>
</tr>
<tr>
<td>Ghana-II</td>
<td>2.39 (0.82, 6.99)</td>
</tr>
<tr>
<td>Malawi</td>
<td>3.19 (0.80, 12.79)</td>
</tr>
<tr>
<td>Guinea-Bissau-III</td>
<td>4.33 (1.54, 12.19)</td>
</tr>
<tr>
<td>Overall</td>
<td>2.00 (1.50, 2.67)</td>
</tr>
</tbody>
</table>
Figure 3. The mortality rate ratio for DTP-vaccinated versus DTP-unvaccinated children in studies with no survival bias
Figure 4. The mortality rate ratios for BCG-vaccinated versus BCG-unvaccinated, DTP-vaccinated versus DTP-unvaccinated and measles-vaccinated versus measles-unvaccinated children in the 10 studies with no or limited survival bias
13. Breiman RF, Streatfield PK, Phelan M, Shifa N, Rashi M, Yunus M. Effect of infant immunization on childhood mortality in
rural Bangladesh: analysis of health and demographic surveillance data. Lancet 2004; 364:2204-11
15. Fine PEM, Smith PG. `Non-specific effects of vaccines´ - an important analytical insight, and a call for a workshop. TMIH 2007;12:1-4
30. Aaby P, Ravn H, Andersen A. Combined BCG and DTP vaccinations may reduce infant mortality more than the WHO-schedule of BCG first and then DTP. A re-analysis of demographic surveillance data from rural Bangladesh. Draft manuscript provided to the SAGE review committee.
Supplementary material: Bias assessment and selection of studies

The SAGE review put a major emphasis on assessing bias in the studies included in the review. The assessment of bias was informed by thinking about a hypothetical ‘target trial’ assessing the same comparison as the observational studies. Bias was assessed within seven domains: 1) bias due to confounding (including frailty bias); 2) bias in participation into the study (including inception bias) – were all eligible children included and did follow-up start at the time of intervention?; 3) bias in measurement of intervention (including survival bias); 4) bias due to departure from intended interventions (performance bias) – were critical co-interventions balanced over intervention groups? If all children received a co-administered vaccine (other than OPV with DTP) the study was excluded; 5) bias in measurement of outcome (detection bias) – with all-cause mortality as the main outcome there was no problem in this domain; 6) bias due to missing outcome data (attrition bias) – not considered to be problems in this domain; and 7) bias in selection of the reported results (reporting bias) – the reviewers had problems in assessing this but assumed that all studies had ‘moderate risk of bias’ (6). This is a useful overview of bias domains for observational studies.

The GAVCS and the SAGE reviews differed with regard to several important aspects: First, as a result of point 4 above, the SAGE review excluded studies in which all children received DTP and BCG simultaneously (10). In the GACVS review most studies had in fact administered BCG and DTP simultaneously (16,17). Second, the SAGE review favoured the shortest follow-up period reported in the paper as this would be likely to represent the most direct effect of being vaccinated versus unvaccinated. This is also in contrast to the GACVS review in which follow-up was to 2 years of age even though that would mix the effects of several vaccines (10,12). This latter approach assumes that vaccines have constant effects over time. Specific effects of specific vaccines may well be relative constant through childhood. However, NSEs may be related to reprogramming of innate immune responses; for example, BCG has been shown to reprogram monocytes via epigenetic changes promoting stronger pro-inflammatory responses (5). Hence, subsequent vaccinations may change the programming. Potential important non-specific effects will therefore be most visible while a given vaccine is the most recent vaccination and the assessment should therefore not mix the effect of several vaccines over an extended period of follow-up.
Supplementary Table 1. Studies in the SAGE-review assessing the effect of DTP-vaccination compared with no DTP vaccination. Stratified based on how ‘unvaccinated’ was assessed

<table>
<thead>
<tr>
<th>Study (name used by SAGE)</th>
<th>Age group</th>
<th>Assessment of ‘unvaccinated’; % excluded for no information</th>
<th>Mortality rate per 1000 person-years (deaths/follow-up time)</th>
<th>Bias index (i.e. MRR for unvaccinated vs Vaccinated children)</th>
<th>MRR (95% CI) for DTP-vaccinated versus DTP-unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh @ (13,30)</td>
<td>6 weeks-9 months</td>
<td>Default; none</td>
<td>67 (329/1783651 days)</td>
<td>20 (362/6677421 days)</td>
<td>3.40 (2.93-3.95); 0.52 (0.31-0.87)</td>
</tr>
<tr>
<td>Burkina Faso (12)</td>
<td>0-7 months, 6 months follow-up</td>
<td>Default; none</td>
<td>120 (281/28128 person-months)</td>
<td>52 (64/14662 person-months)</td>
<td>2.29 (1.74-3.00); 1.00 (0.60-1.67)</td>
</tr>
<tr>
<td>Papua New Guinea (9)</td>
<td>29 days to 5 months</td>
<td>Default; none</td>
<td>233 (92/144285 days)</td>
<td>31 (38/448418 days)</td>
<td>7.52 (5.15-10.97); 0.48 (0.22-1.09)</td>
</tr>
<tr>
<td>India-I &amp; (19)</td>
<td>6 weeks to 8 months</td>
<td>Health centre vaccinations; none</td>
<td>200 (23/1381 person-months)</td>
<td>29 (183/74937 person-months)</td>
<td>6.82 (4.42-10.52); 0.28 (0.20-0.40)</td>
</tr>
<tr>
<td>Ghana-I (20)</td>
<td>0-59 months</td>
<td>Default; none</td>
<td>Data not provided; since estimates for some vaccines versus no vaccine were 0.14 (0.13-0.16) the MRR must be at least 7-fold higher for unvaccinated than for vaccinated children</td>
<td></td>
<td>7.14 (6.25-7.69); 0.15 (0.14-0.16)</td>
</tr>
<tr>
<td>Guinea-Bissau-II with survival</td>
<td>0-7 months, 6 months</td>
<td>Default</td>
<td>183 (92/503 person-years)</td>
<td>68 (130/1906.3 person-years)</td>
<td>2.96 (2.15-4.08); 0.62 (0.41-0.92)</td>
</tr>
<tr>
<td>bias (32)</td>
<td>follow-up</td>
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<tr>
<td>Philippines § (21)</td>
<td>ND</td>
<td>By vaccination card but 2/3 had BCG+DTP; only BCG-vaccinated</td>
<td>All had received BCG</td>
<td>NA</td>
<td>0.87 (0.33-2.29)</td>
</tr>
<tr>
<td><strong>Limited survival bias (see text)</strong></td>
<td></td>
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</tr>
<tr>
<td>Senegal (25)</td>
<td>Day 2 to 24 months</td>
<td>Default; none</td>
<td>86 (372/4318.5 person-years)</td>
<td>58 (174/2985.3 person-years)</td>
<td>1.48 (1.23-1.77)</td>
</tr>
<tr>
<td>India-II (11)</td>
<td>2 to 6 months</td>
<td>Home interview; censored for ‘receipt of first unknown vaccine’</td>
<td>34 (20/594 person-years)</td>
<td>24 (53/2177 person-years)</td>
<td>1.38 (0.83-2.31)</td>
</tr>
<tr>
<td>‘Unvaccinated’ documented by vaccination card or health centre register</td>
<td></td>
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</tr>
<tr>
<td>Guinea-Bissau-I (22)</td>
<td>2-14 months</td>
<td>All vaccinations administered and documented by researchers</td>
<td>51 (20/388.6 person-years)</td>
<td>113 (47/415.7 person-years)</td>
<td>0.49 (0.28-0.85)</td>
</tr>
<tr>
<td>Guinea-Bissau-II § (8)</td>
<td>0-7 months, 6 months follow-up</td>
<td>By vaccination card; 34% (2998/8752) card not seen</td>
<td>109 (95/875.1 person-years)</td>
<td>83 (127/1534.2 person-years)</td>
<td>1.35 (0.97-1.89)</td>
</tr>
<tr>
<td>Guinea-Bissau-III (23)</td>
<td>2-6 months</td>
<td>By vaccination card; 14% (309/2139) card not seen</td>
<td>108 (11/37071 days)#</td>
<td>75 (14/68310 days)</td>
<td>1.45 (0.66-3.19)</td>
</tr>
<tr>
<td>Malawi (24)</td>
<td>7 days-8 months</td>
<td>Health centre register; 4 children not</td>
<td>99% were BCG vaccinated; no deaths for unvaccinated after 3 months. MRR for no BCG versus BCG was 1.45 (0.65-3.23)</td>
<td>1.45 (0.65-3.23)</td>
<td>3.19 (0.80-12.79)</td>
</tr>
<tr>
<td>Country</td>
<td>Ages</td>
<td>Method</td>
<td>Cases</td>
<td>Controls</td>
<td>MRR</td>
</tr>
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</tr>
<tr>
<td>Benin (26)</td>
<td>0-35 months</td>
<td>Health centre Register; based on documented vaccinations</td>
<td>3.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>2.20 (0.93-5.22)</td>
</tr>
<tr>
<td>Ghana-II (27)</td>
<td>6-36 months – 4 months follow-up</td>
<td>By vaccination card; 13% (908/6882) card not seen</td>
<td>80 (31/389)</td>
<td>69 (48/701)</td>
<td>1.16 (0.74-1.83)</td>
</tr>
<tr>
<td>Guinea- Bissau-IV (28)</td>
<td>1.25-6 months</td>
<td>By vaccination card; 47% (1309/2800) card not seen</td>
<td>20 (1/49)#</td>
<td>33 (8/264)</td>
<td>0.67 (0.08-5.38)</td>
</tr>
<tr>
<td>India-III (29)</td>
<td>2-8 months</td>
<td>By vaccination card; 7% (991/14922) card not seen</td>
<td>35 (25/719)</td>
<td>20 (20/1000)</td>
<td>1.74 (0.97-3.13)</td>
</tr>
</tbody>
</table>

Notes: Studies in grey colour were studies which were classified as having ‘very high risk of bias’ according to the SAGE review (see Supplementary Table 2) (6). Where possible we have not included the neonatal period in the comparison of unvaccinated versus vaccinated since mortality is inherently high in the neonatal period and few children will have been vaccinated. Guinea-Bissau-II is presented in the estimate used by SAGE, and we are also presenting Guinea-Bissau-II in a flawed version (not used by SAGE) where we calculated the estimates we would have obtained if the children who had not had their vaccination card inspected had been classified as unvaccinated (in bold) (32). The estimates for DTP-vaccinated versus DTP-unvaccinated cannot be generated from the columns of “unvaccinated” and “vaccinated” as the DTP-unvaccinated are likely to have been BCG-vaccinated. In the Guinea-Bissau-I study there was little BCG vaccination and the DTP-vaccinated versus DTP-unvaccinated is close to the inverse of the MRR for unvaccinated vs vaccinated children in the table.
The Bangladesh study is based on community registers and it is assumed that all information is available since no child is excluded for lack of information. However, there is no documentation that ‘unvaccinated’ was actively verified. The study had problems with registration of early events since children who subsequently moved had highly significantly lower vaccination coverage. The SAGE estimate has used our reanalysis of the Bangladesh data set (30) which focused on comparing mortality between 6 weeks and 9 months of age according to whether the immunization schedule was started with BCG-first, BCG+DTP1-first or DTP1 first. More than 70% started with BCG+DTP1-first and that option was associated with significantly lower mortality. The SAGE reviewers have used the DTP1 group from the BCG-first schedule to assess the effect of DTP after BCG. However, if there are problems with registration of early events it is also likely that information was not collected for some DTP1-vaccinated children who died and they ended up as misclassified deaths in the BCG-group. The estimate of 0.52 (0.31-0.87) should not be used as a true estimate of the effect of DTP among BCG vaccinated children. Within the Bangladesh data set, controlling for the relevant background factors, children who received DTP1 after BCG had 1.78 (1.03-3.06) higher mortality than children who had received BCG+DTP1 (30). Hence, this study should not be used to suggest that DTP after BCG as currently recommended by WHO, has a good effect on child survival.

May have received BCG.

& Health centre provided all vaccinations. Vaccinations coverage was very high. Hence, unvaccinated children would be a small group of frail children in the 6-8 week age range whereas DTP vaccinated children would tend to be 5-6 months old on average. Hence, the comparison is between different age groups and between healthy and frail children but not between different vaccination groups. The researchers themselves did not make a comparison between DTP-vaccinated and DTP-unvaccinated children, but the SAGE reviewers included this age-unadjusted comparison as an estimate of the effect of DTP. This comparison is “unnatural” both because it is age-unadjusted and because the control group does not reflect the general population of children (15).

§ Two-thirds of the children had received BCG and DTP simultaneously. Hence, the comparison is not DTP-vaccinated versus DTP-unvaccinated but DTP+BCG versus BCG-vaccinated only. The study should not have been included in the DTP analysis.

diamond The SAGE review used the estimate 1.74 (1.10-2.75) for the Guinea-Bissau D study rather than the estimate of 1.84 (1.10-3.10) found in the original publication (8). Since the SAGE review prioritised adjusted estimates it would have been more correct to use the estimate of 2.50 (1.31-4.78) obtained when adjusting for mid-upper-arm-circumference (8).

diamond§ The SAGE review used the estimate 1.11 (0.30-4.12) which is based on dividing the age unadjusted estimates for “DTP after BCG” with the estimate for “Only BCG”. The estimate was then dismissed as “very high risk of bias” as the comparison was age-unadjusted
and covered the MV period. The paper itself (29) calculated an age and weight-adjusted estimate of 1.36 (0.63-2.92) for DTP versus unvaccinated which only covered the period until 9 months of age.
### Supplementary Table 2: Studies of DTP used in the SAGE review (6)

<table>
<thead>
<tr>
<th>Country; setting</th>
<th>Design and comments</th>
<th>No. of children</th>
<th>Age group covered</th>
<th>Information on unvaccinated</th>
<th>Vaccines</th>
<th>Other vaccinations during follow-up</th>
<th>The basis for the SAGE estimate (6)</th>
<th>SAGE overall assessment of bias (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>&quot;Unvaccinated&quot; is a default group or poorly defined</strong></td>
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</tr>
<tr>
<td>Bangladesh (13); rural</td>
<td>Routine surveillance</td>
<td>37,894</td>
<td>6-9 mo</td>
<td>No-information</td>
<td>BCG, DTP, MV</td>
<td>71% received BCG and DTP simultaneously; control group undefined</td>
<td>BCG+DTP1 vs. BCG; adjusted age, gender, others</td>
<td>Overall bias: High risk of bias (no adjustment for child’s health)</td>
</tr>
<tr>
<td>Burkina Faso (12); rural</td>
<td>Routine surveillance</td>
<td>9,085</td>
<td>0-24 mo</td>
<td>No-information</td>
<td>BCG, DTP</td>
<td>Proportion who received BCG and DTP simultaneously is not defined; control group undefined; survival bias</td>
<td>BCG+DTP1 vs. BCG; calculated from table 3; adjusted for area, dispensary in village, health services use, diarrhoea, season</td>
<td>Overall bias: High risk of bias (potential selection bias; co-administration of BCG; assumptions about non-vaccination; likely co-intervention with MV)</td>
</tr>
<tr>
<td>Papua New Guinea (9); rural</td>
<td>Routine surveillance</td>
<td>4,048</td>
<td>1-24 mo</td>
<td>No-information</td>
<td>BCG, DTP, MV</td>
<td>Proportion who received BCG and DTP simultaneously is not defined; control group undefined; pigbel vaccine was used with DTP</td>
<td>BCG+DTP1 vs. BCG (mortality between 1-5 months; adjusted for vaccinations, dose response of DTP, background covariates)</td>
<td>Overall bias: High risk of bias (TO ADD (sic))</td>
</tr>
<tr>
<td>India-I (19); rural</td>
<td>Routine service delivery; research supervised system</td>
<td>12,142</td>
<td>6-9 mo</td>
<td>By register</td>
<td>BCG, DTP, MV</td>
<td>Service provision so all vaccines known; few received BCG and DTP simultaneously</td>
<td>BCG+DTP1 vs. BCG; mortality between 1.5-8 month; not adjusted; computed from Table 3;</td>
<td>Overall bias: Very high risk of bias (confounding, particularly by age)</td>
</tr>
<tr>
<td>Ghana-I (20); rural</td>
<td>Routine surveillance</td>
<td>18,368</td>
<td>0-59 mo</td>
<td>No-information</td>
<td>BCG, DTP, MV</td>
<td>Effect of DTP estimated to be 0.15 (0.14-0.16). However, proportion who received BCG and DTP simultaneously is not defined; control group undefined; major survival bias as children are counted from birth though vaccination only administered later</td>
<td>DTP1 vs. no DTP; mortality up to 60 months; adjusted for mother’s education, poverty status, age</td>
<td>Overall bias: Very high risk of bias (high degree of co-intervention with other vaccines; unable to judge methods for determining vaccination status from publication)</td>
</tr>
<tr>
<td>Country (Year); area</td>
<td>Surveillance method</td>
<td>Study Period</td>
<td>Vaccination History</td>
<td>Surveillance Indicator</td>
<td>Survival Bias</td>
<td>Overall Bias</td>
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<tr>
<td>Philippines (21); rural</td>
<td>Routine surveillance; Only children with BCG vaccination included in study</td>
<td>14,537 0-30 mo</td>
<td>No unvaccinated in study</td>
<td>BCG, DTP</td>
<td>63% received BCG and DTP simultaneously (59)</td>
<td>BCG+DTP1-3 vs. BCG; mortality up to 30 mo; landmark approach; adjusted for maternal education, LBW, ownership of TV/radio, age, household cluster; Overall bias: Very high risk of bias (selection of children too long after vaccination)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senegal (25); rural</td>
<td>Routine surveillance</td>
<td>4,102 Day 2-24 months</td>
<td>No information</td>
<td>BCG, DTP, MV</td>
<td>2/3 received BCG and DTP simultaneously; however DTP effect is for children who did not receive BCG+DTP</td>
<td>BCG+DTP1 vs. BCG; before 24 months; adjusted for age, village, birth year, season; Overall bias: High risk of bias (no adjustment for child’s health)</td>
<td></td>
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</tr>
<tr>
<td>India-II (11); rural</td>
<td>Routine surveillance in vitamin A trial</td>
<td>10,274 1 wk-5mo</td>
<td>No-information</td>
<td>BCG, DTP</td>
<td>Proportion who received BCG and DTP simultaneously is not defined; control group undefined.</td>
<td>BCG+DTP1-3 vs. BCG; mortality up to 6 mo; adjusted for OPV, season, birthweight, prior live births, BCG, propensity score; Overall bias: High risk of bias (likely confounding; retrospective collection of vaccination data)</td>
<td></td>
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<tr>
<td>Guinea-Bissau-I (22); Rural area; First introduction of DTP. Few had received BCG</td>
<td>Introduction of DTP in 20 villages; unvaccinated were children who were travelling, too sick to get vaccinated and children examined on days when vaccines were not available for logistic reasons.</td>
<td>1657 2-8 mo</td>
<td>Vaccination provided by project</td>
<td>DTP, BCG</td>
<td>The proportion receiving additional doses of DTP during follow-up increased from 14% to 40% (average 28%) during project; the proportion receiving MV increased from 2 to 18% (average 11%).</td>
<td>DTP1-3 vs. no DTP; mortality up to 8 mo, adjusting for period, season, gender, BCG; Overall bias: High risk of bias (no adjustment for child’s health; assumptions about non-vaccination)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea-Bissau-II (8); rural</td>
<td>Survey in 100 villages</td>
<td>8752 0-13 mo</td>
<td>Vaccination card; maternal history</td>
<td>BCG, DTP, MV</td>
<td>Additional vaccinations were provided during follow-up; 65-71% vaccinated</td>
<td>DTP1 vs. no DTP; age up to 6 mo at visit, mortality within 6 months of follow-up; adjusted for length of follow-up, age, other vaccines; Overall bias: High risk of bias (likely confounding; assumptions about non-vaccination; co-intervention with MV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country</td>
<td>Study Design</td>
<td>Setting</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Vaccination Card</td>
<td>Vaccination Status</td>
<td>Mortality Analysis</td>
<td>Bias Assessment</td>
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<tr>
<td>Guinea-Bissau-III (23); urban</td>
<td>Prospective community trial with low-birth-weight children with assessment of vaccination status at 2 and 6 months of age.</td>
<td>1830</td>
<td>2-6 mo</td>
<td>Vaccination card seen at 2 mo and 6 mo</td>
<td>BCG, DTP</td>
<td>Vaccination status known for all children. Most unvaccinated children received DTP during follow-up</td>
<td>BCG+DTP1 vs. BCG+delayed DTP1; mortality up to 6 mo; LBW children who received BCG at birth; adjusted for arm circumference, gender</td>
<td>Overall bias: High risk of bias (likely confounding; DTP co-intervention in the no-DTP group)</td>
</tr>
<tr>
<td>Malawi (24); rural</td>
<td>Routine monthly surveillance.</td>
<td>767</td>
<td>0-17 mo</td>
<td>Vaccination card</td>
<td>BCG, DTP, MV</td>
<td>With monthly visits and control of health centre records unlikely that many vaccines have been missed; the study included the children seen at the monthly home visit for whom vaccination records are assumed to be complete</td>
<td>DTP1 vs. no DTP; mortality up to 8 mo; only children who were present; adjusted for age, maternal HIV status</td>
<td>Overall bias: High risk of bias (no adjustment for SES or child’s health; assumptions about non-vaccination)</td>
</tr>
<tr>
<td>Benin (26); rural</td>
<td>Case control study of community deaths. Only deaths with vaccination status available.</td>
<td>74 deaths + 230 controls</td>
<td>4-35 mo</td>
<td>Vaccination card</td>
<td>BCG, DTP, MV</td>
<td>Known from vaccination card</td>
<td>DTP+ OPV (1 dose) vs. no DTP, no OPV; adjusted for socioeconomic score, weight for age, other vaccinations</td>
<td>Overall bias: High risk of bias (likely confounding; unknown BCG co-administration; likely co-interventions including MV)</td>
</tr>
<tr>
<td>Ghana-II (27); rural</td>
<td>Trial of vitamin A supplementation; vaccination status assessed at enrolment. General and sex-specific mortality rates available. Only children receiving placebo used in this analysis.</td>
<td>11,722</td>
<td>6-60</td>
<td>Vaccination card</td>
<td>DTP, MV</td>
<td>Most vaccines given out of sequence. Analysed for DTP after MV</td>
<td>BCG+DTP1 2 vs. BCG; 4 months of follow-up; calculated based on Table 5; adjusted for age, zone, weight, ownership of radio</td>
<td>Overall bias: Very high risk of bias (selection of children into the study; high proportion of co-administration with BCG; high proportion of co-administration with MV)</td>
</tr>
<tr>
<td>Guinea-Bissau-IV (28); urban population in</td>
<td>Survey before war, mortality during war.</td>
<td>1491</td>
<td>1-17 mo</td>
<td>Vaccination card before war</td>
<td>BCG, DTP, MV</td>
<td>42% of DTP-unvaccinated received DTP during follow-up</td>
<td>DTP1-3 vs. no DTP; mortality between 6-20 mo; adjusted for age, gender</td>
<td>Overall bias: Very high risk of bias (follow-up begins after DTP vaccinations, and</td>
</tr>
<tr>
<td>rural area</td>
<td>India-III (29)</td>
<td>Routine surveillance</td>
<td>4,138</td>
<td>1-12 mo</td>
<td>Vaccination card</td>
<td>BCG, DTP, MV</td>
<td>Analysis is by sequence of vaccination</td>
<td>BCG+DTP1-3 vs. BCG; mortality up to 12 months; not adjusted; computed from Table 4</td>
</tr>
</tbody>
</table>