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Testing the hypothesis that diphtheria–tetanus–pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries

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ABSTRACT

Background: Measles vaccines (MV) have sex-differential effects on mortality not explained by protection against measles infection.

Objective: The authors examined whether whole-cell diphtheria–tetanus–pertussis (DTP) vaccine has sex-differential and non-specific effects.

Data sources and eligibility: Following previous reviews and a new search, the effect of DTP on mortality up to the next vaccination was assessed in all studies where DTP was given after BCG or DTP was given after MV and there was prospective follow-up after ascertainment of vaccination status.

Setting: High-mortality countries in Africa and Asia.

Methods: The initial observation of negative effect of DTP generated six hypotheses, which were examined in all available studies and two randomised trials reducing the time of exposure to DTP.

Main outcome: Consistency between studies.

Results: In the first study, DTP had negative effects on survival in contrast to the beneficial effects of BCG and MV. This pattern was repeated in the six other studies available. Second, the two 'natural experiments' found significantly higher mortality for DTP-vaccinated compared with DTP-unvaccinated children. Third, the female–male mortality ratio was increased after DTP in all nine studies; in contrast, the ratio was decreased after BCG and MV in all studies. Fourth, the increased female mortality associated with high-titre measles vaccine was found only among children who had received DTP after high-titre measles vaccine. Fifth, in six randomised trials of early MV, female but not male mortality was increased if DTP was likely to be given after MV. Sixth, the mortality rate declined markedly for girls but not for boys when DTP-vaccinated children received MV. The authors reduced exposure to DTP as most recent vaccination by administering a live vaccine (MV and BCG) shortly after DTP. Both trials reduced child mortality.

Conclusions: These observations are incompatible with DTP merely protecting against the targeted diseases. With herd immunity to whooping cough, DTP is associated with higher mortality for girls.

ARTICLE SUMMARY

Article focus

- MV has sex-differential non-specific effects for child survival. We examined whether DTP vaccine has negative effects for survival, particularly for girls.
- We tested six hypotheses suggesting that DTP may have negative health consequences if found to be true.
- Furthermore, we conducted two randomised trials reducing the time of exposure to DTP as most recent vaccination by providing a live vaccine shortly after DTP.

Key messages

- All available studies suggest that the effect of DTP on child survival is opposite of the effects of BCG and MV. In the two natural experiments, DTP-vaccinated children had significantly higher mortality than DTP-unvaccinated children.
- Among DTP-vaccinated children, girls have higher mortality than boys in all studies, whereas the tendency is the opposite for BCG- and measles-vaccinated children. DTP administered after MV in randomised trials of MV is associated with increased female but not male mortality.
- Reducing time of exposure to DTP as the most recent vaccination with BCG or MV reduce child mortality.

Strengths and limitations of this study

- Since the healthiest children are vaccinated first, one would expect DTP to be associated with a benefit. However, all the data suggest consistently that DTP is associated with a negative effect for girls.
- A randomised trial of the effect of DTP on overall survival could not be conducted. There is a need to conduct such studies now.

Randomised studies of DTP are warranted to measure the true impact on survival.

INTRODUCTION

Overall, routine vaccinations have beneficial effects on child survival in high-mortality countries.[1] It is assumed that the effect on child survival is proportional to vaccine efficacy and the burden of the targeted infection.[2] However, routine vaccinations may have non-specific effects (NSE) on child survival—that is, effects not explained by prevention of the vaccine-targeted infections.[1 3 4] Such effects are presumably due to vaccines affecting susceptibility to unrelated infections. NSE were initially demonstrated by the high-titre measles vaccine (HTMV) incident. Though protective against measles infection, HTMV was associated with a twofold increased female mortality if the children got diphtheria–tetanus–pertussis (DTP) after HTMV.[5] In contrast, standard measles vaccine (MV) is associated with a reduction in mortality, which is not explained by prevention of acute measles infection,[6–20] and with reduced mortality for girls relative to boys[5 7 13 17 21–31]; for example, the female–male mortality rate ratio (MRR) declined from approximately 1.00 to 0.65 after the first MV campaigns in two rural areas of Senegal.[13 17]

Following the studies of HTMV and standard MV, we examined the NSE of other vaccines.[1 32–81] BCG was associated with a beneficial effect on mortality, whereas DTP had no such effect, the effects being statistically different for the two vaccines.[1] Subsequent studies corroborated that BCG has beneficial effects for girls.[38 40] On the other hand, DTP appeared to have a negative effect for girls.[26]

With the increasing evidence for NSE of vaccines, the Working Group on the Non-specific Effects of Vaccines (WGNSEV) has formulated and prioritised six trials, which would help to optimise the current vaccination schedules so that they maximise the reduction in child mortality in high-mortality areas.[82] Two priority trials to examine the beneficial NSE of BCG and MV have now been completed.[3 65 79] In trials of BCG at birth versus delayed BCG among low birth weight (LBW) children, neonatal mortality was reduced by 48% (95% CI 18% to 67%) (WGNTEV, trial 1).[65 79] In a large trial of an additional dose of MV at 4.5 months of age,[3] per-protocol analysis showed that children receiving two doses of MV at 4.5 and 9 months of age had a 30% lower mortality between 4.5 and 36 months of age than children receiving the currently recommended single dose of MV at 9 months of age (WGNTEV, trial 2). There was a negative interaction with neonatal vitamin A supplementation (VAS): among the children who had not received VAS, the reduction in mortality was 50% between 4.5 and 36 months of age. When cases of measles infection were excluded, the reduction in mortality was 45%. With such strong support for the importance of the beneficial NSE of live vaccines, there are good reasons to examine carefully the possible negative NSE of inactivated whole-cell DTP.

Though several meta-analyses have shown that well-conducted observational studies can provide the same results as randomised trials,[83 84] observational studies have historically ranked lower as evidence for a treatment effect than randomised studies. Hence, it is desirable to conduct randomised studies to test the NSE of vaccines, including the possible negative effect of DTP. However, once a vaccine is recommended by WHO, it becomes difficult to withhold the vaccine in randomised trials to measure the NSE of the vaccine. A possible way to test DTP would be to randomise children to either the current regimen of vaccination at 6, 10 and 14 weeks of age or to delayed vaccination at 6–8 months of age just before measles vaccination at 9 months of age (WGNSEV, trial 6). However, this has been deemed unethical by WHO committees.[85] Hence, the hypothesis that DTP has a negative effect on mortality can only be tested indirectly, first by making logical deductions about epidemiological trends and testing these in other data sets and, second, by testing the implications in randomised trials. The more deductions can be generated and found to be consistent in unrelated data sets, the more likely it is that the underlying hypothesis represents a causal process. We have tested our initial observation that DTP was associated with increased mortality[1] in several other data sets and subsequently generated five new deductions. These six linked observations suggest that DTP increases mortality in girls in areas with herd immunity to whooping cough. Hence, reducing exposure to DTP as the most recent vaccination should reduce child mortality. In two randomised trials, we tested whether reducing exposure to DTP by vaccinating with MV at 4.5 months of age after DTP3 (WGNSEV, trial 2) and by BCG revaccination at 19 months after DTP booster vaccination (WGNSEV, trial 3) reduced child mortality. This proved to be the case in both trials.

METHODS: MATERIALS AND ASSUMPTIONS

The initial hypothesis about a negative effect of DTP on child survival was formulated in a paper published in 2000.[1]

Data sources

There have been very few studies of routine immunisations and their effect on child mortality in high-mortality countries. A previous WHO-sponsored literature review from 2001[2] found only two studies of the effect of DTP on mortality.[1 18] During the last 10 years, several new studies have become available and studies of DTP and mortality have been reviewed three times.[61 81 86] In 2011, we conducted a new PubMed search for publications on 'mortality' or 'death' in relation to one of the vaccine terms 'DTP', 'DPT', 'diphtheria-tetanus-pertussis' or 'pentavalent vaccine' (see supplementary flow diagram). To be relevant, a study had to report total mortality following DTP vaccination in a defined population of children in high-mortality countries (Africa and Asia). The methodological exclusion criteria are discussed below. This search identified 444 papers: based on reading the abstract or paper, 122 papers were classified

as reviews, commentaries and cost-effectiveness analyses without primary data; 99 papers were not from a high-mortality country in Africa or Asia; 117 papers had no mortality data or were not about children; 43 papers were not related to DTP and 33 papers were disease-specific studies or case reports. Hence, we retained 30 studies, and an additional five studies were known from vaccine reviews or other sources.[9 18 47 78 87] These studies are briefly presented in tables 1 and 2. Most of the identified studies were conducted in Guinea-Bissau or were reanalyses of data from longitudinal studies in Africa, including studies from Congo,[22] Gambia,[31] Ghana,[74] Malawi,[30 53] Senegal[5] and Sudan[22] (tables 1 and 2). Seven studies were a result of WHO's Global Advisory Committee on Vaccine Safety request that other groups reanalyse existing data sets to examine the effect of DTP on child mortality.[44–49 58] Fourteen studies had information on DTP provided before MV (table 1); two studies were partly overlapping,[3 52] so only one of them was used in the hypothesis-specific tables.[52] Three studies had specific morbidity but no mortality data (table 1). Nine studies had information on mortality when DTP was administered after MV (table 1). Nine studies were not used for the reasons discussed in the following paragraph. Table 1 indicates whether the studies had general and sex-specific mortality rates or only data from case–control and hospital case death studies with no follow-up.

Study exclusion criteria

We excluded studies that had survival bias and studies in which DTP had been administered with BCG or MV. First, in our previous review of DTP and mortality,[61] studies with retrospective updating of vaccine information had survival bias which produced misleading estimates of the effect of DTP[60 61] (table 2). Second, the recommended vaccination schedule used in Bissau while most of these studies were conducted is depicted in figure 1. Our hypothesis has focused on vaccinations, which have been administered in the sequence recommended by WHO: BCG first, followed by three doses of DTP and oral polio vaccine (OPV) and then MV (figure 1). However, delays in vaccination programmes are common and vaccinations are often given simultaneously: BCG with DTP or DTP with MV.[44 45 47 48 58 77] These combinations have different NSE.[22 28 53 57–59 64 66 77] Hence, studies in which most children received BCG and DTP simultaneously or DTP and MV simultaneously were not included (see table 2).

Analyses

The issues associated with analysing incomplete vaccination data have been presented more fully in an appendix (supplementary material). Many studies have analysed simultaneously the effect of several vaccines over several years, for example, 2 or 5 years of age.[44–48] This would be appropriate if each vaccine had an independent and permanent programming effect on the immune system. However, the NSE of vaccines apparently influence general susceptibility to infectious diseases and that effect seems to be strongest while a vaccine is the *most recent vaccination*.[81] We therefore focused on studies in which the effect of a specific vaccine could be analysed from the time it was administered until the next vaccine was given.

In most survival analyses, the estimates of the effects of vaccines on survival are obtained by comparing *mortality at the same age* for vaccines, which are recommended to be given sequentially. This comparison is inherently biased ('frailty bias') because the healthiest children are likely to receive the recommended vaccine first (see appendix). Comparisons of sequential vaccinations should be interpreted cautiously. However, if a vaccine, in spite of the expected lower mortality, is associated with a higher mortality than the previous vaccine, this is an indication that the vaccine in question may be associated with an absolute increase in mortality. We have also emphasised the comparison of female and male mortality rates among children who have received the same vaccine. In the pre-vaccination era, post-neonatal child mortality was similar for boys and girls in West Africa (figure 2). All data from Africa also suggest that girls and boys are treated equally with respect to vaccination, the age of vaccination and coverage being the same for boys and girls. In these circumstances, a deviation of the female–male MRR from 1.0 for children who have received a specific vaccine as the most recent vaccination would suggest that this vaccine is associated with sex-differential NSE.

Statistical analyses

Estimates are presented as reported in the published papers or have been calculated based on the deaths and person-years reported in the papers. Tests for interaction have been used to assess whether MRR for different vaccines were similar for vaccinated versus unvaccinated children and for girls versus boys.

Presentation

For each of the six deductions, we present a short section on the deduction which generated a new hypothesis and how studies were selected, a section on the observations which followed the hypothesis and a section discussing possible methodological problems and conclusions in relation to the observation. The development of the hypotheses is summarised in figure 3. The sequence of presentation follows more or less the sequence in which these hypotheses were generated: observations I–III deal with the effect of DTP when administered after BCG (or no vaccine) and observations IV and V with the effect of DTP administered after MV. Observation VI relates to the effect on female mortality of changing from DTP to MV vaccination. Finally, we present the two trials, which were conducted to reduce the duration of exposure to DTP as the most recent vaccination.

Table 1 Studies used in the analysis

Country/ reference	Setting	Design and comments	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Other vaccinations during follow-up
Studies of DTP administered after no prior vaccine and before MV: Guinea-Bissau								
DTP introduction[42]	Rural area; first introduction of DTP. Few had received BCG	Introduction of DTP in 20 villages; unvaccinated were children who were travelling, too sick to get vaccinated and children examined on days when vaccines not available for logistic reasons. General and sex-specific mortality rates available.	1657	2–8 months	6 months	Vaccination provided by project	DTP, BCG	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%).
Studies of DTP administered after BCG and before MV: Guinea-Bissau								
Routine vaccinations[1]	Rural	Survey in 100 villages; first results from this study reported in Aaby *et al.*[11] General mortality rates available.	8752	0–13 months	6 months	Vaccination card	BCG, DTP, MV	Additional vaccinations were provided during follow-up; 65%–71% vaccinated
War[26]	Urban population in rural area	Survey before war, mortality during war. General and sex-specific mortality rates available	1491	1–17 months	3 months in war	Vaccination card before war	BCG, DTP, MV	42% of DTP-unvaccinated received DTP during follow-up—only 2% of MV-unvaccinated received MV
Guinea-Bissau, Senegal twins[28]	Female–male twin pairs from several studies	Mortality until next vaccine. Only deaths by sex and vaccination status available.	626 pairs	0–17 months	Within 0–17 months of age	Vaccination card	BCG, DTP, MV	Unlikely since the co-twin would indicate whether other vaccinations had been received
Hospital case death[27]	Hospital mortality	Survey at admission. Only deaths by sex and vaccination status available.	2079	0–17 months	At hospital	Vaccination card	BCG, DTP, MV	Vaccination status assessed at hospitalisation. No additional vaccinations at hospital

Continued

Table 1 Continued

Country/ reference	Setting	Design and comments	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Other vaccinations during follow-up
Hospital-OPV[43]	Hospital mortality	Survey at admission, DTP missing in certain periods. Only deaths by sex and vaccination status available.	719	0–59 months	At hospital	Vaccination card	DTP, OPV	Vaccination status assessed at hospitalisation. No additional vaccinations at hospital
Trial of vitamin A supplementation (VAS) at birth[52]	Urban	Prospective community trial. General and sex-specific mortality rates available	4345	0–8 months	8 months	BCG provided by project; Vaccinations from health centres, home visits, verbal autopsy	BCG, DTP	All children who died at 0–1.5 months of age had BCG as most recent vaccination. Female–male MRR at 0–1.5 months has been taken to be indicative of the BCG effect. Children with DTP as most recent vaccination are unlikely to have received MV during follow-up as all MV in study area controlled by project
Low birth weight cohort[64 65]	Urban	Prospective community trial with assessment of vaccination status at 2 and 6 months of age. General and sex-specific mortality rates available.	1830	2–6 months	4 months	Vaccination card seen at 2 and 6 months	BCG, DTP	Vaccination status known for all children. Most unvaccinated children received DTP during follow-up

Continued

Table 1 Continued

Country/ reference	Setting	Design and comments	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Other vaccinations during follow-up
Early MV trial[3]	Urban	DTP3-vaccinated children randomised at 4.5 months of age to receive MV or maintain DTP3 as most recent vaccination. General and sex-specific mortality rates available.	6648	4.5–9 months	4.5 months	All vaccines documented	DTP3, MV	No vaccine until MV was due at 9 months of age since they had all received DTP3 before enrolment
Studies of DTP administered after BCG and before MV: studies from other countries								
Nigeria[9]	Rural	Randomised study of MV versus DTP. Only deaths by vaccination status available.	52		18 months	Vaccination provided by project	DTP, MV	Unlikely
Benin[18]	Rural	Case–control study of community deaths. Only deaths by vaccination status available.	74 deaths + 230 controls	0–35 months	Maximum 3 years	Vaccination card	BCG, DTP, MV	Known from vaccination card
Malawi[53]	Rural	Routine monthly surveillance. General and sex-specific mortality rates available	767	0–17 months	Within 0–17 months	Vaccination card	BCG, DTP, MV	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

Continued

Table 1 Continued

Country/ reference	Setting	Design and comments	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Other vaccinations during follow-up
Gambia[31]	Rural	No individual vaccination information collected. Coverage for children <5 years known from two surveys. Vaccination cards of dead children collected at verbal autopsy.	537 deaths under 5 years	0–17 months	Within 0–17 months	Vaccination cards collected from dead children	BCG, HBV, DTP, MV	Vaccination status known from vaccination card of children who died. All children who died at 5–8 months of age had DTP as most recent vaccination and nearly all children who died at 12–17 months had MV as most recent vaccination.
Bangladesh[54]	Urban	Trial: children hospitalised with diarrhoea received DTP1 at discharge and were randomised to VAS/ placebo. DTP2 and DTP3 were provided during follow-up together with VAS/ placebo. General and sex-specific mortality rates available.	200	1–12 months	6 months after DTP	Vaccination provided by project	DTP	
Studies of DTP administered after MV								
Guinea-Bissau[5 6]	Urban	Trial of HTMV. General and sex-specific mortality rates available	242	4–8 months	3–5 years of age	Vaccination provided by project	DTP, IPV, MV	Most vaccines were controlled by project
Senegal[7]	Rural	Trial of HTMV. General and sex-specific mortality rates available	1579	5 months	3–5 years of age	Vaccination provided by project	DTP-IPV, MV	Unlikely that many vaccines have been given since project controlled all vaccinations in area

Continued

Table 1 Continued

Country/ reference	Setting	Design and comments	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Other vaccinations during follow-up
Senegal[23]	Rural	Routine use of HTMV. General and sex-specific mortality rates available	944	5–7 months	3 years of age	Vaccination provided by project	DTP-IPV, MV	Unlikely that many vaccines have been given since project controlled all vaccinations in area
Congo[22]	Urban	Trial of HTMV. General and sex-specific mortality rates available	1023	Cohort 1: 3.5–9.5 months; cohort 2: 6 months	30 months	Vaccination provided by project	DTP, MV	Additional DTP vaccinations unlikely to have been given since most had received DTP3 in cohort 2.
Sudan[22]	Rural	Trial of HTMV. General and sex-specific mortality rates available	510	5 months	31 months	Vaccination provided by project	DTP, MV	Likely that additional DTP vaccinations might have been given to those missing DTP3
Two-dose MV trial[51]	Urban	Standard MV given at 6 months; control group received IPV. Both groups received MV at 9 months. General and sex-specific mortality rates available	8511	6–9 months	Mostly 3–6 months	Vaccination provided by project	IPV, DTP, MV	Those missing DTP3 likely to have received DTP during follow-up. Control children unlikely to have received MV before 9 months of age as all MV administered by project.
Guinea-Bissau[57]	Urban	Survey at admission; only children with MV. Only deaths by sex and vaccination status available.	779	6–17 months	At hospital	Vaccination card	DTP, MV	No additional vaccinations at hospital

Continued

Table 1 Continued

Country/ reference	Setting	Design and comments	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Other vaccinations during follow-up
Ghana[74]	Rural	Trial of VAS; vaccination status assessed at enrolment. General and sex-specific mortality rates available.	11 722	6–60 months	2 years	Vaccination card	DTP, MV	Most vaccines given out of sequence. Analysed for DTP after MV
Guinea-Bissau[78]	Urban	Trial of vitamin A with MV	455	9–36 months	27 months	Vaccination card	DTP, MV	Those missing DTP3 likely to have received DTP during follow-up
Morbidity studies								
Guinea-Bissau: Morbidity[55 56]	Urban, cohort followed each week	Incidence of cryptosporidium and rotavirus	200	0–23 months	6 months or to 24 months of age	Vaccination card	BCG, DTP, MV	Perfect information on vaccination since only children with card seen after episode has been used.
Guinea-Bissau[75]	Urban	Risk of hospitalisations for measles infection	12 107	6–59 months		Vaccination card	BCG, DTP, MV	

DTP, diphtheria–tetanus–pertussis; HBV, hepatitis B virus; HTMV, high-titre measles vaccine; MV, measles vaccine; OPV, oral polio vaccine.

Table 2 Studies not used in the analysis due to simultaneous vaccination (see study exclusion criteria, p. 3)

Country/ reference	Setting	Design	No. of children	Age group	Length of follow-up	Information on unvaccinated	Vaccines	Reasons for not being included in the mortality analyses
Senegal[11]	Rural	Trial of HTMV	1579	5–10 months	5 months	Children not attending	DTP–IPV	Cohort had received DTP and BCG simultaneously. MRR =1.60 (0.76 to 3.37) for DTP2–IPV versus absent.[11]
Bangladesh[44]	Rural	Routine surveillance	37 894	1–59 months	Ongoing	No information	BCG, DTP, MV	71% received BCG and DTP simultaneously; control group undefined
India[49]	Rural	Routine surveillance in vitamin A trial	10 274	1 week to 5 months	2 weeks	No information	BCG, DTP	Proportion who received BCG and DTP simultaneously is not defined; control group undefined. Female–male MRR =1.39 (0.74 to 2.61) for DTP-vaccinated children
Papua New Guinea[46]	Rural	Routine surveillance	4048	1–24 months	1 month	No information	BCG, DTP, MV	Proportion who received BCG and DTP simultaneously is not defined; control group undefined; pigbel vaccine was used with DTP
Cebu, the Philippines[58]	Rural	Routine surveillance	14 537	0–30 months	6 months	No unvaccinated in study	BCG, DTP	63% received BCG and DTP simultaneously[59]
Senegal[48]	Rural	Routine surveillance within trial of acellular and whole-cell pertussis vaccine	11 369	0–24 months	1 week, 3 months	No information	BCG, DTP, MV	100% received BCG and DTP simultaneously; control group undefined; survival bias in one of the two cohorts
Burkina Faso[45]	Rural	Routine surveillance	9085	0–24 months	6–8 months	No information	BCG, DTP	Proportion who received BCG and DTP simultaneously is not defined; control group undefined; survival bias
Ghana[47]	Rural	Routine surveillance	17 753	4–59 months	12 months	No information	BCG, DTP, MV	Proportion who received BCG and DTP simultaneously is not defined; control group undefined; survival bias
Ghana[81]	Rural	Routine surveillance	18 368	0–59 months	12 months	No information	BCG, DTP, MV	Effect of DTP estimated to be 0.15 (0.14 to 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

DTP, diphtheria–tetanus–pertussis; HTMV, high-titre measles vaccine; IPV, inactivated polio vaccine; MV, measles vaccine.

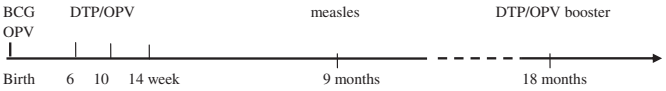

Figure 1 Vaccination schedule in Guinea-Bissau. Note: the vaccination schedule in Guinea-Bissau was changed in 2008. Pentavalent vaccine (DTP + hepatitis B virus + Hib) has replaced DTP, yellow fever is administered with measles vaccine and the booster doses of DTP and OPV have been removed. DTP, diphtheria–tetanus–pertussis; OPV, oral polio vaccine.

RESULTS

Developing the hypothesis: DTP has negative NSE and sex-differential effect on child survival

As described under the Methods section, 444 studies were screened for inclusion and 35 were retained as potentially relevant to the final analyses (tables 1 and 2).

Observation I. Contrasting effects of DTP, BCG and MV

Deduction and study selection

Unexpectedly, a study from rural Guinea-Bissau reported differential effects of DTP, BCG and MV within the same population[1]; mortality was low after BCG, increased after the first DTP vaccination (DTP1) and reduced again after MV. We tested the pattern of contrasting MRR for different vaccines in all studies that reported data on the primary DTP vaccination (after BCG and before MV) as well as BCG and/or MV vaccinations (see table 1).

Observation

A case–control study from Benin[18] had previously reported the same tendencies in relative mortality for the three vaccines (figure 4). We tested these trends in a further five studies. All studies found the same pattern: mortality was low after BCG and MV but increased after DTP vaccination (figure 4, supplementary table 2). The difference in effect on survival of BCG versus DTP was statistically significant in six of six studies and for DTP versus MV in three of five studies. DTP vaccination compared with no DTP vaccination was associated with increased mortality in all seven studies, being significant in three studies.

In most studies, DTP-unvaccinated children had received BCG. However, in the first 2 years of the study of the introduction of DTP in the rural areas of Guinea-Bissau,[42] only 36 children had received BCG. If the BCG-vaccinated children were excluded, DTP-vaccinated children had a mortality rate of 14.7 per 100 person-years (27/183.4) compared with 5.3 per 100 person-years (9/169.4) for the DTP-unvaccinated children, the adjusted MRR being 2.62 (95% CI 1.08 to 6.35); the negative effect was similar for girls (MRR =2.63 (95% CI 0.76 to 9.05)) and boys (MRR =2.60 (95% CI 0.85 to 7.94)) (unpublished data). Hence, DTP may be associated with increased mortality either when it is given to previously unvaccinated children or to children who have previously received BCG.

Methodological considerations and implications

In the initial study,[1] the trends were strong enough to document a differential effect of BCG and DTP, even though we did not limit the analysis to the most recent vaccination but used a landmark approach with multivariable analysis to estimate the effect of BCG and DTP in the same age range. A stricter censoring of the observational period to the age range in which DTP predominates would have provided stronger estimates: for example, the MRR estimate for DTP2 and DTP3 was reported to be 1.38 (95% CI 0.73–2.61)[1] but became 1.75 (95% CI 0.86 to 3.59) when follow-up was restricted to 9 months to reduce the confounding effect of MV, which is usually given at 9 months of age.[50]

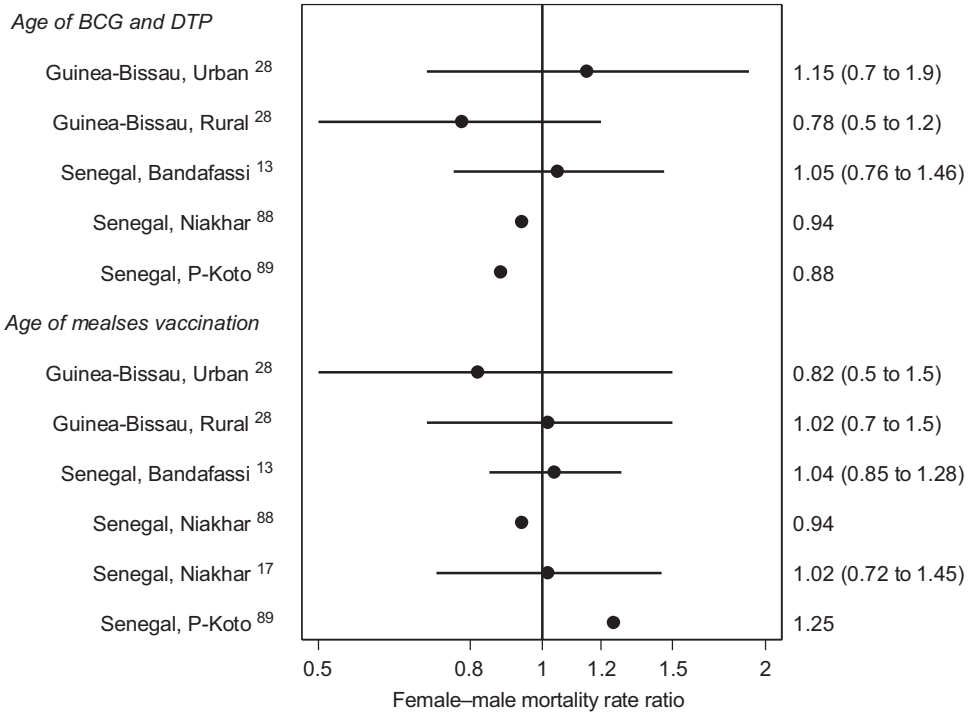

Figure 2 Female–male mortality ratios in community studies from the prevaccination era in West Africa (see supplementary table 1).

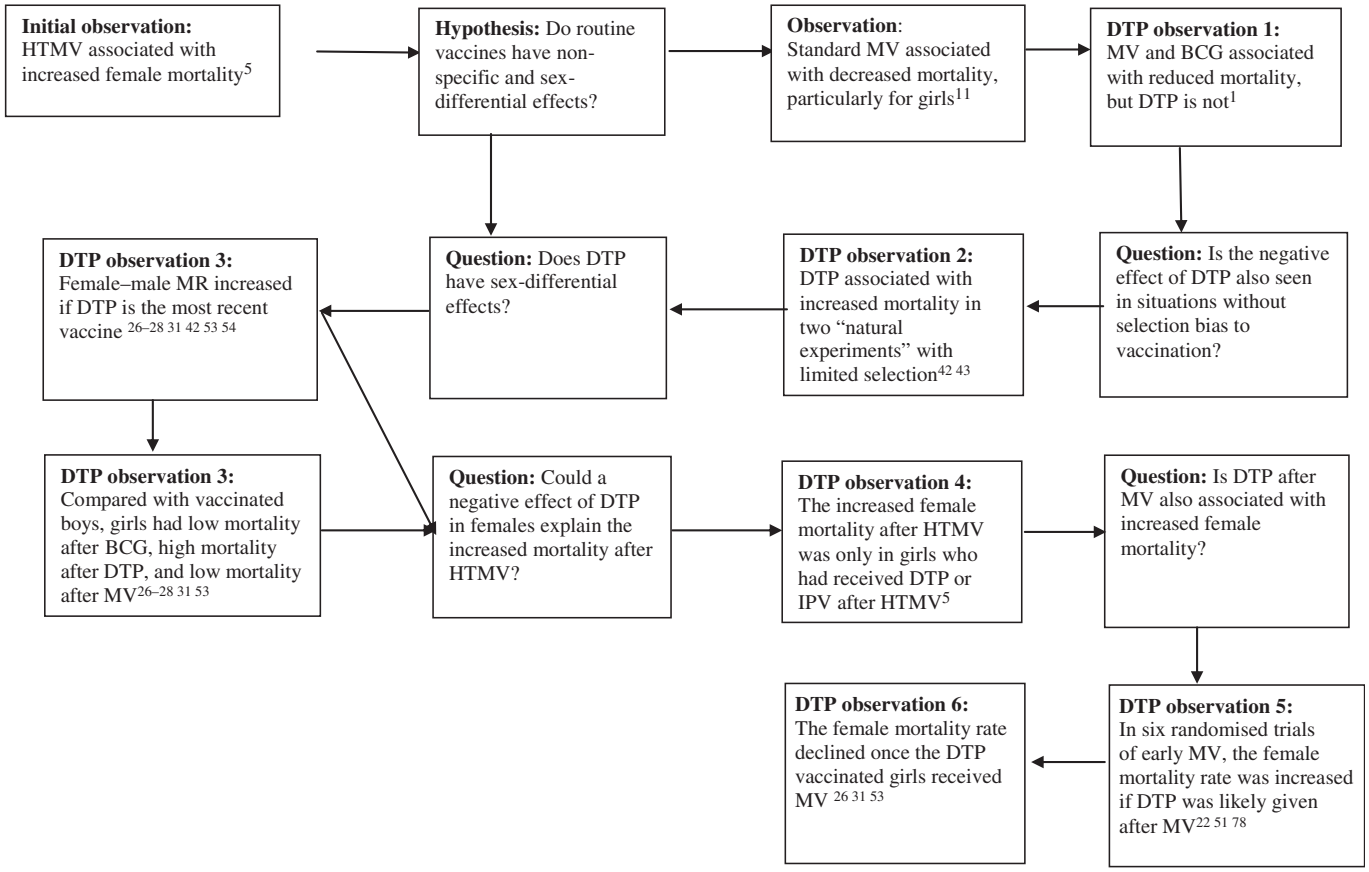

Figure 3 The research process. DTP, diphtheria–tetanus–pertussis; HTMV, high-titre measles vaccine; MV, measles vaccine.

DTP and OPV are usually given together, and it is therefore difficult to distinguish the effects of these vaccines. However, when we first introduced DTP in rural areas of Guinea-Bissau, the effect was particularly strong during the first year when no OPV was available and only DTP was administered.[42] In a second study,[43] DTP administered with OPV had a worse effect than OPV alone. Furthermore, when only OPV was given

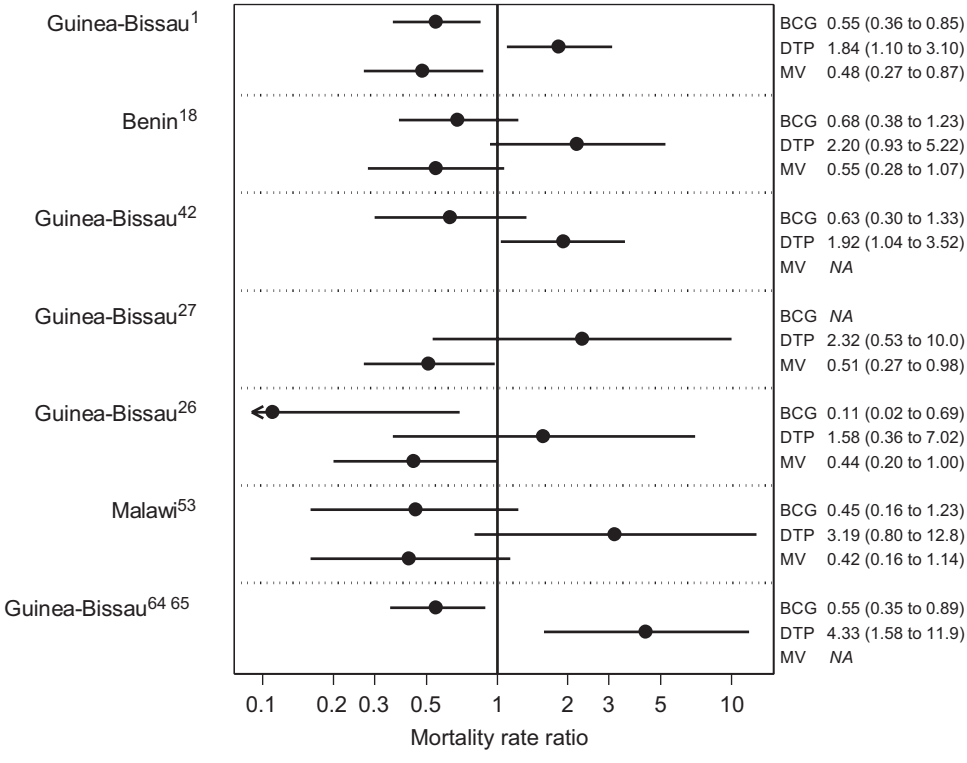

Figure 4 Mortality ratios for different routine vaccinations within the same study (observation I) (see supplementary table 2). DTP, diphtheria–tetanus–pertussis; MV, measles vaccine.

during a polio eradication campaign, the impact of OPV was, if anything, beneficial.[36] Hence, we assume that any negative effect of combined DTP and OPV vaccination is due to DTP.

It has been proposed that a negative effect of DTP could be due to sick children getting vaccinated first because they come more frequently to be treated at health centres.[18 90 91] However, both nurses and mothers are usually reluctant to vaccinate sick children.[42 46 67] If the increased mortality among DTP-vaccinated children occurred because sick children had been preferentially recruited for the DTP group, then there should have been a non-random clustering of deaths in the DTP group. This was not the case.[26 42] For example, in the study of the introduction of DTP,[42] it was noted that the median time to death was 95 days in the DTP group and 80 days in the unvaccinated group. The survival curves in the studies comparing DTP-vaccinated and DTP-unvaccinated children also indicate that there is no clustering of early deaths in the DTP group.[1 42 53 64] Consistent with the tendency of nurses and physicians not to vaccinate sick children, current data suggest that DTP vaccination is associated with a strong positive selection bias, with healthier and wealthier children coming first for vaccination.[1 42 53 64] Due to 'frailty bias' among the DTP-unvaccinated children, DTP should be expected to be associated with a beneficial effect on child survival. The opposite effect of DTP observed in all studies in figure 4 is therefore worrying. Furthermore, BCG and MV are associated with a beneficial effect, and it is therefore unlikely that a negative selection bias for being vaccinated explains the effect of DTP. Hence, since DTP is associated with a higher mortality than BCG, the data in figure 4 clearly suggest that DTP is associated with increased mortality. BCG, DTP and MV have different effects on survival, and since BCG and MV have been found in randomised trials to have a beneficial NSE, DTP apparently has a negative NSE.

Observation II. Comparing DTP-vaccinated and DTP-unvaccinated children in studies with limited selection bias

Deduction and study selection

The consistently opposite effect of inactivated DTP compared with live BCG and MV suggested that mortality might be increased after DTP vaccination. A small randomised trial of MV used DTP as a control vaccine; three of 27 DTP-vaccinated children died during 18 months of follow-up compared with none of 26 measles-vaccinated children.[9] No large-scale randomised study has examined the impact of DTP vaccine on survival. In the absence of randomised trials, we looked for studies with limited selection bias with respect to who received or did not receive DTP. Among the studies indicated in table 1, only two studies could be said to have *limited self-selection* for DTP vaccination.[42 43]

Observations

First, the Bandim Health Project introduced DTP in 20 villages in rural Guinea-Bissau in the mid-1980s, at a time when vaccines were not generally available in rural areas. This is the only study in the global literature of what happened when DTP was introduced in a high-mortality country. Controlling for background factors, the DTP-vaccinated children had a twofold higher mortality (MRR=1.92 (95% CI 1.04 to 3.52)) during 6 months of follow-up compared with DTP-unvaccinated children, even though unvaccinated children had worse nutritional status.[42] Some children were travelling when the team visited the villages every sixth months, some had fever and were therefore not vaccinated, and vaccines were not available at some visits.[42] Unvaccinated children had limited access to vaccination elsewhere until our next visit.

In a second study, DTP was not available in several periods in Bissau city in 2001. Some children, who were due to receive DTP and OPV, received only OPV when they came for vaccination to a health centre, whereas others received both vaccines. We compared the case death of hospitalised children at the only paediatric ward in Bissau according to their vaccination status. Children who had received both OPV and DTP as their most recent vaccines had a significant threefold higher case death compared with children who had received OPV but not DTP when they had come to a health centre. The case death was threefold higher for both boys and girls, though this difference was not statistically significant for either boys or girls individually (unpublished data). Controlling for background factors did not change the estimate.[43 67]

Methodological considerations and implications

Several observational studies have estimated the effect of DTP without survival bias (figure 4). Since these studies did not find the expected benefit for DTP-vaccinated compared with DTP-unvaccinated children, DTP may have negative effects on child survival. The negative effect of DTP in the two studies with limited self-selection for DTP vaccination was not due to sick children being more likely to receive DTP vaccination.[90] DTP-vaccinated children had slightly better nutritional status in both studies and few[42] or no vaccines[43] were given during follow-up. Hence, these two studies provide further support for the hypothesis that DTP is associated with an increase in mortality rate.

Observation III. Sex-differential effects of DTP

Deduction and study selection

HTMV, MV and BCG have sex-differential effects.[5 11 40] We therefore examined whether DTP had sex-differential effects. Nine of the 13 studies of DTP before MV (table 1) had information on sex-specific mortality rates.[1 9 18 43]

Observations on mortality

The studies of the relative mortality of girls and boys after DTP administered before MV are summarised in figure 5 (supplementary table 3). Girls had higher mortality than boys in all nine studies, the difference being statistically significant in four studies. In nearly all

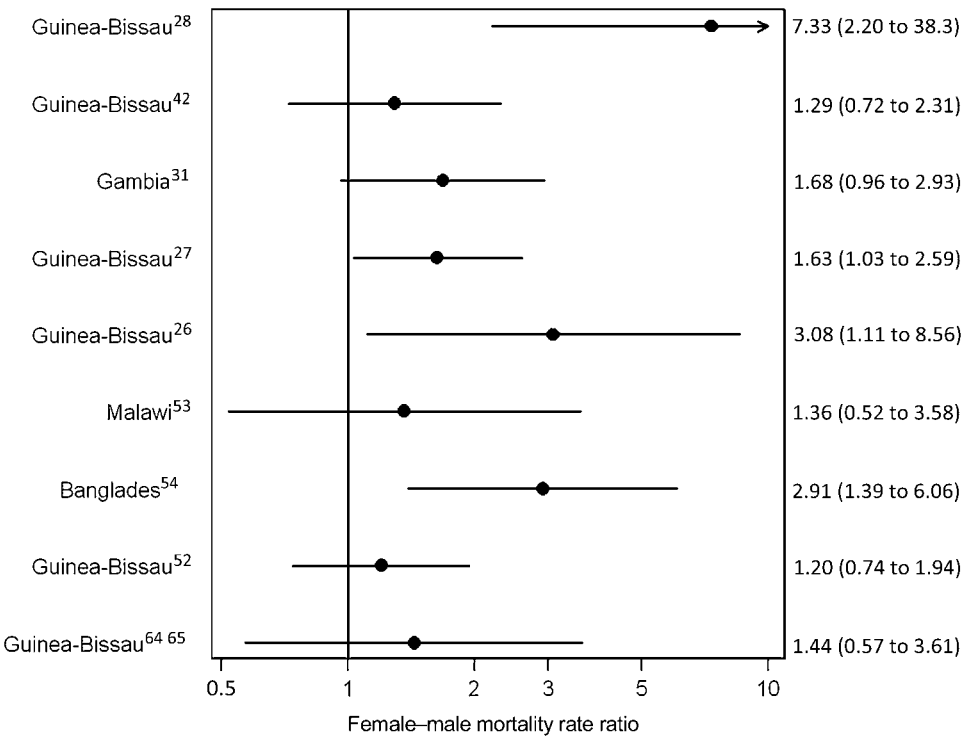

Figure 5 Female–male (F/M) mortality ratios for diphtheria–tetanus–pertussis (DTP)-vaccinated children in studies of DTP administered after no previous vaccine[42] or after BCG (other studies) and before measles vaccine (observation III) (see supplementary table 3).

studies, DTP was administered after BCG. In the two studies in which some children had received VAS, the female–male MRR was higher among those who had received VAS than among those who had received placebo (supplementary table 3). In the study of vaccination status and in-hospital mortality,[27] the sex-differential effect of DTP applied to all major disease categories, including pneumonia, diarrhoea and presumptive malaria. The three studies with mortality data for unvaccinated children suggested a slightly lower mortality for unvaccinated girls than for unvaccinated boys, but the difference was not statistically significant.

In all studies that had information on DTP as well as BCG or MV, the female–male MRR was low after BCG and MV vaccinations and high after DTP (figure 6). The difference in the female–male MRRs between DTP and MV was statistically significant in five of five studies (supplementary table 4).

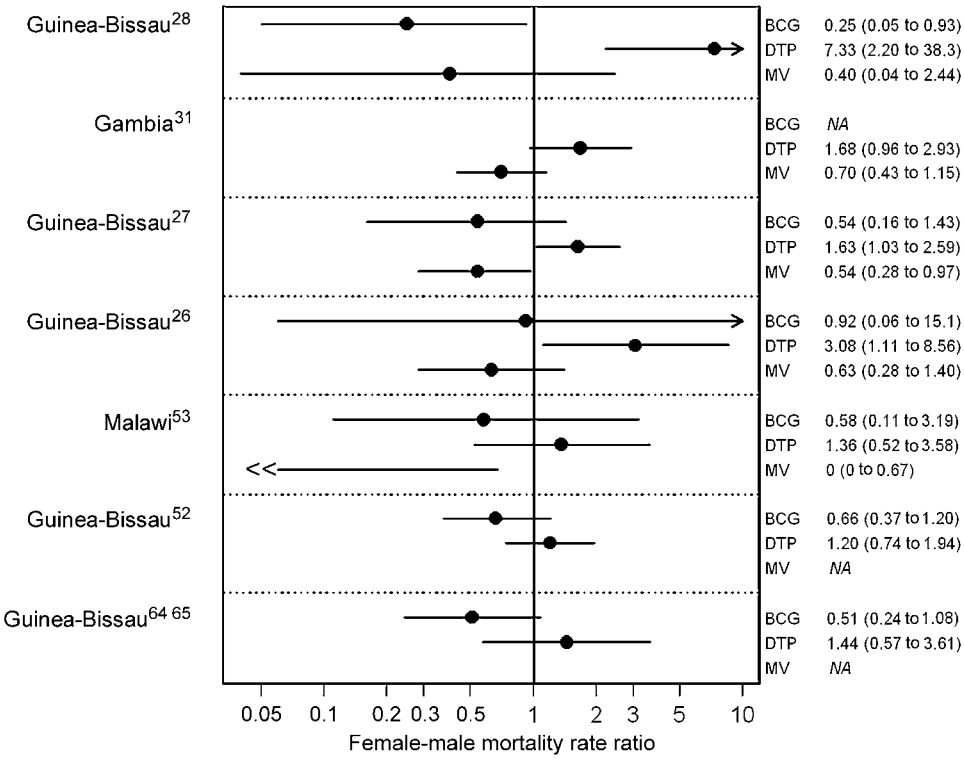

Figure 6 Female–male mortality ratios for diphtheria–tetanus–pertussis (DTP)-, BCG- and measles vaccine (MV)-vaccinated children (observation III) (see supplementary table 4).

Observations on morbidity

In community studies with complete information on vaccinations, the female–male incidence rate ratio among DTP-vaccinated children was 6.25 (95% CI 2.06 to 18.9) for cryptosporidium, 1.93 (95% CI 0.89 to 4.21) for rotavirus infection, 1.51 (95% CI 1.04 to 2.20) for infection with other diarrhoea-causing enteropathogens and 1.32 (95% CI 1.03 to 1.70) for infection with enteropathogens not causing diarrhoea.[55 56] In one study of measles infection, the female–male RR for hospitalisation varied significantly depending on the most recent vaccination, being 1.21 (95% CI 0.82–1.77) for DTP before MV and 0.28 (95% CI 0.11–0.68) for MV alone.[75]

Methodological considerations and implications

Several trials of early MV before 9 months of age have had a control group of children who were not measles vaccinated before 9–10 months of age.[7 22 51] These studies were not included in the present analysis of the sex-differential effect of DTP as most studies used another vaccine as a 'control' vaccine. In the studies using inactivated polio vaccine (IPV) as the control vaccine, the female–male MRR was 1.52 (95% CI 1.02 to 2.28) between enrolment and the receipt of MV.[51] In Senegal, where the control group received placebo at the same time as DTP–IPV at 5 months of age, the female–male MRR was 1.41 (95% CI 0.53 to 3.76) between 5 and 10 months of age. However, since most children in Senegal had received DTP and BCG simultaneously, the study was not included in figure 5.

It could be suggested that girls have a higher mortality than boys in the age group in which DTP predominates and that the sex-differential effects of DTP vaccination are age specific rather than vaccine related. We therefore compared mortality or morbidity rates of DTP and MV-vaccinated boys and girls within the same age group in the studies with relevant data[26–28 55] (figure 7). In all studies, the female–male mortality or morbidity ratio was high after DTP and low after MV, the difference being statistically significant in three of five studies (supplementary table 5). Hence, the pattern is more likely to be vaccine specific than age specific.

The higher relative mortality of DTP-vaccinated girls compared with DTP-vaccinated boys in these studies is not merely a trivial repetition of the same study design with the same potential biases. Very different study designs were used, including studies of female–male twin pairs,[28] vaccination cards collected after death,[31] vaccination status measured just before a war period with high mortality,[26] vaccination status assessed at hospitalisation[27] and children receiving DTP at discharge from a hospital.[54] It cannot be explained by nutritional status because girls did not have worse nutritional status than boys at DTP vaccination (data available upon request). The contrasting female–male MRRs between DTP and BCG or MV also excludes the possibility that the higher female–male MRR after DTP was due to a generally higher female mortality rate in Africa. Since most of the present studies (figures 5 and 6) also documented increased mortality of DTP-vaccinated children relative to BCG- and MV-vaccinated children (figure 4), the higher female than male mortality rates or case death ratios for DTP-vaccinated children presented in supplementary tables 3–5 cannot be explained by a beneficial effect of DTP for boys. Hence, these studies support the hypothesis that DTP is associated with increased female mortality when DTP is given after BCG.

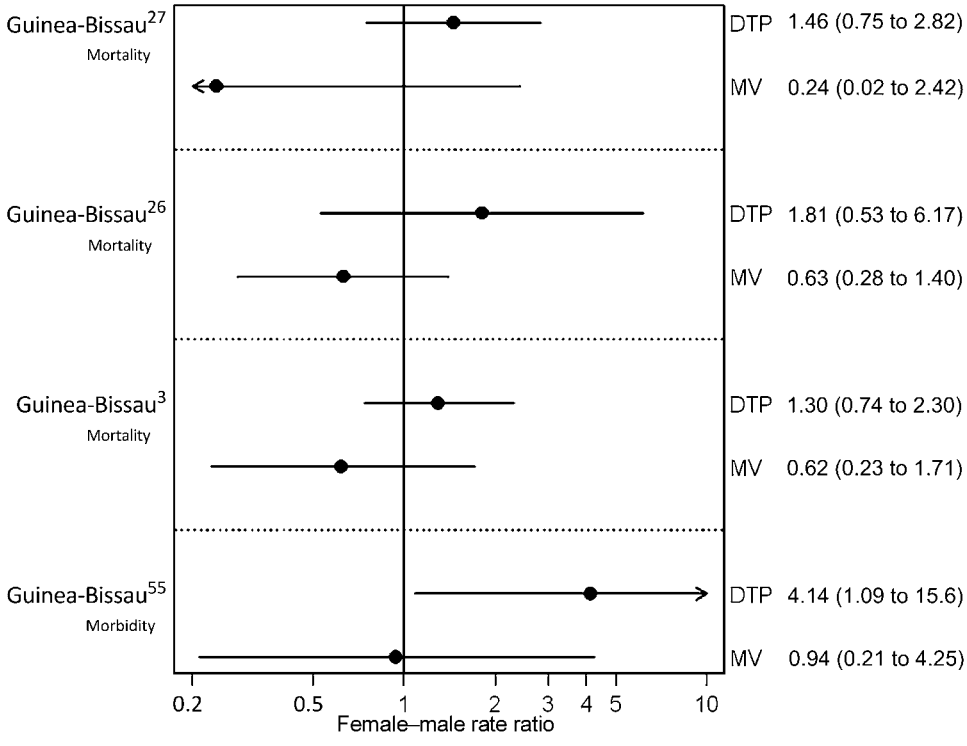

Figure 7 Mortality or morbidity patterns among diphtheria–tetanus–pertussis (DTP)-vaccinated and measles-vaccinated children of similar age (observation III). One additional study of female–male twins could not be presented graphically because one group had no deaths; this study had significant differential effects of DTP and MV (see supplementary table 5).

Observation IV. HTMV trials and DTP

Deduction and study selection

The consistent sex-differential effects of DTP suggested a solution to an earlier enigma. In the late 1980s, randomised trials found that HTMV administered at 4–5 months of age was associated with a twofold higher mortality in girls.[5] Control children received IPV or placebo at 4–5 months of age plus standard-titre MV at 9–10 months of age. Though girls have a lower mortality after standard MV (observation III), HTMV-vaccinated girls had a higher mortality than HTMV-vaccinated boys and a higher mortality than control girls after 9–10 months of age (when control children had received standard MV). HTMV was administered early, and most children received DTP/IPV *after* MV. We examined whether DTP/IPV administered after HTMV could explain the increased female mortality in all the HTMV trials with information on vaccinations after HTMV.[5 22 92] Another HTMV study with mortality data was performed in Haiti,[29] but DTP vaccination status was not reported.

Observations

In a reanalysis of all the West African trials, we found no increase in the female–male MRR for children who did not receive the planned DTP/IPV vaccination after HTMV (female–male MRR =0.96 (95% CI 0.69 to 1.34)). In contrast, twice as many girls as boys died among children who received DTP/IPV after HTMV.[5] Girls in the HTMV group who received DTP/IPV at 9–10 months of age had an MRR of 2.35 (95% CI 1.41 to 3.91) from 9 months to 5 years of age compared with control girls receiving standard MV at the same age.[5] Hence, the real cause of increased female mortality after HTMV was probably that most children received DTP *after* being given HTMV at 4–5 months of age, while control children received DTP *before* being given standard MV at 9 months of age.

We tested this further in a reanalysis of the two other African studies of HTMV with mortality data.[22 92] First, in Congo, children did not receive DTP after HTMV and girls had no excess mortality, the female–male MRR being 0.40 (95% CI 0.13 to 1.27). Second, in Sudan, children did receive DTP after HTMV, and the female–male MRR was 3.89 (95% CI 1.02 to 14.83) from 5 months to 3 years of age. This study collected data on vaccination status at enrolment but not on vaccinations during follow-up. Excess female mortality was found only among children who had not received all doses of DTP prior to enrolment and so presumably received missing doses during follow-up. The excess female mortality increased significantly with the number of doses of DTP missing at enrolment and therefore likely to be given during follow-up. Hence, DTP is likely to have caused the increased female mortality.

Methodological considerations and implications

The hypothesis that DTP causes excess female mortality after BCG generated the deduction that the excess mortality in the HTMV trials could be due to a similar process of DTP vaccinations causing excess female mortality after HTMV. HTMV-vaccinated girls who received DTP/IPV after HTMV were found to have higher mortality than both HTMV-vaccinated boys who received DTP/IPV after HTMV and control girls who had received MV later in infancy. Since girls who received DTP/IPV after HTMV had higher mortality than control girls receiving MV at 9–10 months of age, the increased female–male MRR after HTMV cannot be ascribed to reduced mortality among boys who received DTP/IPV after HTMV. Hence, HTMV may have been withdrawn for the wrong reason. The increased mortality among girls after HTMV appears to have been caused by the administration of inactivated vaccines after HTMV and not by a direct effect of HTMV itself.

Observation V. DTP after measles vaccination associated with increased female mortality

Deduction and study selection

The Sudan trial[22] suggested that the number of missing DTP vaccinations prior to MV explained the subsequent excess mortality of girls in this trial. We therefore tested whether missing doses of DTP at enrolment was associated with increased female mortality in other trials of MV, which had documented DTP vaccination status at the time of enrolment, and where DTP and MV were not administered simultaneously. Apart from the Sudanese study,[22] information was available from five other MV trials: one from Congo[22] and four from Guinea-Bissau.[51] The four trials from Bissau covered all MV trials conducted in Bissau before we introduced the requirement that children had to have received DTP3 prior to enrolment.[3] Furthermore, we reviewed observational studies of DTP administered after MV (tables 1 and 2). Several studies have information on DTP and MV administered simultaneously,[22 31 53 57 66 77] but only three studies had information on DTP administered after MV.[31 57 74]

Observations

In the six randomised trials of early MV compared with children who had received DTP3 at enrolment, mortality was significantly increased among children who were missing DTP3 at enrolment (and presumably received additional doses of DTP after MV) with a MRR of 1.60 (95% CI 1.14 to 2.24) (figure 8). However, the negative effect was found only for girls. Compared with children who had received DTP3 prior to MV, girls likely to receive DTP after MV had a MRR of 2.36 (95% CI 1.43 to 3.89), whereas there was no difference for boys. The effect was significantly different for girls and boys (p=0.031, test of homogeneity) (supplementary table 6).

The observational studies also suggested that DTP after MV is associated with increased female mortality. In a large trial of VAS after 6 months age in Ghana,[74] the female–male MRR was 1.62 (95% CI 1.02 to 2.63) during the 2 years of the study among children who had received only one to two doses of DTP and were likely to receive DTP during follow-up. The excess female mortality in this study was also stronger among the children who received

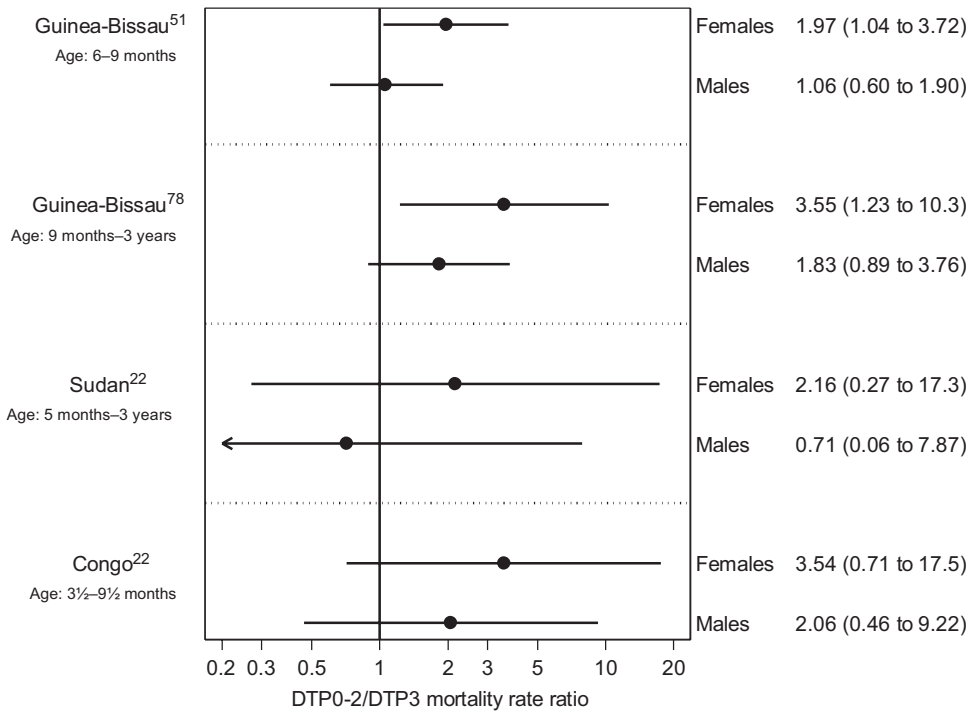

Figure 8 Mortality after enrolment in measles vaccination trials according to diphtheria–tetanus–pertussis (DTP) status at enrolment: missing DTP vaccinations versus fully DTP vaccinated (observation V) (see supplementary table 6 with two additional small studies which could not be presented due to undefined estimates).

VAS rather than placebo. At the paediatric ward in Bissau, children who had received DTP after MV rather than MV alone as the most recent vaccination had an increased in-hospital mortality and the trend was stronger for girls, though the effect was not statistically significant in this small study.[43] In a Gambian community study, vaccination cards were collected in connection with a verbal autopsy interview: measles-vaccinated girls who died were 3.34 (95% CI 1.15 to 9.74) fold more likely to have received DTP3 after MV than girls who did not die.[31]

Methodological considerations and implications

The six measles trials did not register all vaccinations during follow-up. However, most children did receive the missing doses of DTP: at least 85% received missing doses of DTP in Guinea-Bissau.[51] In general, children missing doses of DTP might have less compliant mothers or have less access to healthcare and therefore have higher mortality during follow-up. However, these children were enrolled in trials and had the same access to healthcare, and it seems unlikely that minor delays in the administration of DTP can explain the differences in mortality. Furthermore, the effect was found only for girls, making it unlikely that the overall effect is due to selection bias. The few other studies with information on mortality for children who had received DTP after MV also suggested a stronger negative effect for girls. Hence, the data suggest that administration of DTP after MV increases mortality and that this effect is stronger in girls than boys.

Observation VI. Mortality declines when DTP-vaccinated girls receive MV

Deduction and study selection

Without a control group, it is difficult to ascertain the overall effect of DTP on child mortality. However, the increased female–male mortality ratio after DTP and the reduced ratio after MV (figure 6) suggested that mortality would decline for girls once they receive MV. We therefore assessed the change in mortality levels for boys and girls in the transition from DTP to MV. There were three community studies among the studies in observation III (figure 6) with information on both DTP and MV mortality rates.[26 31 53]

Observations

In the transition from DTP to MV, mortality decreased for girls in all three studies (figure 9). This pattern is illustrated for two of the studies in figure 10. For boys, mortality increased in two studies and declined less markedly than for girls in one study. The effect of MV was statistically different for boys and girls in two of the three studies (supplementary table 7).

Methodological considerations and implications

In two studies, we used data for individuals with known vaccination status within the respective age groups. In the Gambia, it was known that the coverage was very high but individual vaccination data were not available and the comparison could therefore only be made for all children in the age groups. We analysed the community data sets used in figure 6, and girls experienced a pronounced decrease in overall mortality when MV replaced DTP. As discussed above, this change could not be ascribed to an age effect (figure 7).

Testing the hypothesis: the negative effect of DTP may be reduced with a subsequent vaccination with MV or BCG

The six observations in this paper support the hypothesis that DTP has negative NSE and sex-differential effect on child survival. It has been considered unethical to test DTP in a randomised study.[85] Instead we tested

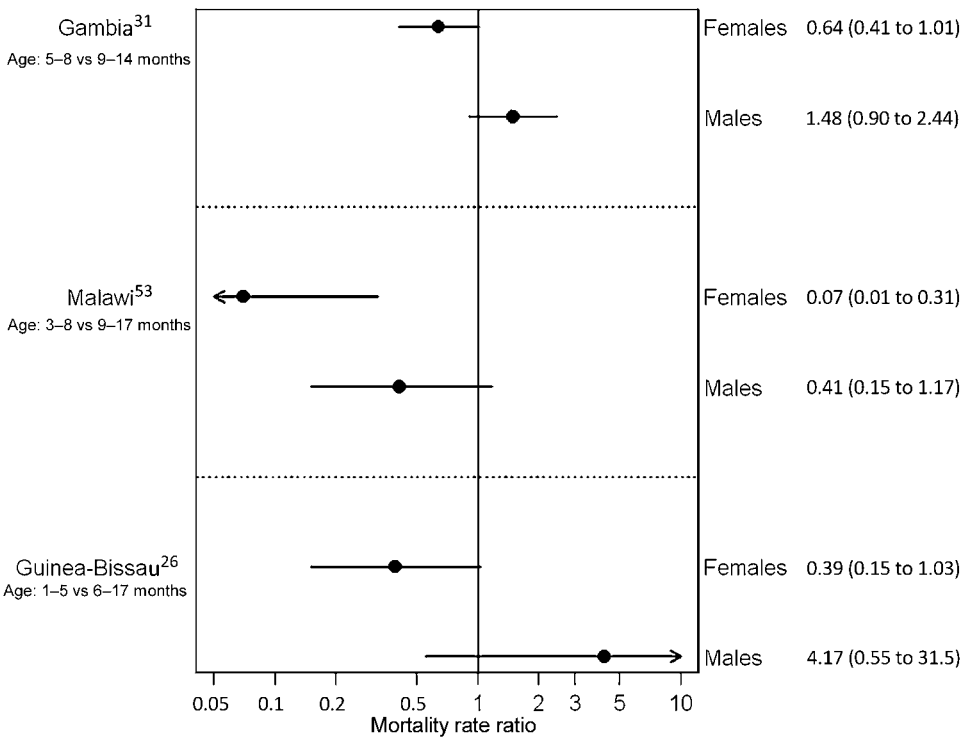

Figure 9 Mortality rate ratios for measles vaccine age group versus diphtheria–tetanus–pertussis age group (observation VI) (see supplementary table 7).

an implication of the hypothesis in two randomised trials.[3 69]

Deduction

If overall mortality is increased when DTP is the predominant vaccine (observations I, II and VI), it might reduce mortality if a live vaccine were given shortly after DTP to change the immunological profile and reduce exposure to DTP as most recent vaccine. As shown in figure 10, which presents data from The Gambia and Malawi, mortality is increased between 2 and 8 months of age (especially in girls), the age at which DTP is likely to be the most recent vaccine, and around 18 months of age, the age at which booster DTP vaccination has been administered in The Gambia. Hence, mortality might be reduced if MV were administered earlier than 9 months of age or a BCG revaccination was given shortly after the 18 months booster dose of DTP.

First test: early measles vaccination

We conducted a randomised trial of an additional dose of MV at 4.5 months of age to test whether early MV is associated with reduced mortality for girls.[3] To prevent the problem with increased mortality among girls getting DTP after MV,[51] only children who had already received DTP3 were enrolled in the trial.[3 68] Compared with the control children who had DTP3 as the most recent vaccination, MV tended to have a beneficial effect for girls, the MRR between 4.5 and 9 months of age being 0.46 (95% CI 0.19 to 1.11) for girls and 0.94 (95% CI 0.44 to 2.01) for boys.[3] Many of the children in this trial had received VAS at birth, and VAS had a negative effect

Figure 10 Age- and sex-specific mortality curves from Gambia and Malawi.[31 53]

on child survival. VAS at birth is not WHO policy, and among children who had not received VAS at birth, early MV compared with DTP3 as the most recent vaccination was associated with a threefold reduction in mortality between 4.5 and 9 months of age (MRR =0.33 (95% CI 0.13 to 0.86)), the estimate being 0.30 (95% CI 0.07 to 1.31) for girls and 0.36 (95% CI 0.10 to 1.26) for boys.

Second test: booster BCG vaccination

Since booster DTP and OPV are usually administered at 18 months of age in Guinea-Bissau, we enrolled children at 19 months of age in a trial of BCG revaccination.[69] Among children who had received booster DTP before enrolment, BCG revaccination was associated with a threefold reduction in mortality, the MRR being 0.36 (95% CI 0.13 to 0.99)[69] with a similar effect in girls and boys. Children who had not received booster DTP before enrolment were encouraged by health personal to come back and receive missing DTP vaccinations. In this group, BCG revaccination was associated with increased mortality, the MRR being 1.78 (95% CI 1.04 to 3.04). In general, the effect of BCG revaccination may have been better for girls since girls had a significantly lower risk of hospitalisation than boys after BCG revaccination (female–male hospitalisation ratio 0.65 (95% CI 0.46 to 0.93)), the annual incidence rates being 3.1% for girls and 4.8% for boys. Among controls, the female–male hospitalisation ratio was 1.00 (95% CI 0.70 to 1.43), the incidence rates being 3.8% for both girls and boys.[69]

Methodological considerations and implications

Since live vaccines usually have a good effect for girls, the proposed changes in vaccination schedule represented attempts to reduce the negative effects by administering live vaccines after inactivated DTP. Both randomised studies showed an effect consistent with our hypothesis; bias is unlikely to have had any effect. Both studies also showed that there may be unimagined interactions with other immune interventions; in the MV trial, neonatal VAS removed the beneficial effect of early MV, and in the BCG revaccination trial, getting DTP after BCG revaccination doubled mortality. It is not possible to determine whether the beneficial effect of early MV or BCG revaccination is due to a reduction in the negative effect of DTP or to beneficial NSE of these vaccines. However, both studies suggest that it is beneficial to reduce exposure to DTP as most recent vaccination by using a live vaccine.

DISCUSSION

Main findings

Our observations are not compatible with DTP merely preventing the targeted diseases. Observations I, II and IV–VI indicate that mortality is increased after DTP vaccination compared with children who have BCG or MV as their most recent vaccination. Observations III–VI suggest that DTP has sex-differential effects on mortality, the negative effect of DTP being worse for girls. Hence, most excess mortality associated with DTP is due to increased female mortality. As summarised in table 3, most studies reflect what happens when DTP is administered after BCG or MV. In both situations, DTP is associated with an increase in overall mortality, the effect being particularly negative for girls. One study[42] suggests that the effect of DTP is also negative when it is given to totally unvaccinated children (table 3).

To test the implications of these observations, we conducted two trials to reduce the time of exposure to DTP as the most recent vaccination by administering MV or BCG shortly after the last DTP vaccination. The effect was beneficial in both trials. These results are in glaring contradiction to the increasing number of randomised trials showing that BCG and MV have beneficial NSE in high-mortality countries.[3 65 69 79 81] The randomised trials confirm previous observational studies of the beneficial effects of BCG and MV.

Strengths and weaknesses

In spite of the limitation of observational studies and the variability in how the different data sets were collected, the studies produced consistent results. Most observations were repeated in several studies and generated new deductions, which were tested in all the available data sets. Furthermore, several randomised trials or re-interpretations of randomised trials now support the hypothesis that DTP has negative effects, particularly for girls.[5 51 52 69 72–74]

Table 3 Evidence for the effect of DTP in relation to the sequence of vaccinations

Sequence of vaccines: most recent vaccination before DTP	Effect on overall child mortality	Effect on female mortality	Effect on male mortality
Unvaccinated	Result: increased mortality Evidence: ref.[42]	Result: no significant negative effect Evidence: ref.[42]	Result: no significant negative effect Evidence: ref.[42]
BCG	Result: increased mortality Evidence: observations I, II	Result: increased mortality Evidence: observations I–III	Result: no significant negative effect Evidence: ref.[43]
Measles vaccine	Result: increased mortality Evidence: observations IV, V	Result: increased mortality Evidence: observations IV, V	Result: no effect Evidence: observation V

DTP, diphtheria–tetanus–pertussis.

The inherent tendency is to dismiss observations conflicting with the current disease-specific paradigm as probably due to uncontrolled confounding.[90 93] However, confounding is unlikely to explain the twofold increase in mortality in the only community study of the introduction of DTP,[42] the threefold decrease in hospital case death when DTP was missing,[43] the fourfold increase in mortality when DTP was given after BCG in a trial of early BCG vaccination to LBW children,[64] the twofold increase in mortality when DTP was administered after HTMV[5] or standard MV (supplementary table 6) and the twofold increase in mortality when vitamin A was given to girls likely to receive DTP during follow-up.[74]

Since our group produced most of these studies, it could be said that these observations are due to publication bias because studies were published only if they showed a negative effect of DTP.[93] Although this is not the case, it is difficult to provide evidence other than the fact that all data sets reanalysed in collaboration with other African research groups documented similar patterns.[5 22 31 53 74]

Analytical designs and conflicting interpretations

In the first design, we compared DTP-vaccinated versus DTP-unvaccinated children in a survival model comparing sequential vaccinations at the same age. Given the positive selection bias associated with DTP or, alternatively, the frailty bias associated with not getting DTP, the negative effect of DTP in all studies using a landmark approach or case–control design is noteworthy.[61] Both the contrasting estimate for the different vaccines and the natural experiment studies support the hypothesis that DTP increases mortality. In contrast to observations I and II, some studies using a sequential vaccination comparison have produced beneficial estimates for DTP.[44–49 58] However, several of these studies had survival bias due to retrospective updating of vaccination status (table 2,[60 61]). We obtained similar (falsely) positive estimates for DTP when we reanalysed our own data with survival bias.[62] Furthermore, these studies mostly administered BCG and DTP simultaneously, whereas we administered DTP after BCG as currently recommended by WHO. We have shown in a randomised trial of BCG at birth to LBW children that the negative effect of DTP was much stronger among the children who received BCG at birth and DTP 6–7 weeks later than among children who received BCG and DTP more or less at the same time.[64] Finally, social selection and frailty bias were important in the studies that produced beneficial estimates for DTP.[46 59]

In the second design, we compared the mortality rates of girls and boys, and all studies suggested that mortality is relatively increased for girls when DTP is the most recent vaccination, whereas the opposite is true after BCG and MV vaccinations. These studies are methodologically less complicated as long as we can be sure that the children are unlikely to receive other vaccinations during follow-up. There is no clear indication of a gender-specific selection bias which could explain this pattern, and the fact that the female–male MRR is increased after DTP but reduced after MV and BCG excludes the possibility that these patterns are mainly due to selection bias. It has been suggested that the higher female–male MRR after DTP could be due to reduced male mortality after DTP.[94] However, several of our observations demonstrate that this is not the explanation. First, DTP administered after BCG is associated with increased mortality in both boys and girls (observations I and II). Second, mortality was increased twofold when girls received DTP/IPV after HTMV (observation IV) or after standard MV, while this was not the case for boys (observation V). Third, female mortality is reduced once DTP-vaccinated girls get MV (observation VI).

Interpretation

Our six observations on DTP are consistent with the hypothesis that DTP without simultaneous administration of BCG or MV has a negative effect for girls compared with both DTP-unvaccinated girls and DTP-vaccinated boys.[80] Furthermore, we deduced the hypothesis that DTP after MV might have been the explanation of the increase in female mortality in the HTMV trials, and the results of all the available studies are consistent with this hypothesis (observation IV). We also deduced that if an inactivated vaccine like DTP were associated with higher female–male MRR, similar effects might occur with other inactivated vaccines. Both hepatitis B vaccine and IPV were associated with increased female mortality.[37 51] Though it has not been possible to conduct a randomised trial of delaying the primary series of DTP vaccinations, we did randomise children at 4.5 months of age to early MV in an attempt to reverse the adverse effect of DTP and we achieved a threefold reduction in mortality.[3] Likewise, BCG revaccination after DTP booster vaccination reduced mortality threefold.[69] It is unlikely that DTP has no negative sex-differential effects when such consistent deductions can be generated.

The majority of studies have been conducted in communities with herd immunity sufficient to eliminate deaths from whooping cough, and our studies do not reflect what would happen in a situation without general DTP vaccinations. There are very few community studies that have assessed the impact of pertussis on child mortality in high-mortality countries in the pre-vaccination era. The best community study was conducted in the 1970s in Kenya in the initiation phase of the vaccination programme; the case death was assessed to be 1.3% for all ages and pertussis was 6% of the infant deaths.[95] Given the major increases in mortality in observational studies and the reduction in mortality in the randomised studies, it seems likely that DTP would also be associated with increased mortality for girls in situations without herd immunity.

Possible biological mechanisms of NSE

Our work is based on the hypothesis that a vaccine may affect the immune system in a sex-differential manner

during the period when it is the most recent vaccination.[80] Studies of specific infections suggest that vaccines may enhance or reduce susceptibility to these unrelated infections in a sex-differential manner.[38 55 56 75] Presumably, the NSE could also be due to changes in the control of infections, with vaccines enhancing or reducing the severity of subsequent infections. This possibility has yet to be examined in epidemiological studies.

Immunological studies have shown that vaccines may influence the response to unrelated antigens. In animal studies, inactivated vaccines like DTP are associated with a Th2 profile, whereas live vaccines of the same antigens produce a Th1 profile, and this difference is important for the response to a subsequent challenge.[96–103] This difference may be more important for girls.[102] In animal studies, some infections prime the immune response to other pathogens, resulting in so-called heterologous immunity, which may be either protective or detrimental.[4 104] Some human infections may have a similar importance; for example, we have found in several studies that measles infection may reduce the mortality from other causes after the acute phase of measles infection.[19 105 106] Furthermore, the very rapid reduction in mortality in randomised trials of BCG vaccination at birth suggests that BCG is training the innate rather than the adaptive immune system in a beneficial manner.[79 107] However, it may still be difficult to believe that a single vaccination like BCG, DTP or MV—in an environment with numerous concurrent and competing infections—could have such profound sex-differential NSE on overall mortality. However, the vaccines with strong NSE are all given by parenteral injection, which bypasses the natural immune barriers, and this could have an important influence on their effects on the immune system.

Other factors affecting the immune system may influence the NSE of DTP, for example, season, other vaccines and micronutrient interventions.[70–74 76] We have already alluded to the fact that combinations of DTP and BCG, or DTP and MV, have distinct effects, which cannot be deduced from the patterns described in the present hypothesis.[5]

Conclusions and implications

There are no definite criteria for imputing causality in observational studies, though the Hill criteria[108] are often invoked. The present data on DTP comply with the criteria of strength of association, dose–response relationship,[27 42] temporal sequence, consistency between different studies and specificity of the association. Most people would also request biological plausibility. However, as Hill noted, only biological processes that are already known are plausible.[108] When exploring a phenomenon with unknown biological mechanisms, the main criterion for scientific fruitfulness is consistency of observations. We developed six linked hypotheses, which suggest that DTP is consistently associated with higher female mortality. Furthermore, we conducted two randomised trials to reduce exposure to DTP, and both trials suggested that this was beneficial. If randomised trials are deemed unethical, the present study represents the best possible evidence for the hypothesis that one of the most widely used routine vaccines has negative sex-differential effects. The finding that DTP has adverse effects has several implications.

International organisations and donors have chosen to use DTP3 coverage as the main indication of the effectiveness of national vaccination programmes.[109 110] This leads to DTP vaccination campaigns and to vaccinations being administered out of sequence.[66] Both DTP vaccinations before 9 months of age and DTP administered after MV suggest that DTP is associated with many unnecessary deaths. Given the potential for negative effects from DTP, MV coverage should be used to monitor the effectiveness of national vaccination programmes rather than DTP coverage.

Randomised trials are warranted to assess the 'true' impact of DTP on child survival.[82] First, several observational studies have found that early DTP vaccination is particularly harmful.[55 64] and it would therefore be desirable to examine whether delaying DTP vaccinations reduces this negative effect (WGNSEV, trial 6). However, this design is likely to be particularly controversial,[85] and it may therefore be more strategic to further test whether an early two-dose MV schedule at 4 and 9 months can improve child survival (WGNSEV, trial 2).[3] Second, administering DTP with or after MV may be harmful,[22 44 53 57–59 66 77] and trials are therefore needed to assess whether the vaccination programme should forbid such out-of-sequence vaccinations (WGNSEV, trial 4). Third, SAGE (WHO Strategic Advisory Group of Experts on immunisation) has recently recommended a booster dose of pertussis vaccine given in the second year of life.[111] This recommendation could increase mortality (see figure 10). As suggested by the WGNSEV, the effect of a booster dose of pertussis should be tested in a randomised controlled trial (RCT) measuring the impact on overall survival by providing a live vaccine shortly after booster DTP (trial 3).[82] Alternatively, before being introduced, a booster dose of DTP should be tested in a randomised trial.

We should also consider whether immunity to DTP could be obtained with a different vaccine having no negative effects in a situation with herd immunity.[112] In the current vaccination schedule (see figure 1), DTP is the most recent vaccination for 49.5 months for the first 5 years of life if booster DTP is given and for 7.5 months if no booster is given. This could be reduced to 3–4 months if an additional dose of MV were given at 4 months of age and a live vaccine were given 1 month after a DTP booster. This could be further reduced if the initial series of three doses of DTP were reduced to two doses in countries with a booster dose of DTP.

The WGNSEV recommended six trials to assess the beneficial effects of BCG and MV and the potential negative effects of DTP. The three priority trials (trials

1–3) have been completed and have clearly shown that NSE are important. A small trial similar to trial 4 has also found negative effects of DTP.[77] The two remaining trials (trials 5–6) are less relevant or feasible. Trial 5 would test DTP without aluminium, but this could not be done without major funding to get a new vaccine licensed. Trial 6 tests a delayed DTP schedule, which is controversial and has become less relevant due to the beneficial effect of early MV.[3] Hence, to advance the understanding of the NSE of vaccines, the most important steps may be that other groups repeat the three priority trials.[82] Many new vaccines are being tested and introduced at the moment. It would seem important that potential NSE and sex-differential effect of the new vaccines be tested through carefully controlled before–after studies, phased introduction studies or randomised trials.

The global health community has conducted no trial to document that BCG, DTP, OPV and MV have the intended effects on child survival and only those effects. Randomised controlled trials have now shown major beneficial NSE of BCG and MV[3 65 69 79] and negative effects of HTMV.[5] The common assumption that vaccines have mainly disease-specific preventive effects is no longer tenable. The data presented here suggest that DTP has a negative effect for girls. Experts have recognised that WHO-sponsored studies reporting a beneficial effect of DTP had methodological problems,[113] and Global Advisory Committee on Vaccine Safety has declared that it will keep a watch on the evidence of NSE of vaccines, including a potential deleterious effect of DTP vaccination.[114] Despite this, policy has not changed and studies have not been organised to resolve the issues. In a culture that prides itself on being evidence based, such inconsistencies between policy and evidence should not be acceptable. We can only hope that the global health community will seek to establish the much-needed evidence for the current vaccination policies to ensure that immunisation confers maximum benefit on children in high-mortality countries.

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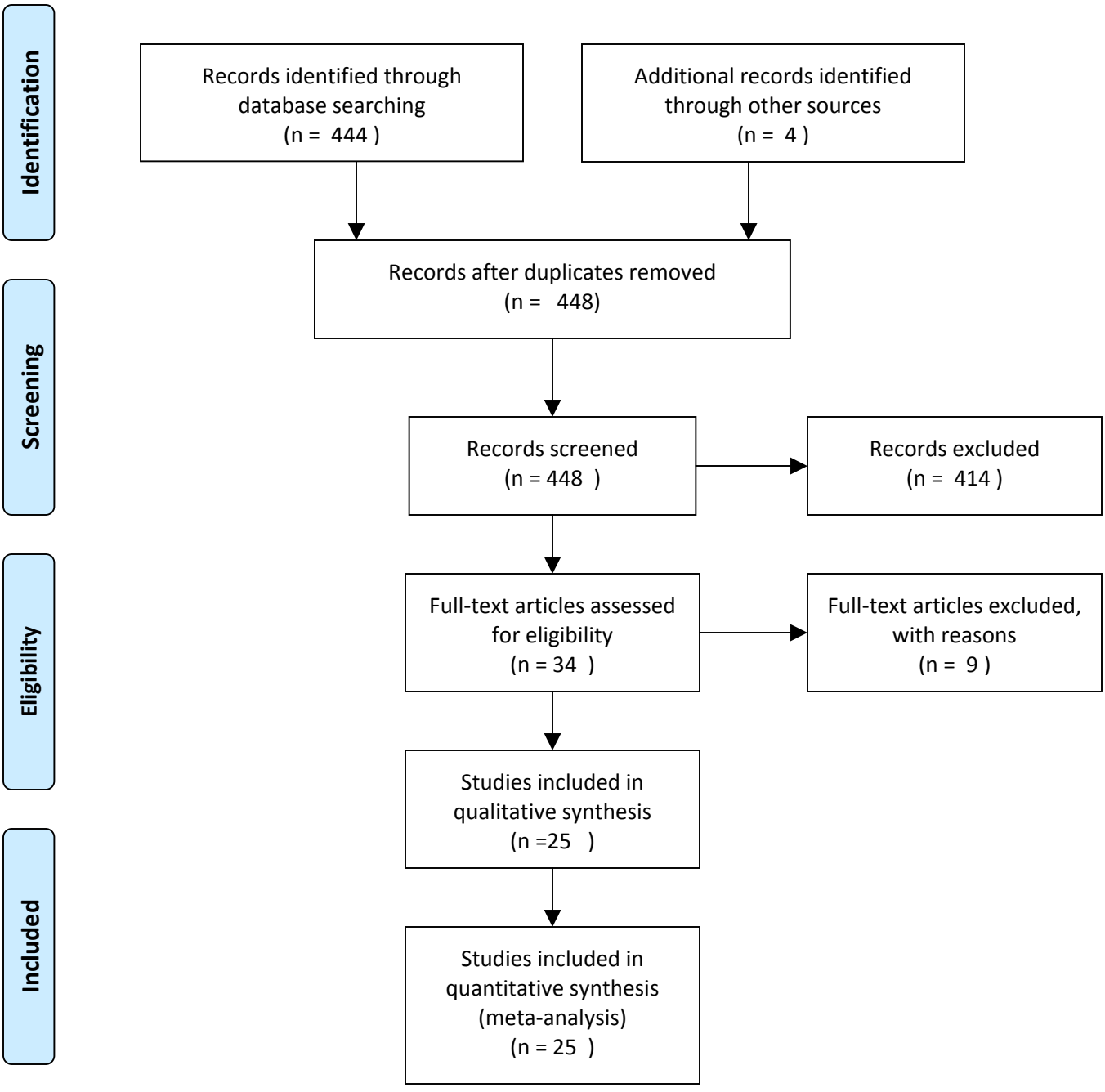
PRISMA 2009 Flow Diagram DTP review



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Supplementary table 1. Female-male mortality ratios in community studies from the pre-vaccination era in West Africa (Observation III)

Study (ref)	Period	Age group	Mortality rate (deaths/person-years)		Female-male mortality ratio
			Girls	Boys	
Age of BCG and DTP					
Bissau, Urban (28)	1978-81	1-8 mo	21.0%(30/142.6)	18.3%(29/158.5)	1.15 (0.7-1.9)
Bissau, Rural (28)	1979-83	1-8 mo	7.7%(32/416.0)	9.8%(42/427.1)	0.78 (0.5-1.2)
Senegal, Bandafassi (13)	1981-86	1-8 mo	11.2%(65/581.8)	10.6(61/574.8)	1.05 (0.76-1.46)
Senegal, Niakhar (88)	1963-67	0-11 mo	193/1000	206/1000	0.94
Senegal, P-Koto (89)	1963-67	0-11 mo	112/1000	128/1000	0.88
Age of measles vaccination					
Bissau, Urban (28)	1978-81	9-17 mo	11.5%(21/182.6)	14.0%(27/193.4)	0.82 (0.5-1.5)
Bissau, Rural (28)	1979-83	9-17 mo	12.2%(49/400.2)	12.0%(46/383.3)	1.02 (0.7-1.5)
Senegal, Bandafassi (13)	1981-86	9-59 mo	7.6%(171/2250.9)	7.3%(174/2387.1)	1.04 (0.85-1.28)
Senegal, Niakhar (88)	1963-67	12-23 mo	198/1000	210/1000	0.94
Senegal, Niakhar (17)	1985-86	9-23 mo	7.3%(62/847.5)	7.2%(62/866.3)	1.02 (0.72-1.45)
Senegal, P-Koto (89)	1963-67	12-23 mo	140/1000	112/1000	1.25

Supplementary table 2: Mortality ratios for different routine vaccinations within the same study (Observation I)

Study (ref)	Length of follow-up in study	Mortality rates per 1000 person-years or case fatalities (%) and mortality ratios (MR) for different vaccine groups			Significance testing, p-value	
		BCG	DTP	Measles (MV)	BCG versus DTP	DTP versus MV
Guinea-Bissau: (1)	Mortality during 6 months	BCG 3.8% (125/3301) NoBCG 4.9% (97/1973) MR 0.55 (0.36-0.85)	DTP 4.8% (62/1295) NoDTP 4.0% (72/1822) MR 1.84 (1.10-3.10)	MV 1.8% (19/1036) NoMV 4.0% (94/2378) MR 0.48 (0.27-0.87)	0.005	0.001
Benin (18)	Case control study 3 years of age	MR 0.68 (0.38-1.23)	MR 2.20 (0.93-5.22)	MR 0.55 (0.28-1.07)	0.027	0.013
Guinea-Bissau: DTP-introduction (42)	6 months	BCG 62/10/161.9) [1] NoBCG 95 (80/846) [1] MR 0.63 (0.30-1.33)	DTP 113 (47/415.7) No DTP 51 (20/388.6) MR 1.92 (1.04-3.52)	**	0.038	NA
Guinea-Bissau: Hospital case fatality (27)	End of hospitalisation	*	DTP 16.1% (28/174) noDTP 9.1% (1/11) MR 2.32 (0.53-40.0)	MV 7.0%(18/259) NoMV 13.7% (19/139) 0.51 (0.27-0.98)		0.188
Guinea-Bissau (26)	Case fatality during 3 months of war	BCG 2.4% (9/375) [1] NoBCG 9.4% (3/32) [1] MR 0.11 (0.02-0.69) [1]	DTP 4.2% (19/455) NoDTP 2.6% (2/78) MR 1.58 (0.36-7.02)	MV 2.6% (25/958) NoMV 5.5% (12/220) MR 0.44 (0.20-1.00)	0.024	0.138
Malawi (53) @	1 week to 8 mo for BCG and DTP 9-17 mo for MV	BCG 157 (17/108.0) NoBCG 389 (13/33.4) MR 0.45 (0.16-1.23)	DTP1 188 (20/106.6) NoDTP1 157 (17/108.0) MR 3.19 (0.80-12.8)	MV 35 (11/314.0) NoMV 69 (12/174..2) MR 0.42 (0.16-1.14)	0.021	0.019
Guinea-Bissau: LBW cohort (64,65)#	Neonatal for BCG 2-6 mo for DTP	BCG 358 (27/75.4) NoBCG 646 (48/74.3) MR 0.55 (0.35-0.89)	DTP 100 (20/200.7) NoDTP 49 (5/102.3) MR 4.33 (1.58-11.9)#	**	<0.001	

Notes: The control group for BCG would usually be unvaccinated children except in the randomised trial of BCG to LBW children (64) where the controls had received OPV according to current recommendations; the control group for DTP would be BCG-vaccinated and a few unvaccinated children except in the study of introduction of DTP (42) in which virtually none of the children had received BCG; the control group for MV would mainly be DTP vaccinated children. The first study from Bissau (1) and from Benin (18) estimated effects in a model with all vaccinations, whereas the remaining studies have estimated the effect of the "most recent vaccination". NA = not applicable

1. Data not reported in the published paper

* Unvaccinated children did not have a vaccination card and therefore the status as BCG-unvaccinated could not be documented by a vaccination card as for the other vaccines;

**The studies were limited to children under 6 or 9 months of age who had not received measles vaccine.

@ The rates are for all children but the MRs are only for children present in the community and for whom the information on vaccinations would be most accurate.

Estimate for BCG is from a randomised comparison of BCG at birth versus BCG later as currently recommended for LBW children (65). The estimate for DTP is adjusted for mid-upper-arm-circumference; the estimate is only from the BCG at birth arm of the trial since most children in the control arm of the study received DTP before or simultaneously with BCG (64)

Supplementary table 3. Female-male (F/M) mortality rates and mortality ratios for DTP-vaccinated children (Observation III)

Study (ref)	Vaccine	Mortality rate per 1000 person-years (deaths/person-years) or case fatality % (deaths/cases)		F/M for DTP	F/M Unvaccinated
DTP as most recent vaccine		Female	Male		
Guinea-Bissau: Female-male twin pairs (28)	DTP1-3	22 died first	3 died first	7.33 (2.20-38.3)	NA
Guinea-Bissau: DTP-introduction (42)[1]	DTP1-3	126 (27/215.0) [1]	100 (20/200.4) [1]	1.29 (0.72-2.31)	0.86 (0.35-2.08)
Gambia (31)	DTP1-3	67 (32/475.0)	40 (20/498.3)	1.68 (0.96-2.93)	NA
Guinea-Bissau: Hospital case fatality (27)	DTP1-3	17.8% (35/197)	10.9% (29/264)	1.63 (1.03-2.59)	No vaccination card*
Guinea-Bissau: Case fatality during 3 months of war (26)	DTP1-3	6.1% (14/228)	2.2% (5/227)	3.08 (1.11-8.56)	0.92 (0.06-15.1)
Malawi (53)	DTP1	218 (11/50.4)	176 (9/51.1)	All 1.24 (0.51-2.99) Present: 1.36 (0.52-3.58) ¤	0.55 (0.16-1.87)
Bangladesh (54)	DTP1-3 @	22.5% (16/71)	7.8% (10/129)	2.91 (1.39-6.06)	NA
Guinea-Bissau: Vitamin A at birth trial (52)$	DTP1-3 &	41 (36/872)	34 (31/901)	1.20 (0.74-1.94)	NA (all children received BCG at enrolment)
Guinea-Bissau: LBW cohort (64,65)#	DTP1-2	115 (13/113.3)	80 (7/87.4)	1.44 (0.57-3.61)	NA

Note: Include only studies of DTP provided before measles vaccination. Most DTP-vaccinated had received BCG prior to DTP except in the study of introduction of DTP (42) in which virtually none of the children had received BCG. NA = not applicable

1. Data not reported in the published paper

* Children who brought no vaccination card to the hospital could be unvaccinated but could also have forgotten or lost the card.

¤ The rates are for all children but the MRs are only for children present in the community and for whom the information on vaccinations would be most accurate.

@ Mortality between DTP1 and 6 months after DTP3. The F/M mortality ratio was 4.17 (1.39-13) for children receiving DTP1-3 with Vitamin A and 2.08 (0.76-5.73) for children receiving DTP1-3 with placebo.

& The F/M mortality ratio was 1.78 (0.92-3.42) after DTP for children who had received VAS with BCG at birth and 0.73 (0.35-1.52) after DTP for children who had received placebo together with BCG at birth.

Estimate for BCG is from a randomised comparison of BCG at birth versus BCG later as currently recommended for LBW children (65). The estimate for DTP is adjusted for mid-upper-arm-circumference; the estimate is only from the BCG at birth arm of the trial since most children in the control arm of the study received DTP before or simultaneously with BCG (64)

$ The study (52) is partially overlapping with the trial of early measles vaccination (3) and the result from this trial has therefore not been presented separately (MRR=1.30 (0.74-2.30))

Supplementary table 4. Female-male (F/M) mortality ratios (MR) for DTP, BCG and MV vaccinated children (Observation III)

Study (ref)	Female vs male mortality rates or case fatalities after DTP	F/M MR for DTP	Female vs male mortality rates or case fatalities after BCG	F/M MR for BCG	DTP versus BCG	Female vs male mortality rates or case fatalities after MV	F/M MR for measles vaccine	DTP versus MV
Guinea-Bissau: Female-male twin pairs (28)	22 F vs 3 M died first	7.33 (2.20-38.3)	3 F vs 12 M died first	0.25 (0.05-0.93)	P<0.001	2 F vs 5 M died first	0.40 (0.04-2.44)	P=0.001
Gambia (31)	F: 67 (32/475.0) M: 40 (20/498.3)	1.68 (0.96-2.93)	NA	NA	NA	F: 38 (26/689.2) M: 55 (40/722.3)	0.70 (0.43-1.15)	P=0.02
Guinea-Bissau: Hospital case fatality (27)	F: 17.8%(35/197) M: 10.9%(29/264)	1.63 (1.03-2.59)	F: 12.1% (4/33) M: 22.5% (11/49)	0.54 (0.16-1.43)	P=0.07	F: 6.2% (14/225) M: 12.7% (29/249)	0.54 (0.28-0.97)	P=0.003
Guinea-Bissau: Case fatality during 3 months of war (26)	F: 6.1% (14/228) M: 2.2% (5/227)	3.08 (1.11-8.56)	F: 1.9% (1/35) M: 2.3% (1.43)	0.92 (0.06-15.1)	p=0.44	F: 2.1% (10/472) M:3.1% (15/486)	0.63 (0.28-1.40)	P=0.01
Malawi (53) ¤	F: 218 (11/50.4) M: 176 (9/51.1)	All 1.24(0.51-2.99) Present: 1.36 (0.52-3.58)¤	F: 134 (7/52.2) M: 180 (10/55.7)	All 0.80 (0.30-2.10) Present: 0.58 (0.11-3.19)¤	P=0.40	F: 13 (2/151.1) M: 47 (7/149.8)	All 0.28 (0.06-1.37) Present: 0 (0-0.67)¤	P=0.002
Guinea-Bissau: Vitamin A at birth trial (52)&	F: 41 (36/872) M: 34 (31/901)	1.20 (0.74-1.94)	F: 94 (18/192) M: 140 (28/200)	0.66 (0.37-1.20)	p=0.12	NA	NA	NA
Guinea-Bissau: LBW cohort (64,65)#	F: 115 (13/113.3) M: 80 (7/87.4)	1.44 (0.57-3.61)	F: 1.7% (11/667) M: 3.3% (16/493)	0.51 (0.24-1.08)	P=0.087	NA	NA	NA

Notes: Most DTP-vaccinated had received BCG prior to DTP except in the study of introduction of DTP (42) in which virtually none of the children had received BCG. In the original study (1) suggesting a negative impact of DTP we did not report sex-specific estimates; we subsequently reported that the effect of BCG was 0.45 (0.23-0.86) and 0.63 (0.38-1.04) whereas the effect of DTP compared with no DTP was 2.31 (1.16-4.59) for girls and 1.45 (0.81-2.59) for boys. The divergent estimates were significant for girls (p=0.009) but not for boys suggesting a relative worse effect of DTP for girls than for boys (1).

NA = not applicable/not available;

¤ The rates are for all children but the MRs are only for children present in the community and for whom the information on vaccinations would be most accurate.

& Among children who had received VAS with BCG at birth, the F/M mortality ratios were 0.64 (0.26-1.54) after BCG and 1.78 (0.92-3.42) after DTP; for children who had received placebo together with BCG at birth the F/M mortality ratios were 0.69 (0.31-1.54) after BCG and 0.73 (0.35-1.52) after DTP

Estimate for BCG is the case fatality for neonatal mortality among LBW children receiving BCG in randomised trial comparing BCG at birth versus BCG later as currently recommended for LBW children (65). The estimate for DTP is adjusted for mid-upper-arm-circumference; the estimate is only from the BCG at birth arm of the trial since most children in the control arm of the study received DTP before or simultaneously with BCG (64)

Supplementary table 5. Mortality or morbidity patterns among DTP-vaccinated and measles vaccinated children of similar age (Observation III)

Study outcome and age group compared (ref)		Female and male mortality/incidence rates or case fatalities and female/male mortality ratios (F/M MR/IR)		Ratio for MV/DTP for girls
		DTP most recent vaccine	Measles vaccine (MV) most recent vaccine	
Deaths in female-male twin pairs (28)	9 to 17 months	7 F vs 0 M died first	1 F vs 5 M died first	P=0.009, test of homogeneity
Hospital case fatality (27)	6 to 8 months	F: 14.7% (16/109) M: 10.1% (15/149) F/M MR 1.46(0.75-2.82)	F: 3.7% (1/27) M: 15.4% (2/13) F/M MR 0.24 (0.02-2.42)	0.25 (0.03-1.82)
Guinea-Bissau: Case fatality during 3 months of war (26)	6 to 20 months	F: 7.1% (7/99) M: 4.4% (4/92) F/M MR 1.81(0.53-6.17)	F: 2.1% (10/472) M: 3.1% (15/486) F/M MR 0.63 (0.28-1.40)	0.30 (0.12-0.77)
Early MV trial (3)	4.5 to 9 months	F: 35 (27/765.5) M: 27 (21/775.2) F/M MR 1.30 (0.74-2.30)	F: 16 (6/376.0) M: 26 (10/390.0) F/M MR 0.62 (0.23-1.71)	0.46 (0.19-1.11) No neonatal VA: 0.30 (0.07-1.31)
Incidence of cryptosporidium (55)	6 to 11 months	F: 1391 (9/6.5) M: 417 (3/7.2) F/M IR 4.14 (1.09-15.6)	F: 292 (3/10.3) M: 334 (4/12.0) F/M IR 0.94 (0.21-4.25)	0.20 (0.05-0.89)

Note: The age groups are the ones used in the original presentation of the data

Supplementary table 6. Mortality after enrolment in measles vaccination trials according to DTP status at enrolment (Observation V)

Study (ref) Observation interval		Girls			Boys			All
		Missing doses of DTP (DTP0-2)	Fully vaccinated for DTP (DTP3)	MRR (DTP0-2/DTP3)	Missing doses of DTP (DTP0-2)	Fully vaccinated for DTP (DTP3)	MRR (DTP0-2/DTP3)	MRR (DTP0-2/ DTP3)
Guinea-Bissau-A (51)	4-9 mo	6.0% (3/50.2)	0% (0/0.5)	ND	8.7% (6/68.9)	0% (0/1.3)	ND	ND
Guinea-Bissau-B (51)	4-9 mo	17.8% (10/56.2)	0% (0/0.9)	ND	7.4% (4/53.7)	0% (0/0.6)	ND	ND
Guinea-Bissau-C (51)	6-9 mo	7.5%(29/387.8)	3.8%(14/368.3)	1.97 (1.04-3.72)	6.4%(25/390.3)	6.0%(21/348.6)	1.06 (0.60-1.90)	1.42 (0.93-2.17)
Guinea-Bissau-D (78)	9-36 mo	6.1% (8/131,1)	1.7% (6/352.9)	3.55 (1,23-10.3)	3.2% (5/156.3)	3.3% (11/333.3)	0.97 (0.34-2.80)	1.83 (0.89-3.76)
Sudan (22)	5-36 mo	6.0% (8/133.1)	2.8%(1/35.9)	2.16 (0.27-17.3)	1.4%(2/145.4)	1.9%(1/51.9)	0.71 (0.06-7.87)	1.58 (0.35-7.19)
Kinshasa, Congo (22)	3½-9½ mo	10.0% (3/30.0)	2.8%(3/106.2)	3.54 (0.71-17.5)	10.6%(3/28.4)	5.1%(6/116.7)	2.05 (0.51-8.21)	2.54 (0.91-7.15)
Total				2.36 (1.43-3.89)#			1.11 (0.69-1.77)#	1.60 (1.14-2.24)

Notes: Trials in Guinea-Bissau: A= Medium EZ-trial (51); B=Medium and high-titre EZ-trial (51); C=2-dose MV trial (51); D=trial of MV with vitamin A (78); ND=Not defined. #The test of homogeneity for the estimates for boys and girls was p=0.031.

Supplementary table 7. Mortality rates (deaths/person-years) in different age groups dominated by specific vaccines (Observation VI)

Study (ref)	All children or vaccine groups	Age groups compared	DTP age group	MV age group	MRR (MV/DTP)
Girls					
Gambia (31)	All children¤	5-8 mo vs 9-17 mo	6.7% (32/475.4)	4.3%(45/1040.0)	0.64 (0.41-1.01)*
Malawi (53)	DTP1 vs MV&	3-8 mo vs 9-17 mo	21.1% (9/43)	1.4% (2/143)	0.07 (0.01-0.31)
Guinea-Bissau: War (26)	DTP vs. MV&	1-5 mo vs. 6-17 mo	5.4% (7/129)@	2.1% (10/472)@	0.39 (0.15-1.03)*
Boys					
Gambia (31)	All children¤	5-8 mo vs. 9-17 mo	4.0% (20/498.3)	5.9% (65/1094.9)	1.48 (0.90-2.44)*
Malawi (53)	DTP1 vs MV&	3-8 mo vs. 9-17 mo	14.6% (6/41)	6.1% (9/148)	0.41 (0.15-1.17)
Guinea-Bissau: War (26)	DTP vs. MV&	1-5 mo vs. 6-17 mo	0.7% (1/135)@	3.1% (15/486)@	4.17 (0.55-31.5)*

Note: ¤ Mortality rate for all children in the indicated age group; & Mortality rate for children known to have received the vaccine which predominates in the relevant age group; @ Fatality ratios during the war; *A comparison of the change for boys and girls, respectively, is significant ($p<0.05$) in a homogeneity test.

Appendix: ***Analysis of incomplete vaccination data***

In the analysis of vaccination data, the completeness of the data and the mode of analysis as well as the study designs and associated biases have been critically important (113,114). The way data on vaccinations is collected in low-income countries has implications for the analysis. Data on vaccinations will most often be incomplete. In longitudinal studies, information on vaccination status has typically been collected at home visits with a certain interval between visits, for example, 1, 3, 6 or even 12 months. It is unlikely that information is obtained for all children and some vaccinations administered between visits will not be known. A few projects have provided all vaccinations for a certain period or collected information from the health centres (18, 44, 46, 48, 53) but few have successfully collected information from all children, including those who travelled or died (31). The one exception would be a recent study from Cebu, The Philippines, which collected post-mortem vaccination information from 99% of the children who died (58). As many mothers in Africa will throw away the vaccination card or move from the area if a child dies, information on vaccinations is likely to be better for children who survive.

Few studies excluded the children with "no information", instead these children were usually assumed to be unvaccinated controls (60,61). Hence, controls will include absent children who may be absent because they are sick or travelling, and this group may therefore be a high-risk group with a particularly high mortality (Table 1). For example, in one study from Malawi, the estimated impact of DTP1 on mortality changed from 0.99 to 3.19 when the comparison was restricted to unvaccinated children present in the community (53). Furthermore, such controls are likely to include children who would not have been included in a randomised clinical trial (RCT) due to chronic disease or malformation.

Retrospective updating and landmark approaches. Methodological issues because of incomplete vaccination data are discussed in detail elsewhere (60-63,113,114). In brief, when incomplete data are analysed with *retrospective updating* of vaccination status as a *time-varying* covariate, *survival bias* is introduced because information is better for surviving children. Some dead vaccinated children will be misclassified as unvaccinated giving a high mortality rate in the unvaccinated group. This will exaggerate the estimated survival benefit of vaccinations (60). For these reasons (60), we did not include studies with retrospective updating in the present examination of mortality levels after DTP (Table 1); these studies are reviewed elsewhere (61).

To avoid survival bias, vaccination dates should only be taken into consideration from the date they were registered. The status as "not vaccinated" should be based on assessment; "no information" should *not* be interpreted as "not vaccinated". These restrictions lead to a *landmark approach* (60) in which vaccination status is a *fixed covariate for the period of follow-up.* In other words, mortality is analysed from the date of data collection until the next time vaccination data are collected, disregarding vaccinations given during follow-up. The landmark approach is not affected by survival bias, though *misclassification of vaccination status* due to new vaccinations during follow-up may be important as discussed below.

Most recent vaccination. The relevance of data analysed with the landmark approach depends on how many children might have received other vaccinations during follow-up; the unvaccinated group may receive the vaccine being examined and the vaccinated group may receive a subsequent vaccination during follow-up. If the unvaccinated and vaccinated groups receive only the examined vaccine, say further doses of DTP, the estimates of the effect of DTP vaccine on mortality will be conservative, i.e. biased towards 1.0 (1, 60). However, if alternative vaccines are also given, the resulting bias is unpredictable depending on the effect of the additional vaccines on mortality. We therefore emphasised periods in which additional

vaccinations are unlikely. Hence, we are ideally analysing the effect of a specific vaccine in the restricted period in which it is the "*most recent vaccination".*

Study design issues

We have mostly used two lines of enquiries to examine the possible non-specific effects of DTP. These approaches are associated with very different biases.

Comparison of sequential vaccinations. We compared DTP-vaccinated versus DTP-unvaccinated children, i.e. totally unvaccinated or BCG-only vaccinated children. This comparison is inherently biased since vaccination statuses which are scheduled to be sequential are compared at the same age in a survival analysis. The selective factors which determine which children are vaccinated first are therefore critically important for what is being compared. It has been suggested several times that the weakest children are vaccinated first, because they got vaccinated when consulting at a health centre (18,45,48). The estimated effect of the most recent vaccination might therefore be negative. However, there is no well documented case of weak children being vaccinated first. Instead, most studies, including those from Guinea-Bissau, have found that vaccinated children have better nutritional status and that children with better nutritional status are vaccinated first (1,19,53,64). Furthermore, all studies suggest that vaccination coverage is associated with better maternal education (1,44,49,58), which is associated with better survival. Hence, all the current evidence suggests that low-risk children are vaccinated first, and the weak or "frail" children get delayed vaccination or no vaccination (63). This bias, which we call *frailty bias*, would leave the weak children to die in the unvaccinated or current-vaccination group, while moving the follow-up time for the low-risk children to the group of the next vaccination (58,59). The longer the comparison is extended the more the previous-vaccination group will consist of frail children, and the more biased the comparison will be (59). In situations with high vaccination coverage, comparison of BCG-vaccinated versus unvaccinated, DTP versus BCG, or MV versus DTP, can only be conducted over a short age range (44,59). Frailty bias would tend to bias the estimates of the effect of all vaccines on mortality towards zero, i.e. better than the true effect.

Comparison of sequential vaccinations in a survival analysis would therefore inherently tend to provide a beneficial survival estimate for the most recent vaccinations even in the absence of any impact on survival. Such comparisons are therefore only informative when they provide an unexpected negative estimate for a specific vaccine, or contrasting estimates for different vaccines (1,64). Contrasting effects of different vaccines are an indication of non-specific effects unless it can be shown that different forms of selection or frailty bias are important for different vaccines. The available evidence suggests that these determinants are the same for different vaccines.

In comparisons of sequential vaccinations, the healthy newly-vaccinated children are compared with the weak not-yet vaccinated children at a specific age. Such frailty bias is not controlled by adjusting for common determinants of mortality level (maternal education, socio-economic status). Frailty bias is only fully controlled in randomised studies (82). Since randomised studies are not currently acceptable, we might seek *"natural experiments"* with limited self-selection for vaccination, for example, when vaccines were missing during a certain period (43), vaccines were only available in a certain period (37), or vaccines were stopped due to a war (21, 26). In such situations, vaccinated and unvaccinated children may be compared as they would in a randomised trial without the unvaccinated children being inherently more frail. However, such "natural experiments" with DTP are rare (42, 43).

Comparison of female and male mortality rates. In the pre-vaccination era, post-neonatal child mortality was similar for boys and girls in Africa (Figure 2). All data from Africa also suggest

that girls and boys are treated equally with respect to vaccination and the age of vaccination, and that the coverage is the same for boys and girls. In these circumstances, a deviation of the female-male mortality rate ratio (MRR) from 1.0 for children who have received a specific vaccine as the most recent vaccination would suggest that this vaccine is associated with sex-differential non-specific effects. We therefore conducted several studies examining the female-male MRR. It should be noted that a comparison of female-male mortality rates is not affected by the same survival, selection and frailty biases as comparison of sequential vaccinations. As long as biases are the same for boys and girls, and are proportional to the mortality level, they would not affect the estimated MRR for girls compared with boys. The more likely methodological problem in analyses of the female-male MRR of DTP vaccinated children is that the children could have received the subsequent MV during follow-up. If that were the case, it would lead to a conservative estimate because MV is associated with lower female mortality (11) and would dilute the effect of DTP. To prevent such misclassification, we have tried to restrict follow-up to age groups (less than 9 months) or situations (hospital admissions, war) in which it is unlikely that MV would have been provided during follow-up - in these situations we can be fairly sure that DTP has remained the "most recent vaccination".

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Hypothesis formulated in ref 1. Page 4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4-5 Table 1
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4-5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5 Table 1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Table 1
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5-6 Appendix
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5-6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Discussion
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6, Table 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figures 4-10 and supplementary tables 2-7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Pages 6-12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pages 6-12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Pages 7-13
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12-15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14-15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	16

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