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Observational studies of non-specific effects of Diphtheria-Tetanus-Pertussis vaccines in low-income countries: Assessing the potential impact of study characteristics, bias and confounding through meta-regression

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ABSTRACT

Introduction: It has been suggested that some vaccines have effects beyond protection against the diseases they target, called non-specific effects (NSEs). In 2016, a systematic review by Higgins et al., commissioned by the WHO Strategic Advisory Group of Experts (SAGE) on immunization, estimated the relative risk (RR) of all-cause mortality after whole-cell Diphtheria-Tetanus-Pertussis (DTwP) vaccination to be 1.38 (95% CI: 0.92–2.08), and described these potential NSEs as inconsistent. However, the selection of studies for meta-analysis, based on their proneness to bias and confounding, was debated.

Objective: To identify study characteristics and postulated risks of bias and confounding that might have impacted the RR of all-cause mortality after DTwP vaccination in observational studies conducted in low-income countries.

Methods: Based on methodological considerations on study design and analysis, we systematically assessed all 17 DTwP studies from the Higgins et al. review for risk of selection bias, exposure and outcome misclassification, confounding and differential co-interventions. We used meta-regression to assess the impact of study characteristics and the postulated risks of bias and confounding on the RR estimates, and looked for outlying and influential risk estimates. Permutation tests were performed to control for false-positive findings.

Results: The overall RR of all-cause mortality after DTwP vaccination including all but one outlying and influential study was 1.32 (95% CI: 0.83–2.08). Based on uni-variable meta-regression, we found that study location ($p = 0.01$), studies using the landmark approach ($p = 0.015$) and studies at high risk of exposure misclassification ($p = 0.036$) were significantly associated with increased RR estimates whereas studies at high risk of selection bias ($p = 0.059$) showed borderline significance. The results further suggest these effect modifiers are clustered in studies conducted in West-Africa.

Conclusion: The increased RR of all-cause mortality after DTwP might be confined to West-African countries and/or certain postulated risks of bias might have inflated these RRs.

1. Introduction

Whereas prophylactic vaccines are known to reduce the burden of the targeted infectious disease, a number of randomized clinical trials and observational studies suggested that some vaccines also affect the risk of morbidity and mortality due to causes not directly associated with the pathogen targeted by the vaccine. Specifically, it has been hypothesized that, until a different vaccine is given, live vaccines induce a protective non-specific immune response, whereas inactivated vaccines cause a harmful non-specific

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, confidence interval; DTwP, whole-cell Diphtheria-Tetanus-Pertussis; MCV, measles containing vaccine; NSE, non-specific effect; ROBINS-I, Risk Of Bias In Non-randomized Studies – of Interventions; RR, relative risk; RRR, relative risk ratios; SAGE, Strategic Advisory Group of Experts.

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immune response [1]. Such heterologous or non-specific effects (NSEs) are also called 'off-target effects' and there is currently a growing epidemiological and immunological interest in this topic [2,3]. The beneficial NSEs of Bacillus Calmette-Guérin (BCG) vaccine and standard titre measles containing vaccine (MCV) have been studied and documented since the middle of the 20th century, and different mechanisms have been proposed to explain these effects [4]. Amongst these, the mechanism that has gained much traction is concept of 'trained immunity', the phenomen by which innate immune cells adapt to infections and other stimuli by acquiring memory-like properties [5].

In the late 1990s, detrimental NSEs were first suggested when Aaby and colleagues studied the effect of whole-cell Diphtheria-Tetanus-Pertussis (DTwP) on all-cause mortality in Guinea-Bissau, West-Africa [6]. In the subsequent decades, more of such studies were conducted in other low-income countries such as Malawi [7], Papua-New Guinea [8] or India [9], with conflicting results. It was later postulated that the NSEs may depend on other factors such as gender [10], age at vaccination [11], co-administration with other vaccines [12] and vitamin A supplementation status [13].

Extensive reviews of studies on NSEs of vaccines illustrated how such studies are very challenging and prone to bias (sources of systematic error resulting in a incorrect estimate of the true effect) and confounding (a distortion of the true effect by the presence of another variable) [14–16]. Farrington et al. suggested the main sources of bias to be frailty bias (a form of selection bias by which children at lower or higher risk of mortality are differentially selected for vaccination) and survival bias (a form of differential exposure misclassification by which deceased children are more likely to be misclassified as unvaccinated compared to non-deceased children). One particular aspect that received a lot of attention is the method by which the vaccination status is ascertained. In studies where exposure is ascertained by retrospectively updating guardian held vaccination cards (with the vaccination status being recorded to change from unvaccinated to vaccinated at the date of vaccination), survival bias may be introduced if the vaccination status is not updated when a child dies after vaccination and before the next household visit. The risk of survival bias is particularly high for studies conducted in West-African countries where there is a tradition to destroy the belongings of diseased children, including their vaccination cards [14]. The landmark approach has been introduced to mitigate this risk of survival bias. In the landmark approach, vaccination status is only updated during visits where the child is alive (with the vaccination status being recorded to change from unvaccinated to vaccinated at the date of the visit) [17]. However, applying the landmark approach when the risk of survival bias is low is not recommended as it will result in precision loss [14,18].

Recently, a systematic review was commissioned by the WHO Strategic Advisory Group of Experts (SAGE) on immunization to summarize and ascertain the strength of the evidence of the effect of BCG, DTwP and MCV on non-specific and all-cause mortality in children under 5 years of age [19]. Most evidence originated from observational studies, which were classified as having 'low', 'moderate', 'high' to 'very high' risk of bias using the ROBINS-I tool (Risk Of Bias In Non-randomized Studies – of Interventions) [20]. The review found all studies to be either at 'high' or 'very high' risk of bias. Studies at 'very high' risk of bias were discarded from the meta-analysis. The meta-analysed relative risk (RR) for all-cause mortality was estimated to be 0.47 (95% CI: 0.32–0.69) for BCG and 0.51 (95% CI: 0.42–0.63) for MCV, suggesting that receipt of the live vaccines BCG and MCV were both associated with reductions in all-cause mortality. On the other hand, the meta-analysis suggested that receipt of the inactivated DTwP was associated with a possible increase in all-cause mortality with a meta-analysed RR of 1.38 (95% CI: 0.92–2.08). However, given the wide range of risk estimates it was concluded that the DTwP results were inconsistent, but raising sufficient concerns to warrant further research [19]. As a way forward, suggestions were made to conduct clinical trials or re-analyze available clinical trial data [19], to further carry-out well designed observational studies in a wide range of geographic locations [2,15] and to assess the likely magnitude and direction of different sources of bias and confounding on the effect estimates and conclusions [14].

In response to the Higgins et al. review [19], Aaby et al. [21] called into question the exclusion of studies at 'very high' risk of bias without taking the direction of the bias into consideration and challenged the conclusion that the DTwP results were inconsistent. Aaby and colleagues defined a 'bias index' as the mortality rate ratio comparing children without any vaccine reported to children with at least one vaccine reported and argued that a high 'bias index' suggests the presence of selection or survival bias. They further argued that the meta-analysis should have been done on studies with a low 'bias index', which would have yielded a meta-analysed RR of 2.00 (95% CI: 1.50–2.67), implying deleterious NSEs of DTwP.

In this work, we do not discuss which studies should or should not have been included in the meta-analysis by Higgins et al. [19,21]. Instead, we followed the recommendation by Farrington et al. [14] to assess the likely impact of study characteristics and different sources of bias and confounding on the DTwP effect estimates and conclusions. To this end, we first assessed all studies for risk of bias and confounding and subsequently, used meta-regression to assess the impact of study characteristics and the postulated risks of bias and confounding on the RR estimates of all-cause mortality after DTwP vaccination.

2. Methods

2.1. Assessment of the risk of bias and confounding

We systematically assessed the 17 DTwP-related RR estimates from the Higgins et al. review [19] for risk of selection bias, exposure misclassification, outcome misclassification, confounding and risk of differential co-intervention with BCG, MCV and polio vaccination. Risks were assessed as *low* versus *high*. This assessment was informed by operational and methodological recommendations regarding collection of data, design and analysis of observational studies by the Working Group on NSEs of Vaccines [14,15]. The criteria used for the assessment are summarized in Table 1. We additionally listed two key study characteristics that have been linked to potential biases and confounding: use of the landmark approach for exposure ascertainment (as this approach is expected to mitigate survival bias [17]) and region of study conduct (as the risk of survival bias is believed to be highest in West-African countries where there is a tradition to destroy the belongings of diseased children [14]).

2.2. Meta-analysis and meta-regression

Following the same approach used in the Higgins et al. review [19], we applied standard meta-analysis to the (log-transformed) DTwP RR estimates using random effects inverse variance weighted averages with a moment estimate of between-studies variances [22] and quantified the between-study inconsistency using the I^2 statistic, which is the proportion of total variation in the estimates of treatment effect that is due to between-study heterogeneity compared to chance [23]. We performed meta-analysis for studies categorized in the Higgins et al. review [19] as at 'high' and at 'very high' risk of bias separately, and overall.

Table 1
Criteria for the assessment of the risk of bias and confounding in observational studies of non-specific effects (NSEs) of vaccines.

	Low risk	High risk
Selection bias		
	Data were collected through a population-based health and demographic surveillance system recording all births and deaths	Study participants were to a certain extent self-selected (e.g. through clinic attendance) or the study is a re-analysis of a clinical trial imposing inclusion criteria
Exposure misclassification		
	Follow-up visits were frequent (every 2 weeks or less), or the vaccination status could be updated post-mortem through inspection of the vaccination card or the vaccination information was centrally recorded and held	Vaccinations (which might happen between visits) were recorded on guardian-held vaccination cards with exposure status being ascertained by the study team through consulting the vaccination cards during family visits. The less frequent these visits, the higher the risk of exposure misclassification
Outcome misclassification		
	Deaths were collected through a population-based health and demographic surveillance system, or through a hospital-based study, or the researchers of the study reported that all deaths were recorded	Deaths might not have been recorded due to extreme conditions, such as war
Confounding		
	Important confounders (e.g. sex, age, child's health, socio-economic status, health care access) were controlled for by design (e.g. only enrolling patients with access to health care, vaccinees only study) or by type of analysis (e.g. matching, multiple regression, propensity scores)	Important confounders were not controlled for
Differential BCG co-intervention		
	Study was on BCG vaccinated subjects only or BCG was always given before DTwP	Study also included subjects not vaccinated with BCG or BCG was sometimes co-administered with DTwP
Differential MCV co-intervention		
	The length of follow-up did not exceed the recommended age at measles vaccination or subjects were censored at measles vaccination	The length of follow-up exceeded the recommended age at measles vaccination or subjects were not censored at measles vaccination
Differential polio co-intervention		
	DTwP and polio vaccines were always co-administered	DTwP and polio vaccines were not always co-administered, or no information regarding co-administration could be found

NSE, non-specific effect; BCG, Bacillus-Calmette Guérin; DTwP, whole-cell Diphtheria-Tetanus-Pertussis; MCV, measles containing vaccine

Outlying RR estimates were identified using studentized deleted residuals r (i.e. the difference between the observed RR of study i and its predicted RR based on all studies except i) whereas influential RR estimates were identified using standardized DFBETAs (i.e. the change in the overall effect estimate when excluding study i from the meta-analysis). Risk estimates that were both outlying and influential were excluded from the meta-analysis, Particularly, RR estimates were excluded when both $|r| > 2.5$ and $|\text{DFBETAs}| > 2/\sqrt{n}$, where n is the number of risk estimates and $|\cdot|$ indicates the absolute value [24].

We performed meta-regression to explore the potential impact of the postulated biases and confounding (see Table 1) and the study characteristics 'region' (West-Africa: yes – no) and 'exposure ascertainment approach' (landmark approach: yes – no). After excluding outlying and influential risk estimates, we fitted meta-regression models based on which we obtained relative risk ratios (RRRs) or the ratio of the RR of all-cause mortality in DTwP-vaccinated vs DTwP-unvaccinated children when the covariate is present compared to when the covariate is absent. RRRs > 1 indicate that the DTwP RR estimate is larger when the covariate is present compared to absent. However, the risk of false-positive findings is high given the limited number of studies and the large number of potential effect modifiers we wished to explore. Therefore, we used permutation tests to control for the risk of false positive findings and obtained p-values adjusted for this risk [25]. Then, starting from these adjusted p-values, we obtained 95% Wald CIs of the RRRs. We conducted uni-variable as well as multivariable meta-regression. Model building was performed manually using forward selection (i.e. starting from the intercept-only model and adding most significant covariates one by one till no model improvement can be made based on F-statistics at 5% significance level) while still inspecting all fitted models. All analyses were performed in R 3.4.0 [26].

3. Results

Of the 17 DTwP RR estimates, 11 were from studies conducted in Africa of which 5 were conducted in Guinea-Bissau and 5 in other West-African countries, 3 studies were from India, and the remaining 3 from Bangladesh, The Philippines and Papua New Guinea. For illustrative purposes, Table 2 shows the assessment of the risk of bias and confounding for two very different studies with regards to study population, study design, and results of the risk assessment. The risk assessment for all 17 studies is given in Supplementary material #1. Seven studies were judged to be at high risk of selection bias, eight to be at high risk of exposure misclassification and only one study (conducted during war) was judged to be at high risk of outcome misclassification (Fig. 1). Ten studies used the landmark approach, three of which were at low risk of exposure misclassification. Five estimates were based on the retrospective updating approach for exposure ascertainment while for the two remaining estimates the distinction between landmark and retrospective updating approach was not relevant (one case-control study and one study on in-hospital case fatality). The majority of the estimates (10/17) were classified as being at high risk of confounding. For several studies, the risk of differential co-intervention with BCG (11/17), MCV (7/17) and polio vaccination (8/17) was assessed to be high.

The DTwP RR estimates of the various studies were very diverse (Fig. 1). One study [28], categorized as at 'very high' risk of bias in the Higgins et al. review, was identified as outlying and influential ($|r| > 2.5$ and $\text{DFBETAS} > 2/\sqrt{17}$), and was therefore excluded from further analyses. The mean DTwP RR estimates varied approximately from a 4-fold decrease (RR = 0.28) to a 4-fold increase (RR = 4.33). The DTwP RR estimates of the studies categorized as at 'very high' risk of bias in the Higgins et al. review were not very different from studies at 'high' risk of bias. When using all but the outlying study, the meta-analysed DTwP RR was 1.32 (95% CI: 0.83–2.08, $I^2 = 84.4\%$) and very similar to the estimate obtained by Higgins et al. when excluding studies at 'very high' risk of bias (RR = 1.38, 95% CI: 0.92–2.08, $I^2 = 70.9\%$) (Fig. 1). When analyzing only the studies at 'very high' risk of bias, the meta-analysed DTwP RR was 1.18 (95% CI: 0.42–3.30, $I^2 = 87.2\%$).

The results of the uni-variable meta-regression are given in Table 3. Region of study conduct (West-Africa: yes - no) was the most significant effect modifier associated with higher RR estimates of all-cause mortality after DTwP (RRR = 2.69; 95% CI: 1.27–5.7, p = 0.01), followed by use of landmark approach (RRR = 2.97; 95% CI: 1.24–7.16, p = 0.015) and risk of exposure mis-

Table 2
Assessment of the risk of bias and confounding: two examples.

	Velema, Benin 83–87 [27]	Aaby, Guinea-Bissau 02–08 [11]
Description	*A case-control study by which all children aged 4–35 months who died in 1986–87 were compared to 4 individually matched controls who survived*	*Re-analysis of a cohort of low birth weight (LBW) children to study the impact of DTwP vaccine on mortality between 2-month and 6-month visits. "The LBW cohort was initiated to study the impact of BCG at birth and neonatal vitamin A on infant mortality in a randomized trial"*
Selection bias	*LOW: Cross-sectional demographic surveys were performed. Every household was given a unique number and for each individual within the household name, date of birth and sex were recorded*	*HIGH: LBW children were enrolled at the maternity ward or at the first health centre contact after birth*
Exposure misclassification	*LOW: Vaccination cards were kept centrally. Vaccination card could not be found for 10 children (1 case and 9 controls for a case-control study with 1–4 matched controls per case), which were assumed unvaccinated. Landmark approach is not relevant*	*LOW: At each visit, it was registered whether the mother-held vaccination card was seen and all vaccination dates were noted. For the current analysis, only the vaccinations noted during the 2-month visit were used. "It was possible to see the vaccination card of 99% of the children, and only these children were included in the analyses of the effect of DTwP on survival". The landmark approach was applied*
Outcome misclassification	*LOW: Not an issue for case-control studies*	*LOW: Re-analysis of clinical trial to study the impact of BCG at birth and vitamin A on infant mortality*
Confounding	*HIGH: Case-control study matched for age, sex and village of residence and additionally adjusted for socio-economic status and weight for age in the analysis. However, the study showed a strong protective effect conferred by regular contact of the child with the village health worker. "In order to improve vaccination coverage, those children presenting for curative care at the health centre were encouraged to accept vaccination (referring to DTwP + polio) either immediately or as soon as an opportunity could be found*	*HIGH: The RR estimate was derived from rates only adjusted for sex and arm circumference*
Differential BCG co-intervention	*HIGH: Half of the children were vaccinated with BCG. The selected DTwP RR estimate compared DTwP-1 dose vaccinated to DTwP unvaccinated children*	*LOW: The RR estimate was derived from mortality rates in children with BCG at birth, comparing DTwP-vaccinated to DTwP-unvaccinated children*
Differential MCV co-intervention	*HIGH: Follow-up period from 4 months to 35 months of age and ±40% of the children received measles vaccination*	*LOW: Of the 2320 LBW children (both the 'BCG at birth' and 'delayed BCG arm'), 13 received measles vaccine at 4.5–5 months of age as part of the trial of early measles vaccination*
Differential polio co-intervention	*LOW: DTwP and polio were always co-administered*	*LOW: DTwP and polio were co-administered (ClinicalTrials.gov, NCT00146302)*

classification (RRR = 2.28; 95% CI: 1.06–4.92, p = 0.036). Risk of selection bias was found to be borderline significant (RRR = 2.88, 95% CI; 0.97–5.35, p = 0.059). Based on the bi-variable meta-regression models, we did not find evidence for differences in DTwP RRs between presence/absence of the other potential effect modifiers after adjustment for region of study conduct (Table 3). Observe that these results suggest collinearity between region of study on the one hand and use of landmark approach, risk of exposure misclassification and risk of selection bias on the other hand; none of the significant effects from the uni-variable meta-regression models remained significant in the corresponding bi-variable models.

4. Discussion

The topic of potential detrimental NSEs of DTwP vaccination has remained controversial. The SAGE-commissioned systematic review conducted by Higgins et al. [19] was heavily debated, mainly because of the choice of studies to be included in the meta-analysis [21]. In this work, we did not challenge which studies on the NSEs of DTwP should have contributed to the meta-analysis but took advantage of all available information from the 17 studies from the Higgins et al. review to assess the potential impact of study characteristics and bias and confounding on the RR estimates of all-cause mortality after DTwP vaccination. To this end, we first systematically assessed all studies for risk of bias and confounding. The study characteristics of special interest were 'method of exposure ascertainment' and 'region of study conduct'. Then, in a second phase, we used meta-regression to assess the impact of the study characteristics and risk of bias and confounding on the DTwP RR estimates.

Although Higgins et al. [19] also performed a bias assessment (using the ROBINS-I tool), we did our own assessment (based on methodological considerations for observational studies on NSEs – Table 1), providing detailed justifications of our assessment to allow for scrutiny by other researchers. Using meta-regression, we found that some postulated sources of bias and confounding impacted the RR of all-cause mortality after DTwP vaccination whereas others did not (Table 3). This illustrates the importance of qualitatively assessing whether potential sources of bias and confounding might have impacted the DTwP RR estimates, and not merely suggesting the possible presence of bias and confounding [14]. Only considering the potential presence of bias while ignoring the potential direction of the bias, was the main argument from Aaby et al. [21] to challenge the results and conclusion of the meta-analysis by Higgins et al. [19]. Instead, Aaby et al. [21] concluded that DTwP vaccination has deleterious NSEs based on a meta-analysis of studies with a high 'bias index', defined as the mortality rate ratio comparing children without any vaccination reported to children with at least one vaccination reported. While we acknowledge the importance of considering the potential direction of the bias, the vast majority of DTwP estimates from the Higgins et al. review [19] refers to comparisons of vaccinees only (i.e. comparisons of DTwP vaccinated children to children vaccinated with other vaccines) and therefore, it is questionable whether the 'bias index' as defined by Aaby et al. [21] is relevant for the DTwP RR estimates included in the Higgins et al. review.

Based on our analyses, we found that studies conducted in West-Africa (p = 0.01) were most strongly associated with increased RR estimates of all-cause mortality after DTwP vaccination. Also studies that adopt the landmark approach were significantly associated with increased RR estimates (p = 0.015). In addition, we found that studies at high risk of exposure misclassification were significantly (p = 0.036) associated with increased RR estimates whereas studies at high risk of selection bias showed borderline significant increased RR estimates (p = 0.059). All these effect modifiers tend to cluster in studies conducted in West-Africa, as suggested by the collinearity of the bi-variable meta-regression models.

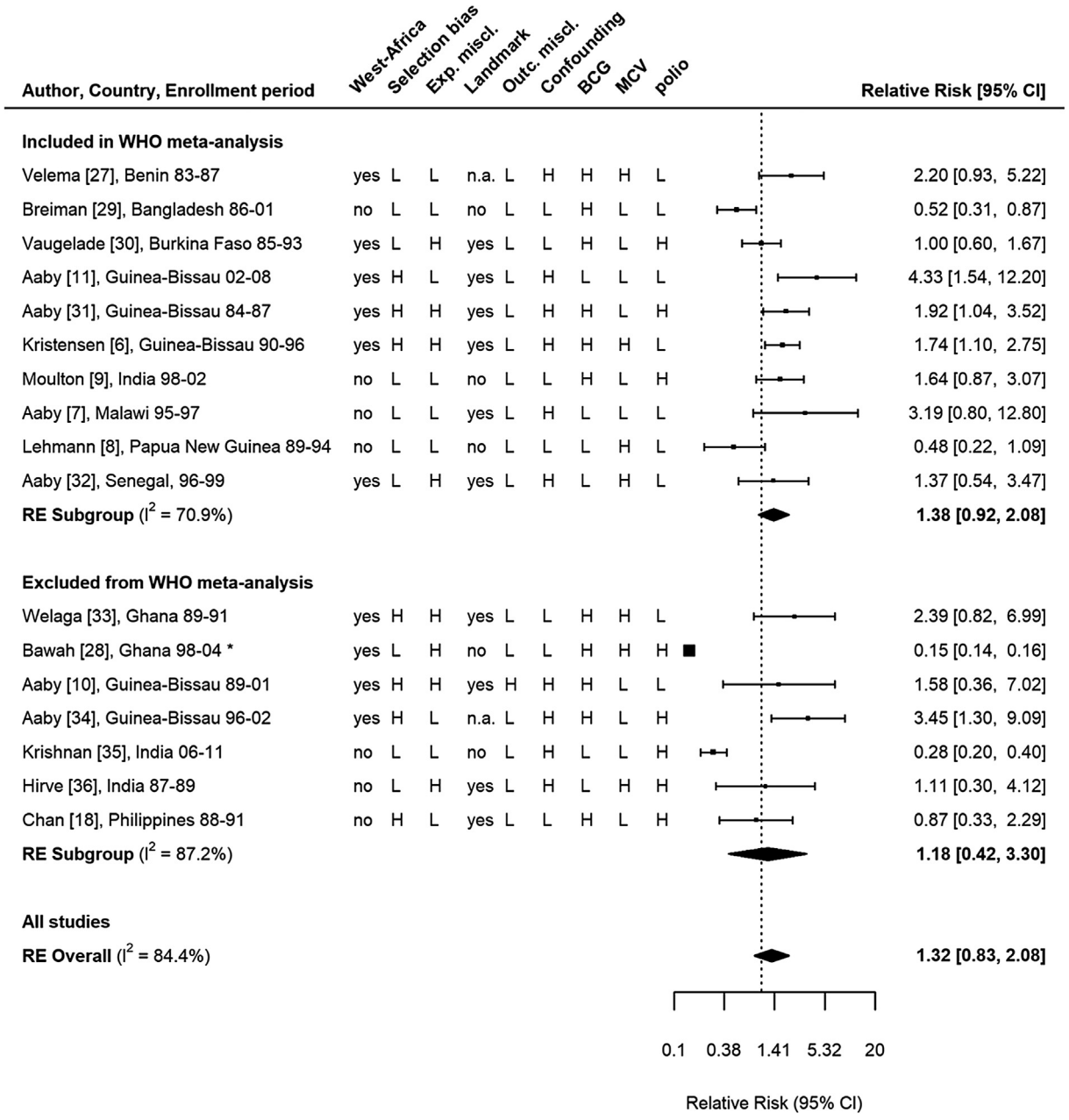

Fig. 1. Forest plot with relative risks of all-cause mortality comparing children with to children without DTwP vaccination. (RE = random effects meta-analysis model, n.a. = not applicable, L = low risk, H = high risk). * Excluded from meta-analysis as the study was outlying and influential. (See above-mentioned references for further information.)

Table 3
Results of uni-variable meta-regression and bi-variable meta-regression after adjustment for region.

	Uni-variable		Bi-variable (Variable + Africa)		
Variable	RRR[+] [95% CI][o]	p-value [o]	RRR[+] [95% CI][o]	p-value [o] Variable	p-value [o] Africa
Region (West-Africa vs other)	2.69, [1.27, 5.7]	**0.01**			
Risk of selection bias (high vs low)	2.28, [0.97, 5.35]	**0.059**	1.56, [0.6, 4.07]	0.367	0.127
Risk of exposure misclassification (high vs low)	2.28, [1.06, 4.92]	**0.036**	1.56, [0.57, 4.26]	0.389	0.115
Exposure ascertainment approach (landmark vs other)	2.97, [1.24, 7.16]	**0.015**	2.4, [0.71, 8.18]	0.16	0.684
Risk of outcome misclassification (high vs low)	1.23, [0.18, 8.21]	0.831	0.81, [0.14, 4.73]	0.812	0.014
Risk of confounding (high vs low)	1.69, [0.71, 4.05]	0.239	1.14, [0.43, 3.01]	0.785	0.025
Risk of differential BCG co-intervention (high vs low)	1.09, [0.42, 2.82]	0.854	0.91, [0.4, 2.09]	0.823	0.022
Risk of differential MCV co-intervention (high vs low)	1.48, [0.61, 3.57]	0.383	1.12, [0.39, 3.21]	0.829	0.009
Risk of differential polio co-intervention (high vs low)	0.75, [0.31, 1.77]	0.508	0.9, [0.38, 2.16]	0.82	0.025

[+] RRR = Relative Risk Ratio, ratio of the relative risk (RR) of all-cause mortality in DTwP vaccinated vs DTwP unvaccinated children when the covariate is present compared to absent.

[o] Based on permutation test to control for false-positive findings [25].

Selection bias by which frail children are being preferentially vaccinated (frailty bias), would lead to increased DTwP RR estimates. This might happen when frail children were more likely to visit health care centers and hence, were more likely to be opportunistically vaccinated compared to healthy children. Exposure misclassification by which deceased children are more likely to be misclassified as unvaccinated compared to non-deceased children (survival bias), would lead to decreased DTwP RR estimates. Many studies at high risk of exposure misclassification (survival bias) adopted the landmark approach, which is expected to mitigate the issue of survival bias. It is therefore puzzling why studies at high risk of survival bias yielded higher DTwP RR estimates compared to studies at low risk of survival bias whereas one would have expected them to yield lower estimates (in case survival bias was not corrected) or similar estimates (in case survival bias was corrected). A possible explanation might be the clustering of the different DTwP RR effect modifiers within the same studies.

Postulated sources of bias and confounding that were not associated with increased RR of all-cause mortality after DTwP vaccination were high risk of confounding, high risk of outcome misclassification and high risk of differential co-intervention with BCG, MCV and polio vaccines. Based on a post-hoc analysis, we indeed found that confounder adjustment had remarkably little impact on the estimated DTwP RR estimates; for 9 studies for which both adjusted and unadjusted risk estimates were available (or could be derived), confounder adjustments resulted in virtually no change (n = 3), a small increase (n = 4) or a small decrease (n = 2) in risk estimates (Supplementary material # 2).

Our approach has limitations. We categorized various risks of bias and confouding as *low* versus *high*. Despite our attempt to be systematic, we cannot exclude that some researchers might disagree with our assessment. Therefore, we were fully transparent and provided detailed descriptions of our risk of bias and confounding assessment (Supplementary material #1). Second, we based our assessment on the published literature and the quality of our assessment is conditional on the quality and completeness of the published information. Third, the categorization in *low* versus *high* risk implies limited granularity. However, given the relatively small number of risk estimates available for the meta-regression (n = 16), a more granular categorization would not have been possible. Finally, we could not disentangle the potential impact of various sources of bias from a regional effect as studies at high risk of bias tend to cluster in studies conducted in West-Africa.

It is important to stress that our analyses explored the potential impact of study characteristics and postulated sources of bias and confounding on the RR of all-cause mortality after DTwP vaccination. They did not assess the presence of a specific source of bias, nor did they provide evidence that a particular source of bias or confounding truly affected the RR estimate. Our analyses merely suggest that observed deleterious NSEs of DTwP vaccination might be confined to studies conducted in West-African countries and/or that certain sources of bias cannot be ruled out as a potential explanation of the observed increased risks of all-cause mortality after DTwP vaccination. In this respect, it is also important to note that, until now, there is no immunological evidence supporting potential detrimental NSEs of DTwP [3]. Whilst 'trained immunity', the phenomen by which innate immune cells adapt to infections and other stimuli by acquiring memory-like properties [5], provides a potential immunological explanation for NSE, further work needs to be done to bridge the gap between emerging immunological concepts and epidemiological observations.

Some authors have suggested that the hypothesis of NSEs is best tested in randomized clinical trials despite the challenges to ethically justify clinical trials given the established benefits of vaccination. Whichever type of study design, randomized controlled trials or observational studies, testing a safety hypothesis regarding NSEs of vaccines requires high quality data. Our analyses highlighted that selection bias and exposure misclassification require particular attention in any future study on NSEs of DTwP vaccination.

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Contributors

All authors attest they meet the ICMJE criteria for authorship. The idea and contents of the article emerged from discussions among the authors. KB performed the analyses and wrote the first draft. All authors contributed to the subsequent revisions and approved the final version. KB is the guarantor.

Conflict of interest

Kaatje Bollaerts and Thomas Verstraeten received consulting fees from the GSK group of companies for the work reported here. Catherine Cohet is an employee of, and holds shares in, the GSK group of companies.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.11.049.

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