Comparative efficacy/effectiveness of schedules in infant immunisation against pertussis, diphtheria and tetanus: Systematic review and meta-analysis.

Part 1: Diptheria and tetanus vaccines

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Introduction

Rational for this systematic review

While the recommended immunization schedules for pertussis, diphtheria and tetanus vaccines vary among countries, three primary doses are used in most countries, often followed by a booster dose in the second year of life, if programmatically feasible. WHO's Optimizing Immunization Schedules project has set out to encourage countries to take informed decisions on their vaccine schedules. The underlying assumption is that one uniform schedule will not allow taking into account countries' epidemiological, programmatic and financial priorities and constraints. In this context, some countries may, for example, find a two-dose primary schedule financially interesting and will need to know whether it can sufficiently protect children against pertussis, diphtheria and tetanus during the first year of life.

Therefore, this review aims at providing best evidence on clinical effectiveness and safety that different pertussis, diphtheria and tetanus immunization schedules have for children less than five years of age. Previous work on this aspects include a systematic review on clinical effectiveness of pertussis vaccine compared to no vaccination [Jefferson 2003] and a rapid review of schedule comparison, which is accessible on the WHO website [Scott].

This second part of the report focusses on diphtheria and tetanus vaccines.

Goal and objectives

The goal of this review was to inform on the comparative efficacy or effectiveness of different immunization schedules during the first 5 years of life against diphtheria and tetanus among children during the first five years of life.

The process also critically appraises the body of evidence with regard to internal and external validity ("quality") and to identify pieces of evidence missing for conclusion or decision making with regard to the primary and secondary study objectives.

Objectives were to provide best evidence on **primary vaccination** against diphtheria (full antigen) and tetanus for children <18 months of age as to compare

a. the effect of the number of doses on the outcomes (eg, 3 vs 2 doses);

b. the effect of age at initiation of vaccination on the outcomes (eg, 6 weeks vs birth dose);

c. the effect of the length of vaccine dosing intervals on the outcomes (eg, 4 weeks vs 2 months);

d. the effect of any vaccination on the outcomes (compared to no vaccination; absolute effectiveness);

and to provide best evidence, on **booster vaccination** against diphtheria (full antigen) and tetanus among children <5 years of age as to compare

e. the effect of age at the booster on the outcomes (eg, 12 vs 18 months) ;

f. the effect of any booster on the outcomes (compared to no booster; absolute effectiveness);

where the outcomes were assessed among <5-year-old children.

Methods

We conducted a systematic review of the published literature and of unpublished recent studies, followed of a critical appraisal and synthesis of the existing evidence.

Systematic review

Search strategy for retrieval of records

The following data bases were searched for relevant records:

a. Medline 1966 to November 2012
b. EMBASE 1988 to November 2012
c. African Index Medicus
d. WHO international Clinical Trials Registry Platform Search Portal
e. the European Public assessment Report (EPAR) listings of the European Medicines Agency (EMEA)
f. LILACS (Latin American and Caribbean Literature on Health Sciences)
g. Cochrane Controlled Trials Register (CENTRAL) up to November 2012

See **Appendix 1** for search queries. We used a web-based application designed specifically for the screening and data extraction phases of a systematic review (DistillerSR®, Evidence Partner corporate).

We handsearched reference lists of included records, relevant review articles, and related systematic reviews to identify any additional studies for inclusion.

Screening, inclusion and exclusion

Retrieved records were screened according to the inclusion and exclusion criteria. Screening was performed independently by two reviewers using standardized screening forms. The steps for record screening are summarized in **Figure 1**.

For the screening process, no translation of articles was sought. Contacting authors for further information or clarification was not part of the standard procedure. Potentially retrieved articles in German were screened and extracted by one reviewer.

Studies labeled as "exclude" by both reviewers were excluded from the review and the reasons for exclusion documented. Records with disagreements between the two reviewers regarding eligibility were arbitrated by the principal investigator. Records classified as "include" in accordance, moved to the next level of evaluation.

Inclusion criteria

We included any study which completed the following criteria.

a. Study design: Studies that can be described as randomized controlled trial (RCT) with individual or cluster randomization or observational study, in form of a cohort study, case control study or population level surveillance study

b. Type of intervention: Studies evaluating primary or booster immunization against diphtheria (full dosage) and / or tetanus. Both stand-alone or combination presentations were eligible.

c. Comparison: Studies that were designed as direct comparison between groups receiving immunization with two or more different schedules or immunization vs no immunization (the latter in form of *nihil* or placebo). The contrast between different schedules concerned the number of doses, duration of intervals between scheduled doses or age at initiation. Direct comparison implied a single protocol implemented in a defined population using the same vaccine product.

d. *Study population:* No restrictions on country, ethnicity, gender or health status were applied. We included studies that evaluated following age groups:

i. For primary vaccination (objectives 1a-d, 2a-d, 3a-d): immunization among children <18 months of age, outcomes among children <5 years

ii. For booster vaccination (objectives 1e-f, 2e-f, 3e-f): immunization and outcomes among children <5 years of age

e. Outcomes: Studies that evaluated and reported immunogenicity, clinical efficacy or effectiveness, and/or reactogenicity of vaccination, as defined in the following:

i. **Immunogenicity** was taken into account if immune response after vaccination has been assessed by relevant serological measures:

- Percentage of participants showing seroconversion
- Pre-post increase of proportion protected or geometric mean concentration
- Proportion protected or geometric mean concentration after vaccination

For **diphtheria**, the serological assays for immunogenicity included per protocol were Vero cell neutralization test; or enzyme immunoassays (EIA, ELISA) (*low evidence level, as poor inter-laboratory reliability*). Per protocol, post-vaccination antitoxin IgG concentration ≥ 0.01 U/mL among those with concentrations <0.01 U/mL prior to vaccination were considered a criterion of seroconversion. For post-vaccination prevalence of protection, we considered as putatively protected vaccinees with anti-toxin titers (neutralization test) of ≥0.1 IU/ml [WHO 2009].

For **tetanus,** the serological assays for immunogenicity included per protocol was toxin neutralization test or standardized ELISA test. Post-vaccination antitoxin IgG concentration ≥ 0.1 U/mL (ELISA) among those with concentrations <0.1U/mL prior to vaccination were considered a criterion of seroconversion. For post-vaccination prevalence of protection, we considered as putatively protected vaccinees with anti-toxin titers of ≥0.01 IU/ml (neutralization test) or ≥0.1 IU/ml (standardized ELISA) [WHO 2006].

ii. **Clinical efficacy/effectiveness** was taken into account as comparison (difference or ratio) of disease incidence, disease-specific mortality or overall mortality.

For **diphtheria,** the classification of cases for evaluation of clinical efficacy or effectiveness comprised:

Clinical case: In the absence of a more likely diagnosis, an upper respiratory tract illness with an adherent membrane of the nose, pharynx, tonsils, or larynx. (CDC definition)

Confirmed case: A clinical case with identification of *C. diphtheria* by culture or PCR on nose or throat swab, histopathologic diagnosis of diphtheria, or epidemiologic linkage to a laboratory-confirmed case of diphtheria.

For **tetanus,** the classification of cases for evaluation of clinical efficacy or effectiveness comprised:

Clinical case: In the absence of a more likely diagnosis, an acute illness with muscle spasms or hypertonia and diagnosis of tetanus by a health care provider; or death, with tetanus listed on the death certificate as the cause of death or a significant condition contributing to death. (CDC definition)

Confirmed case: No definition for confirmed cases of tetanus exists.

Neonatal tetanus was not included as an outcome in this review.

iii. **Reactogenicity** was taken into account if the data reported the proportion of vaccinees experiencing adverse events that occurred within the first week after vaccine administration and that were mild or severe adverse events:
   - local or regional swelling, pain/tenderness, redness
   - or fever (T° > 101.1°F or >38.5°C)
   - or systemic allergic reaction
   - seizures
   - persisting crying
   - HHE (hypotonia/hyporesponsiveness/collapse)

Based on the assumption that pertussis antigens (whole cell or acellular) were more reactogenic than diphtheria or tetanus toxoids, we included only studies evaluating vaccines without a pertussis component.

Exclusion criteria

We excluded studies which

a. evaluated immunization only of children older than 5 years of age and younger than 11 years.
b. compared only different types of vaccine, but not schedules within the same vaccine
c. were cross-sectional studies
d. evaluated immunogenicity only by comparing pre- and post-vaccination (and not between different schedules)
e. closed enrolment before 2009 and which have not been published in a peer-reviewed journal since then
f. closed enrolment after 2009 and for which no precise and detailed public communication trace exists (registration on ClinicalTrial.org or similar, abstract at a scientific international conference)
g. had a full text written in languages other than English, French or German and for which eligibility cannot be assessed based on the abstract
h. were dose finding studies during vaccine development (phase I trials)
i. presented data on vaccines that never have been licensed

Included and additional not-per-protocol studies

Based on criteria related to inclusion criteria defined in the protocol, we grouped eligible articles as "included" or "additional not-per-protocol". The latter group comprised articles that did not fulfil any of the exclusion criteria, contributed relevant evidence with regard to the study objectives, but did not fulfil all inclusion criteria (mainly with regard to outcome definition: case definitions and serological methods).

Data extraction, critical appraisal, grading

Two reviewers extracted data from eligible references as they referred to inclusion and exclusion criteria, results, confounding variables, factors for sub-group analysis and quality indicators.

Two reviewers assessed the included studies for sources of systematic bias, using the guidelines in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions [Altmann] for evaluating RCTs, and the STROBE Statement [Strobe Statement] for assessing the methodological quality of observational studies. Judgments for each criterion were reported as low, moderate or high risk of bias, or "unclear" (if the provided information was insufficient to judge).

Quality, consistency and quantity of the available body of evidence was assessed for each objective. For grading of study design and standard epidemiological considerations, the evidence grading scheme recommended by GRADE working group [Atkins 2004] was used. To increase discrimination, we eventually used the categories low, moderate and high risk of concern for the GRADE categories limitations, inconsistency, indirectness, imprecision etc., which can be translated as low == no serious issue; moderate == serious issue; high == very serious issue.

Inconsistency was not rated, if only one study was available, but this led to a lower GRADE evaluation.

Although immunogenicity was in itself an outcome in this review, we considered immunological studies with specific level of indirectness: "low" if the per-protocol correlate of protection was evaluated, "moderate" if another similar titer cut-off was evaluated, "high" if mean titers were evaluated.

We did not GRADE evidence available from additional not-per-protocol studies, as this would have suggested comparability between studies.

Data synthesis – meta-analysis

We produced descriptive summary tables of included and additional studies, exploring heterogeneity between by study design, outcome definitions, and time point of evaluation. As there was appreciable variability in the studies with regard to interventions, follow-up intervals and assessed outcomes, we did not combine the results in a meta-analysis nor assess reporting biases by evaluating asymmetry in funnel plots.

Results

Study selection

The search identified 5426 records (**Figure 1**). Initial screening excluded 4939 studies, including 2050 duplicates. The remaining 487 full-text articles were screened for inclusion, of which 461 proved ineligible. Fifteen studies were considered eligible, of which 6 eventually were judged not-per-protocol. In addition, 102 potentially relevant references were identified through hand search, of which 96 were excluded, while for 3, no full-text article could be found. Of the 9 eligible articles, 6 were included and 3 considered additional not-per-protocol evidence. In total, 15 articles were included (one with the tetanus component not-per-protocol) and 9 articles considered as not-per-protocol evidence.

Among the 15 included articles and studies, 6 were RCTs and 9 observational studies. Five RCTs evaluated the impact of primary vaccine schedules on immunogenicity against diphtheria and tetanus (one RCT only evaluated diphtheria), with one study also evaluating absolute immunogenicity of a booster dose. One RCT evaluated booster schedules. One clinical trial evaluated absolute immunogenicity against tetanus.

Among the observational studies, all evaluated primary schedules. Seven evaluated primary vaccine schedule impact on immunogenicity, one case-control study absolute effectiveness of different number of doses and one clinical trial absolute immunogenicity of a 2-dose schedule.

All included studies used ELISA serological assays, with the exception of Barkin 1985 (neutralization test on mice for D and on rabbits for T), Giammanco 1998 (not further specified neutralization test for D and T), Kimura 1991 (microcell culture method using VERO cells for diphtheria; passive hemagglutination for tetanus, not per protocol) and Mangay-Angara 1978 (tetanus toxin neutralization in mice). Unless otherwise indicated in Tables 8-11, studies used ELISA assays for serological testing.

Meta-analysis was not considered due to the fact that no outcome was addressed by at least three comparable studies.

Results of individual studies

Primary vaccination

Number of primary doses: Two doses vs. three doses

Evidence is available on the comparison between two and three doses from one RCT and four cohort studies (**Table 8b-A**)

One RCT compared a 3,5- to a 2,4,6-mo schedule and found that 2 doses had induced substantially lower antitoxin concentrations at 1 and 7 mo after primary vaccination against both diphtheria and tetanus [Carlsson 1998]. This however did not clearly translate into clearly lower prevalence of concentrations ≥0.01 IU/ml or ≥0.1 IU/ml). At one month after a booster dose (at age 12 or 13 mo), no difference was found between the two groups.

A cohort study compared immunogenicity against diphtheria of a 2,4,6-mo and a 3,5,11-mo schedule [Giammanco 1998]. At age 7 and 6 mo and after 3 and 2 doses, respectively, the accelerated schedule had elicited significantly higher GMT in the group that had received 3 doses (0.188 vs 0.108 IU/ml).

A cohort analysis of trial groups in Sweden reported on diphtheria titers at age 7 months following a 2,4,6-mo vs. a 3,5-mo schedule [Tiru 2000], and found slightly higher median titers and prevalence of putatively

protective titers (≥0.10 EU/ml) in the 3-dose group (statistical testing not possible, data extracted from cumulative distribution curve).

One small cohort study compared a 2,6 vs. a 2,4,6-mo schedule, at age 8 months [Barkin 1985] and found 2-fold lower titers in the 3 dose group against tetanus (0.26 vs. 0.51 U/ml) and diphtheria (0.13 vs. 0.26 U/ml; the latter was not per protocol, as obtained with animal neutralization test).

One study compared two samples of child populations in Israel who were vaccinated with a 2,4,6 + 12 mo and a 2,3.5 + 10 mo schedule. At one month after the booster (age 13 and 11 mo), diphtheria antitoxin GMT (ELISA) were substantially and significantly lower in the 2+1 group (0.403 vs. 1.043 IU/ml),while prevalence of titers ≥0.1 IU/ml was only slightly lower 91.7 vs. 100%. At age 3 years, no substantial difference was found. For tetanus, antitoxin concentrations were slightly but significantly lower in the 2+1 group (3.45 vs. 4.87 IU/ml), without differences in seroprevalence of putatively protective titers. At age 3 years, titers and seroprevalence were slightly higher in the 2+1 group.

This study also reported results from RIA testing (*not per protocol*), which also showed lower titers against diphtheria in the 2+1 at age 13 months or 3 years, which did not clearly translate into lower seroprevalence.

Three further *not-per-protocol studies* provided additional evidence, two using serological methods not included in the protocol, the third comparing schedules using difference vaccines (**Table 8b-B**).

A small cohort study found that antibody levels obtained after 3 primary doses in 6-weekly intervals were higher than after 2 doses (median titer 0.5 vs 1.5; data extracted from graph) [Laurel 1957].

A small RCT study compared antibody levels obtained after 2 and 3 primary doses, without specifying the schedules used in the participants aged up to 5 years and using a not per protocol assay [Bhandari 1981]. Three primary doses elicited significantly higher antibody levels (about 6-fold for both diphtheria and tetanus).

Additional evidence comes also from a cohort study in Sweden comparing a 2+1 to a 3+1 schedule (3,5,12 mo vs. 2,4,6,15 mo) [Taranger 2000]. Similar or higher titers against diphtheria and tetanus were found with the 2+1 schedule at one month after primary vaccination throughout age 48 months. However, the 2+1 schedule was given with a vaccine containing more D and T toxoid than the one given in the 3+1 schedule, which likely produced the observed difference.

<u>Different ages of vaccination initiation</u>

Evidence came from a Chinese RCT reported in two articles (**Table 8b-A**). Comparing a 3,4,5-mo and a 2,3,4-mo schedule, GMT one month after the three primary doses were higher in the schedule starting later (diphtheria 0.516 vs 0.431 IU/ml, *P*-value <0.05; tetanus 3.02 vs 2.88 IU/ml) [Li 2011 (I)]. The percentage of participants with putatively protective titers (cut-off 0.01 IU/ml for both antigens) was 100.0% in both groups. In a follow-up of this trial, one month after boosting at age 18-20 mo, no substantial or significant difference was found between groups in GMT or prevalence of seroprotection (cut-off 0.01 IU/ml and 0.10 IU/ml) [Li 2011 (II)].

A small cohort study in Japanese children compared vaccination with 3 doses during an early period (age 3-8 months) with during a later period (age 9-23 months), and found similar antibody levels against diphtheria in both groups [Kimura 1991]. After booster, antibody titers were higher in the later-initiated group (10.2 vs. 6.7 IU/ml), however, without statistical significance.

Five *not-per-protocol studies* provided additional evidence (**Tables 8b-B**). Two studies compared schedules using different vaccine products per schedule group. One RCT used radio-immunofluorence assay for serological testing, one cohort study hemagglutinin testing for tetanus and one did not specify testing methods.

One RCT compared a combination vaccine (DTwP-HepB-Hib) with separate injections of DTwP, HepB and Hib, using two different schedules for each group (and 1.5,3,5 mo vs. 3,4,5 mo) [Wong 2008]. At ages 6 and 12 months, diphtheria GMC tended to be higher in the later and shorter schedule (3,4,5 mo-group with separate injections), although without statistical significance, similar finding for percentage of participants with putatively protective titers (≥0.1 IU/ml). By contrast, tetanus GMC were lower in the 3,4,5 mo-group, with a significant difference at age 6 months. The percentage protected participants (≥0.1 IU/ml) was similar in the two groups.

One additional small cohort study compared two long schedules (3,5,12 mo vs. 5,6,15 mo) [Gyhrs 1999]. With the later schedule, significantly higher GMT (about 2-fold) against both diphtheria and tetanus were found at 1 month after the 3rd dose and at age 24 months. One month after the 2nd dose, no differences had been found. The researchers had pointed that the slight increase may be as a result of higher concentration of the toxoid in the vaccine.

A clinical trial in Turkey and Belgium compared a later but shorter 3,4,5-mo schedule with 2,4,5-mo schedule [Hoppenbrouwers 1999] (serological analyses using RIA). The earlier but longer schedule elicited slightly higher titers against diphtheria and significantly higher titers against tetanus (1.84 vs. 1.08 EU/ml).

A small cohort study in Japanese children compared vaccination with 3 doses plus a booster during an early period (age 3-8 months) with initiation during a later period (age 9-23 months), using hemagglutination testing. The results showed higher antibody levels against tetanus after late initiation, before (significant difference) and after booster [Kimura 1991]. No difference was found before or after third dose.

A small study did not report in detail on inclusion or serological methods [Vahlquist 1949]. The author describes diphtheria antitoxin levels after one dose of diphtheria toxoid given at birth, age 2-3 mo or age 6-8 mo. Mean levels at age 6-8 months were 0.02 U/ml or higher and 40%, 33% and 47%, respectively of the three groups had titers of at least 0.02 U/ml.

Birth dose

Two included studies dwelt on the impact of a birth dose in addition to a 2,4,6-mo schedules on diphtheria and tetanus antibody level (**Table 8b-A**). In a small randomized trial reporting only tetanus antibody titers [Dengrove 1986]), no significantly higher titers were achieved with a birth dose. Similarly, another small RCT found similar and not significantly higher titers against diphtheria and tetanus after a birth dose, at age 7 months or age 18 months (after a booster at 17 months). Diphtheria GMC was even significantly higher in the control group at age 7 months (3.00 vs 1.64 IU/ml) [Halasa 2008].

Accelerated vs. long schedules

Evidence comparing a 3+0 to a 2+1 schedule came from four studies (**Table 8b-A**).

A cohort study compared immunogenicity of a 2,4,6-mo and a 3,5,11-mo schedule [Giammanco 1998]. Against tetanus, at one month after the third dose, all participants in both groups had putatively protective neutralizing titers (≥0.01 IU/ml) and titers above the upper test limit. Similarly against diphtheria, at one

month after the third dose (age 7 and 12 mo, respectively), all participants in both groups had putatively protective neutralizing titers (≥0.01 IU/ml) and GMT were 10-fold and significantly lower after the accelerated schedule (0.188 vs. 1.712 IU/ml). However, at age 7 and 6 mo and after 3 and 2 doses, respectively, the accelerated schedule had elicited significantly higher GMT than the long schedule (0.188 vs 0.108 IU/ml).

A synopsis of two studies conducted 1988 and 1990 – before and after schedule change in the UK – compared an accelerated (2,3,4-mo) schedule with a long (3,5,9-mo) schedule [Booy 1992)]. At one month following the last primary dose (age 5 vs. 10 mo), the study recorded about two-fold higher antibody concentrations against both diphtheria and tetanus vaccines in the long schedule (no variance or statistical testing reported, about 100 children per group).

A small clinical trial involving preterm babies (included here data on minimal gestational age 32 weeks) [Conway 1993] also showed that a 3,4,10-mo schedule, compared to a 3,4,5-mo schedule elicited significantly higher (3- to 4-fold) anti-body titres against both diphtheria and tetanus at one month after last primary dose.

Additional evidence was available from two *not-per-protocol studies*. An RCT synopsis compared an accelerated 2,3,4- mo with a long 3,5,8-10-mo schedule and showing higher antibody levels and prevalence of protective titers in the long schedule [Miller 1997]. However, the timing of assessment was not comparable age 4-5 yrs vs. 12-18 mo), such that waning antibodies may explain most of the observed difference.

Another synopsis of cohort studies compared an accelerated 2,3,4- mo with a long 3,5,8-10-mo schedule, using a solid-phase RIA, assessing antibody concentrations at approximately the same age (mean 20 and 22 mo, 12+ mo after third dose) [Ramsay 1993]. The group with the long schedule showed higher antibody titers against both diphtheria and tetanus, however without statistically significant difference. (**Table 8b-B**).

Absolute effectiveness

One case control study estimated the effectiveness of primary and primary plus booster vaccination against diphtheria among children aged 5 years or less in Russia, using a not specified schedule with 3 primary doses in 1-month intervals before age 12 months and a booster at age 2 years [Bisgard 2000]. Vaccine effectiveness compared to zero doses was >90% in both age groups 0-2 years and 3-5 years after at least 2 doses and was ≥99% after 4 doses (**Table 9a-A**).

One clinical trial among children aged 3-8 months evaluated the tetanus immunogenicity of 2 DTwP doses in 6-mo interval compared to diphtheria toxoid only [Mangay-Angara 1978]. GMT at 40 days after the second dose was substantially higher in the vaccinated group (0.24 vs. 0.013 IU/ml) (Table **Table 9b-A**).

Booster vaccination

Schedule impact on immunogenicity

In an RCT (of moderate size and unclear blinding of serological testing) [Scheifele 1999] comparing antitoxin titers after a booster dose (after a not further specified 3-dose primary series before age 7 months), antitoxin concentrations tended to be somewhat higher against both diphtheria and tetanus following a

booster at age 18 months, compared to 12 or 15 months (differences partially significant). However, the prevalence of antitoxin concentrations ≥0.1 U/ml was close to 100% in all groups for both antigens (threshold corresponds to correlate of protection for tetanus).

Absolute immunogenicity

In a small clinical trial involving preterm babies (included here are children with at least 32 weeks gestational age) [Conway 1993], children receiving a booster vaccination showed >16-fold higher diphtheria and tetanus antibody titers than children without booster, at one month after boosting at 18 months after 3 primary doses before age 5 months (**Table 11b-A**).

Result summary and GRADE evidence profiles

Tables 3-5 present GRADE evidence profiles by objectives, if at least two studies addressed a comparable outcome. The following summarizes the retrieved evidence:

Objective a. (effect of the number of doses on the outcomes) was addressed by five studies comparing 2 vs. 3 primary doses. Outcomes were assessed with low or very low level of evidence (limitations, imprecision; but includes evaluations on correlate of protection), suggesting that for both diphtheria and tetanus, 2 doses resulted in substantially lower antitoxin mean titers (factor down to 0.5) than three doses, one to seven months post primary vaccination. Data at one month after a booster dose are inconsistent, with 1of 2 studies reporting lower titers for diphtheria, but not tetanus. Differences did not translate in a substantially decreased prevalence of putatively protective or otherwise dichotomized antitoxin levels.

Appropriate not-per-protocol studies supported lower antitoxin titers after 2 compared to 3 doses soon after the primary series, and, for diphtheria, at age 3 years.

Objective b. (effect of age at initiation of vaccination on the outcomes) was addressed by two studies on birth dose, and two on other schedules. At very low level of evidence, a birth dose prior to a 2,4,6-mo schedule did not provide higher antitoxin GMC against diphtheria or tetanus between age 6 through 9 months or after a booster in the second year or life.

Furthermore, at low level of evidence (some indirectness as not using putatively protective levels, only one moderately large study) one study (Li I and II) suggested that 3,4,5 vs. 2,3,4-mo-schedule provides similar antitoxin seroprevalence above a threshold of 0.01 IU/ml (ELISA) or GMC against diphtheria and tetanus, at one month post third primary or booster dose. At very low level of evidence (cohort with limitations, indirectness, imprecision), only single study suggested that initiation of vaccination with 3 primary doses at age 9-23 months compared to age 3-8 months does not provide higher antitoxin titers against diphtheria or tetanus (assay not per protocol).

Appropriate not-per-protocol studies support the absence of a substantial effect from age at primary series initiation.

Objective c. (effect of length of interval on the outcomes) was addressed by three immunogenicity studies, with an overall very low level of evidence (limitations, indirectness, imprecision). Results suggest that an accelerated schedule results in lower level of antibodies (factor 0.5) after the third dose or during the second year of life, when compared to a long schedule (with an interval of around 6 mo between 2nd and 3rd dose).

One appropriate not-per-protocol study is compatible with higher antitoxin titers after a 2+1 compared to a 3+0 schedule.

Objective d. (effect of any vaccination on the outcomes) was addressed by two studies with overall very low level of evidence (one single case control study with low sample size and large confidence intervals per number of doses; one small clinical trial with unclear allocation procedure). Among children aged 0-2 years, the results suggest that vaccine effectiveness is >90% for one, two or three primary diphtheria doses given

during the first 12 months of life; and among children aged 3-5 years, vaccine effectiveness >90% for two or three primary doses, or a fourth dose at age 2 years. Tetanus toxoid neutralizing titers were 20-fold higher one month after a 2nd dose in 6-mo interval, compared to no vaccination.

Objective e. (effect of booster schedule on the outcomes) was addressed with low level of evidence (one single study). The results suggest that delaying booster vaccination against diphtheria or tetanus to age 18 months, compared to 12 or 15 months, may yield higher antitoxin concentrations, while the differences likely do not translate into higher prevalence of putatively protective concentrations.

Objective f. (effect of any booster vaccination on the outcomes) was addressed by one study (Conway), with overall very low level evidence (one single small RCT with unclear limitations, indirectness). The result suggests substantial increase in diphtheria and tetanus antibody due to booster vaccination at 18 months, following an initial 3,4,5-mo schedule.

Discussion

Summary of findings

This review found that some evidence is available for all questions concerning schedules of primary and booster vaccination against diphtheria and tetanus (number of doses, age at initiation, interval, and absolute effect).

Available evidence carries almost exclusively on immunogenicity. Only one study evaluated clinical effectiveness, and no study evaluated comparative or absolute effects on reactogenicity, as all studies used vaccines combined with pertussis antigens.

In total, 13 articles implying 12 different trials or studies were available, with research starting during the 1970s and reaching into the 2000s. The 8 additional studies (earliest evidence comes from 1957) were considered not-per-protocol due to serological methods or as they compared schedules in secondary analyses of studies initially comparing different vaccines.

The available evidence suggests that

- two versus three primary doses result in substantially lower antitoxin titers after primary series, but
    - this difference does not persist during the second year of life and after boosting; and
    - this difference does not clearly translate into a difference in clinical protection *(overall GRADE 2)*,
- a schedule leaving a long interval (6 months) between second and third dose provides substantially higher antitoxin titers for the second year of life *(GRADE 1)*,
- a birth dose (in addition to a three-dose primary series) does not provide higher antitoxin titers *(GRADE 1)*,
- age of initiation of a 3-dose primary series does not substantially impact on resulting antitoxin titer levels *(GRADE 1)*,
- high vaccine effectiveness is achieved already with one dose for the period of age 0-2 years; and with two doses or more for the period of age 3-5 years *(GRADE 1)*,
- booster vaccination at 18 months of age yields slightly higher antitoxin concentrations than earlier boosting, but this difference does not translate into better protection *(GRADE 2)*
- booster vaccination during the second year of life after a 3-dose primary series substantially increases antitoxin titers *(GRADE 1)*.

Quality of evidence

Despite the availability of several studies on the review questions, the overall evidence available must be considered very low. The reasons for this include that fact that most studies were relatively small and that each question was addressed by only one or two studies; outcomes were indirect (studies mainly report mean titers per group); and that several biases must be suspected in studies.

Although several questions were addressed by RCT, the quality of evidence remains low, as most RCTs were small, did not report on randomization techniques and did not present group characteristics. RCT did not evaluate clinical efficacy and usually did not evaluate correlates of protection.

No cohort study presented detailed group characteristics or adjusted for potential confounding variables. Although confounding may be less serious in immunological studies in children in precise age groups, indication bias may have reduced study validity. A particular case are cohort studies using historical cohorts without reporting on group characteristics, where changes in recruitment strategy, hygiene or nutrition may have influenced results.

Correlates of protection

In this review, we assumed that there are correlates of protection against diphtheria (neutralization titers ≥0.1 U/ml) and tetanus (standardized ELISA ≥0.1 IU/ml or neutralization titers ≥0.01). This allowed grading evidence comparing 2-vs. 3- dose schedules higher than other pieces of evidence. However, expert opinion varies on the cut-offs and serological tests that constitute correlates of protection [WHO 2006 and WHO 2009], as this judgment is based on a limited number of case reports, while no large study evaluating the association between antibody titer and clinical protection is available.

Role of pre-existing antibodies

Although detailed information is not provided by any of the serological studies, DT coverage among the child study populations can be assumed high, resulting in low diphtheria circulation and little occasion for natural immunity at the time of the study. Less clear is in how far infants in the studies have benefitted from high maternal antibody following maternal immunization, as information on DT coverage among women at child-bearing age is not provided in any of the studies. For at least two not-per-protocol studies (Bandhari, Laurell), one must assume absence of maternal immunization. However, according to Dengrove et al., maternal antibody against tetanus did not impact on antitoxin concentrations at age 6 months, which was the timing of assessment in most included and additional studies.

Pre-immunization titers or seroprevalence were reported by six studies on primary immunization. Results tended to suggest low pre-existing immunity or immunity in part of the infant population, with a tendency to better protection against tetanus. In Booy et al. (UK 1990), at age 2 months, diphtheria GMT (ELISA) was 0.006 IU/ml and tetanus GMT (ELISA) 0.14 IU/ml. Similarly, in Conway et al. 1993 (UK early 90s), at age 3 months, 64% and 21% of preterm babies had antitoxin concentrations considered inadequately protective against diphtheria (<0.01 IU/ml) and tetanus (<0.1 IU/ml), respectively (both ELISA). In Li et al., (China, late 1990s?), anti-diphtheria GMT was 0.01 IU/ml and anti-tetanus GMT 0.02 IU/ml at age 2 or 3 months (both ELISA). In Mangay-Angara (Sweden 1970s), pre-immunization GMT against tetanus were 0.011 IU/ml (neutralization test). Highest pre-immunization titers were observed by Barkin (USA early 1980s), with 0.1 U/ml against diphtheria and 0.5-0.8 U/ml against tetanus (both neutralization test).

Further evidence needed – implications for further research:

This review suggests that there is some room for further research producing quality evidence to inform on optimized immunization schedules.

European experiences with a long (2+1) schedule suggest that this is a safe option for populations with low transmission of diphtheria and low risk of infection or appropriate care in case of exposure to tetanus.

Although little high quality evidence is available, the need for further research may be limited here. However, active surveillance or observational studies evaluating clinical protection against diphtheria during the first year of life with this 2-primary dose schedule may be appropriate, as re-emergence of diphtheria has been observed during the last decades. Also, it would be of principal value to better understand serological protection conferred by a 2+1 schedule; therefore, serological studies comparing schedules with close follow-up during the first 2 years of life, using the currently assumed correlate of protection and the appropriate serological tests for both diphtheria and tetanus would be of substantial benefit.

By contrast, evidence appears insufficient for a recommendation for countries with higher risk of transmission or infection and poorer access to care, to use a 2+1 schedule against diphtheria and tetanus, if the justification is to achieve longer persisting immunity with the same number of doses. It would be useful to determine in appropriate studies specifically in such settings, in how far two primary doses can provide clinical protection during the first year of life. As disease incidence may be very low among children <5 years due to the current 3-primary dose schedule, a demonstration project in a larger surveillance with active surveillance may be appropriate.

Furthermore, vaccine effectiveness of a 2+1 schedule among children with specific conditions, such as HIV infection or sickle cell anemia, should be evaluated in targeted studies comparing schedules.

Secondary to this, the clinical efficacy/effectiveness of a booster dose (3+1 vs. 3+0) requires further evaluation. Although it is known that DT antibodies wane after three primary doses, probably below protective levels, no booster is recommended in the EPI. While this has programmatic reasons and would be solved by a 2+1 schedule, it may be worthwhile to establish the principal effect of a booster dose. For serological studies, uncontrolled evaluations have shown the kinetics of antibody with booster, and the additional benefit of controlled serological studies may be limited.

For specific settings such as populations in great distress, the clinical protection provided to infants by only one primary DT dose may be worth evaluating.

Although no evidence is available on the relative schedule effect on reactogenicity, this point appears of minor relevance for further research, as DT vaccines usually are combined with pertussis antigens, for which the greater reactogenicity is established.

Implications for decision making

The results from this review suggest that the elements that will inform the decision between available DT immunization schedules (3+1 vs. 2+1, age at initiation 2 vs. 3 months) include mainly population-specific factors such as current disease risk by age group and the schedule of other antigens in the immunization program, rather than evidence on differences in immunogenicity. In this perspective, it must be kept in mind that information on differences in clinical protection may be relevant for decision making, but is currently scarce and mainly based on not clearly established correlates of protection.

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Figures and Tables

Table set 10. Data from included and additional studies evaluating **booster vaccination schedule impact** on relevant outcomes

Table 10a-A: Included studies on booster vaccination, schedule impact on effectiveness/efficacy – no studies identified

Tables 10a-B: Additional studies – Booster vaccination, schedule impact on effectiveness/efficacy – no studies identified

Table 10b-A: Included studies on booster vaccination, schedule impact on immunogenicity

Tables 10b-B: Additional studies – Booster vaccination, schedule impact on immunogenicity – no studies identified

Table 10c-A: Included studies on booster vaccination, schedule impact on reactogenicity – no studies identified

Tables 10c-B: Additional studies – Booster vaccination, schedule impact on reactogenicity – no studies identified

Table set 11. Data from included and additional studies evaluating **booster vaccination absolute impact** on relevant outcomes

Table 11a-A: Included studies on booster vaccination, absolute vaccine effectiveness/efficacy – no studies identified

Tables 11a-B: Additional studies – Booster vaccination, absolute vaccine effectiveness/efficacy – no studies identified

Table 11b-A: Included studies on booster vaccination, absolute immunogenicity

Tables 11b-B: Additional studies – Booster vaccination, absolute immunogenicity – no studies identified

Table 11c-A: Included studies on booster vaccination, absolute reactogenicity – no studies identified

Tables 11c-B: Additional studies – Booster vaccination, absolute reactogenicity – no studies identified

Appendix 1. Search terms

Appendix 2. Synopsis of inclusion of references from landmark reviews on diphtheria and tetanus vaccines

Figure 1: Flow chart of reference screening
* Includes secondary publication or synthesis of studies included in search

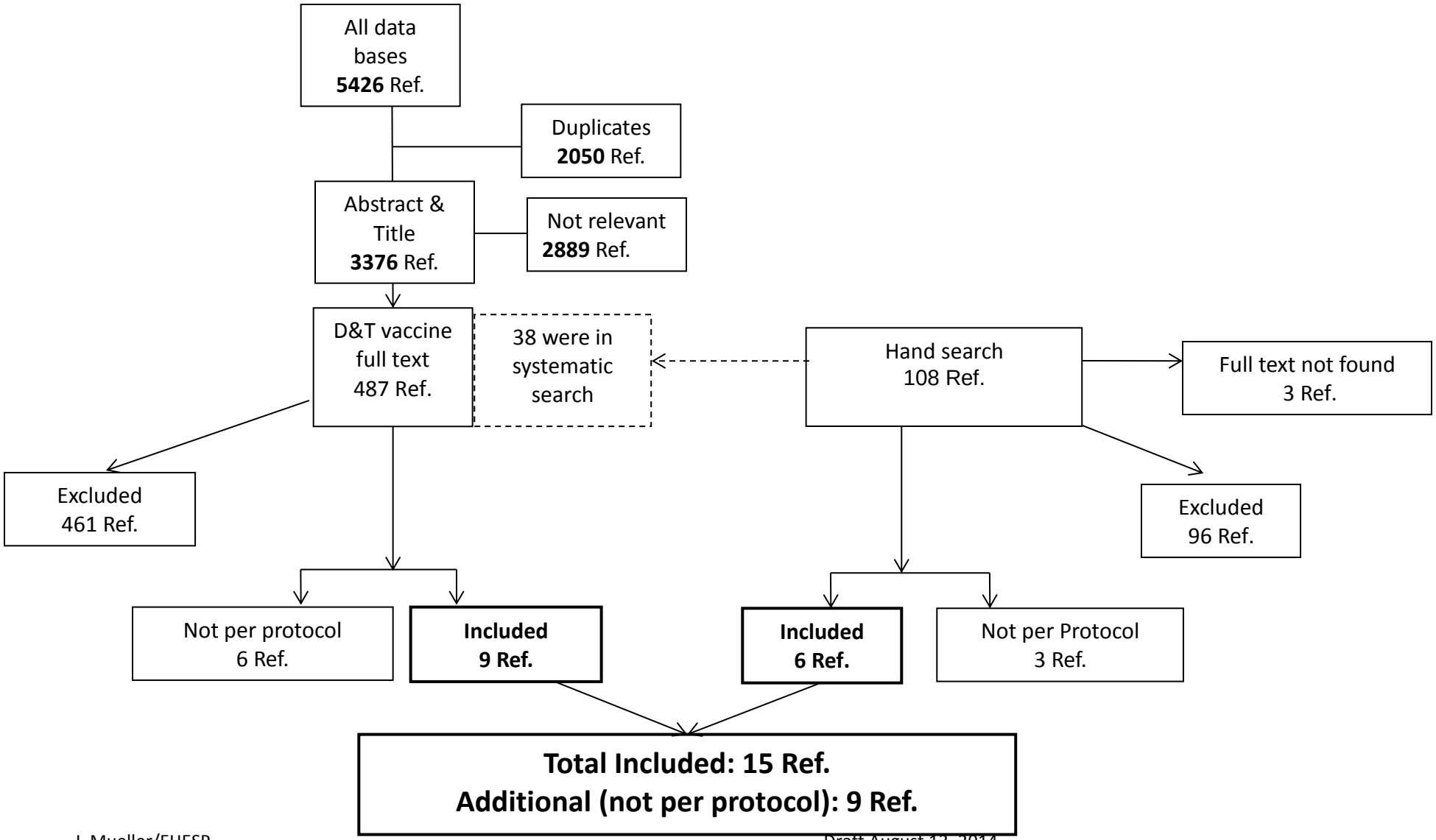
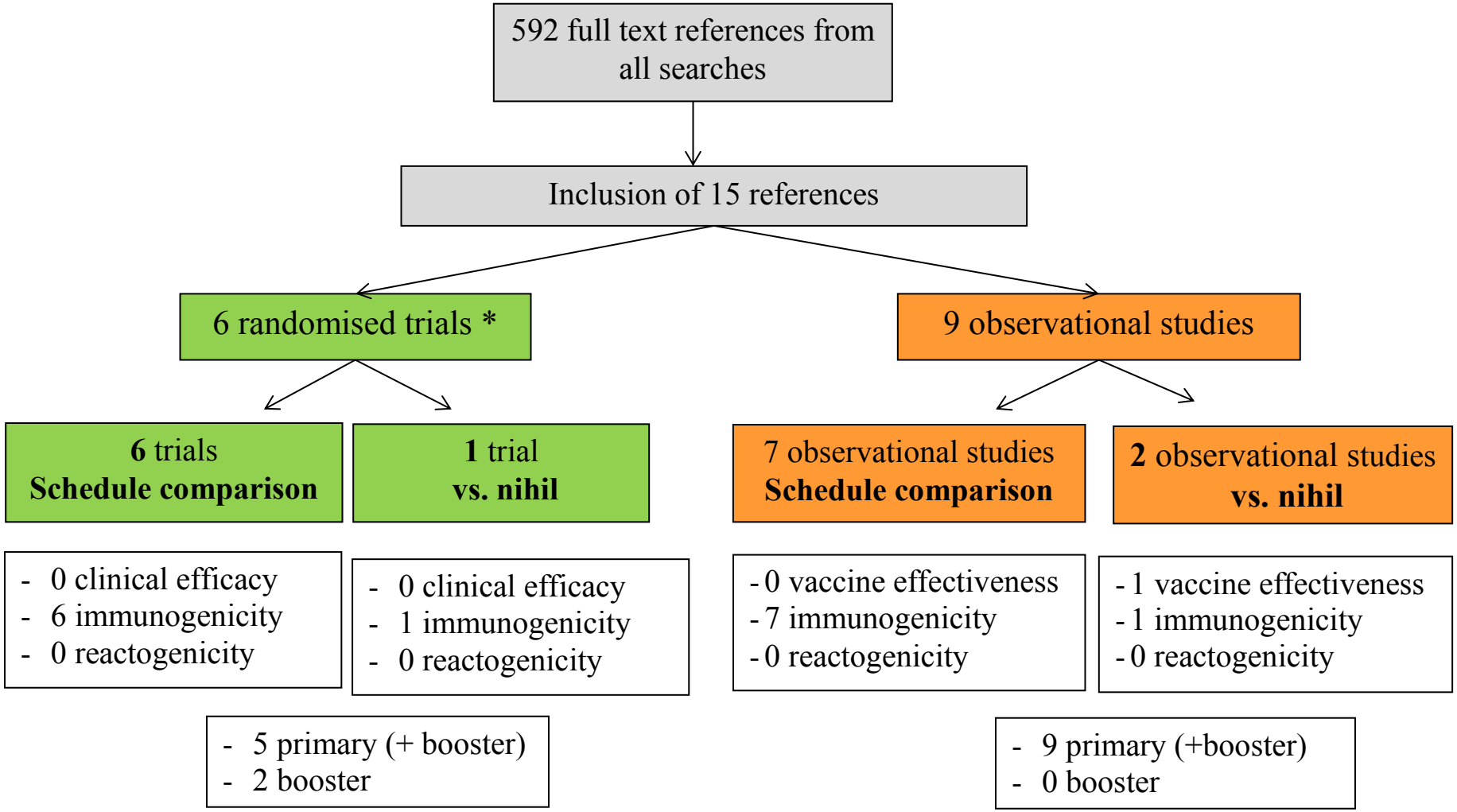


Figure 2. Overview of type of evidence available from included studies



Eff= Effectiveness, Immunogen = immunogenicity, Reactogen = Reactogenicity, VE = Vaccine effectiveness

*one trial studied schedule and vs. nihil comparison; two references concerned the same study

Table 1. Included studies on diphtheria and tetanus vaccination impact

Author	Antigen	Design	Primary/booster	Comparison	Schedule	VE	Immuno-gen.	Reacto-gen.
Barkin 1985. USA	DT	Cohort	primary	schedule (N doses)	2,6 vs. 2,4,6 mo		X	
Bisgard 2000. Russia	D	CC	primary primary+booster	vs. nihil (1-4 doses)	3 doses in ≥1 mo interval booster age 2 yrs	X		
Booy 1992. UK	DT	Cohort synopsis	primary	schedule	2,3,4 vs. 3,5,9 mo		X	
Carlsson 1998. Sweden	DT	RCT	primary primary+booster	schedule (N doses)	2,4,6+13 vs. 3,5+12 mo		X	
Conway 1993. UK	DT	RCT	primary 2+1	schedule	3,4,5 vs. 3,4,10 mo		X	
	DT	RCT	booster	vs. nihil	booster at age 18 mo		X	
Dengrove 1986. USA	T	Cohort	primary	schedule	birth + 2,4,6 mo		X	
Giammanco 1998. Italy	DT	Cohort	primary 2+1	schedule	2,4,6 vs. 3,5,11 mo		X	
Halasa 2008, USA	DT	RCT	primary primary+booster	schedule	birth + 2,4,6 + 17 mo		X	
Kimura 1991	DT	Cohort	primary	schedule	3 doses at 3-8 mo vs. 9-23 mo		X	
Li 2011 (I). China	DT	RCT	primary	schedule	2,3,4 vs. 3,4,5 mo		X	
Li 2011 (II). China	DT	RCT	primary	schedule	2,3,4 vs. 3,4,5 mo		X	
Mangay-Angara 1978. Philippines	T	CT	primary	vs. nihil	2 doses, int. 6 mo, from age 6-8 mo		X	
Scheifele 1999. Canada	DT	RCT	booster	schedule	12, 15 or 18 mo		X	
Swartz 2003. Israel	DT	Cohort	primary	schedule	2,4,6+12 vs. 2,3.5,10 mo		X	

Tiru 1999. Sweden	D	Cohort	primary	schedule (N doses)	2,4,6 vs. 3,5 mo		X	

VE, clinical vaccine efficacy/effectiveness; RCT, randomized clinical trial; CC, case control; D, diphtheria toxoid; T, tetanus toxoid

Table 2. Additional studies on diphtheria and tetanus vaccination impact, not per protocol

Author	Antigen	Design	Primary	Comparison	Schedule	VE	Immuno-gen.	Reacto-gen.
Bhandari 1981. India	DT	RCT	primary	schedule	2 vs. 3 doses		X	
Gyhrs 1999. Denmark	DT	Cohort	primary 2+1	schedule	3,5,12 vs. 5,6,15 mo		X	
Hoppenbrouwers 1998	DT	RCT	primary	schedule	2,4,6 vs. 3,4,5 mo		X	
Laurell 1957. Sweden	D	Cohort	primary	schedule	2 vs. 3 doses		X	
Miller 1997. UK	DT	RCT synopsis	primary	schedule	2,3,4 vs. 3,5,8-10 mo		X	
Ramsay 1993. UK	DT	Cohort synopsis	primary	schedule	2,3,4, vs. 3,4,10		X	
Taranger 2000. Sweden	DT	Cohort	primary primary+booster	schedule	2,4,6+15 vs. 3,5+12 mo		X	
Vahlquist 1949. Sweden	D	Cohort	primary	schedule	1 d at birth, 2-3 or 6-8 mo		X	
Wong 2008. Malaysia	DT	RCT	primary	schedule	1.5,3,5 vs. 3,4,5 mo		X	

VE, clinical vaccine efficacy/effectiveness RCT, randomized clinical trial CC, case control D, diphtheria toxoid T, tetanus toxoid

Table 3. GRADE evidence profile (included studies): primary DT vaccination, 2 vs. 3 primary doses

Quality assessment						Summary of finding	Final Grade: quality of evidence
Number of studies per design	**Limitations (risk of bias)**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Ratio (2 vs. 3 doses)** min – max	**1-4**
Clinical efficacy/effectiveness							
0 studies							
Immunogenicity Diphtheria							
Percentage ≥0.01 IU/ml							
@ 4-6 wks post primary							
1 RCT	Low	-	Moderate	Moderate	Unclear	1.00	1
@ 7 mo post primary							
1 RCT	Low	-	Moderate	Moderate	Unclear	0.93	2
1 Cohort (4 compar.)	Unclear or moderate	-	Moderate	Moderate	Unclear	0.88 – 0.92	1
@ 1 mo post booster (11/13 mo)							
1 RCT	Low	-	Moderate	Moderate	Unclear	1.00	1
1 Cohort	Moderate	-	Moderate	Moderate	Unclear	1.00	1
@ age 3 yrs (2 yrs post booster)							
1 Cohort	Moderate	-	Moderate	Moderate	Unclear	1.02	1
Percentage ≥0.1 IU/ml							
@ 4-6 wks post primary							
1 RCT	Low	-	Moderate	Moderate	Unclear	0.90	1
@ 7 mo post primary							
1 RCT	Low	-	Moderate	Moderate	Unclear	0.96	1
@ 1 mo post booster (11/13 mo)							
1 RCT	Low	-	Moderate	Moderate	Unclear	1.08	1
1 Cohort	Moderate	-	Moderate	Moderate	Unclear	0.92	1
@ age 3 yrs (2 yrs post booster)							
1 Cohort	Moderate	-	Moderate	Moderate	Unclear	0.65	1
GMC							
@ 4-8 wks post primary							
1 RCT	Low	-	High	Moderate	Unclear	0.78	1

1 Cohort	Unclear or moderate	-	High	Moderate	Unclear	0.57	1
@ 7 mo post primary							
1 RCT	Low	-	High	Moderate	Unclear	0.80	1
1 Cohort (4 compar.)	Unclear or moderate	-	High	Moderate	Unclear	0.38 - 0.60	1
@ 1 mo post booster (11/13 mo)							
1 RCT	Low	-	High	Moderate	Unclear	1.05	1
1 Cohort	Moderate	-	High	Moderate	Unclear	0.39	1
@ age 3 yrs (2 yrs post booster)							
1 Cohort	Moderate	-	High	Moderate	Unclear	1.00	1
Immunogenicity Tetanus							
Percentage ≥0.01 IU/ml							
@ 4-6 wks post primary							
1 RCT	Low	-	Moderate	Moderate	Unclear	1.00	1
@ 7 mo post primary							
1 RCT	Low	-	Moderate	Moderate	Unclear	0.97	1
@ 1 mo post booster (11/13 mo)							
1 RCT	Low	-	Moderate	Moderate	Unclear	1.00	1
1 Cohort	Moderate	-	Moderate	Moderate	Unclear	1.00	1
@ age 3 yrs (2 yrs post booster)							
1 Cohort	Moderate	-	Moderate	Moderate	Unclear	1.00	1
Percentage ≥0.1 IU/ml							
@ 4-6 wks post primary							
1 RCT	Low	-	Low *	Moderate	Unclear	1.00	2
@ 7 mo post primary							
1 RCT	Low	-	Low *	Moderate	Unclear	0.74	2
@ 1 mo post booster (12/13 mo)							
1 RCT	Low	-	Low *	Moderate	Unclear	1.01	2
1 Cohort	Moderate	-	Low *	Moderate	Unclear	1.00	1
@ age 3 yrs (2 yrs post booster)							
1 Cohort	Moderate	-	Low *	Moderate	Unclear	1.04	1
GMC							
@ 4-8 wks post primary							
1 RCT	Low	-	High	Moderate	Unclear	0.50	1
2 Cohorts	Unclear or moderate	High	High	Moderate	Unclear	In both groups, all above limit; 0.51	1
@ 7 mo post primary							
1 RCT	Low	-	High	Moderate	Unclear	0.60	1

@ 1 mo post booster (12/13 mo)							
1 RCT	Low	-	High	Moderate	Unclear	0.93	1
1 Cohort	Moderate	-	High	Moderate	Unclear	0.71	1
@ age 3 yrs (2 yrs post booster)							
1 Cohort	Moderate	-	High	Moderate	Unclear	1.19	1
Reactogenicity							
0 studies							

* Putatively protective antitoxin concentration (ELISA against tetanus): ≥0.1 IU/ml
RCT: Carlsson 1998 (ELISA)
Cohorts: Giammanco 1998 (neutralization assays); Barkin 1985 (animal neutralization assays; not per protocol for diphtheria); Tiru 2000 (ELISA), evaluating schedule on 4 different vaccines

Low = no serious issue; moderate = serious issue; high = very serious issue; Final GRADE: 1 = very low; 4 = high

Table 4. GRADE evidence profile (included studies): primary DT vaccination, birth dose vs. no birth dose

Quality assessment						Summary of finding	Final Grade: quality of evidence
Number of studies per design	**Limitations (risk of bias)**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Ratio (birth/no birth dose)** min – max	**1-4**
Clinical efficacy/effectiveness							
0 studies							
Immunogenicity Diphtheria							
GMC							
@ age 7 mo							
1 RCT	Unclear	-	High	High	Unclear	0.55	1
@ age 18 mo, post booster (17 mo)							
1 RCT	Unclear	-	High	High	Unclear	0.66	1
Immunogenicity Tetanus							
GMC							
@ age 6 mo							
1 RCT	Unclear and low	-	High	High	Unclear	0.83	1
@ age 7 mo							
1 RCT	Unclear	-	High	High	Unclear	1.13	1
@ age 9 mo							
1 RCT	Unclear and low	-	High	High	Unclear	0.70	1
@ age 18 mo, post booster (17 mo)							
1 RCT	Unclear	-	High	High	Unclear	1.15	1
Reactogenicity							
0 studies							

RCT: Halasa 2008, Dengrove 1986 (only tetanus reported)

Table 5. GRADE evidence profile (included studies): primary DT vaccination, accelerated vs. long schedule

Quality assessment						Summary of finding	Final Grade: quality of evidence
Number of studies per design	**Limitations (risk of bias)**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Ratio (accelerated vs. long)** min - max	**1-4**
Clinical efficacy/effectiveness							
0 studies							
Immunogenicity Diphtheria							
Percentage ≥0.01 IU/ml							
@ 1 mo post 3rd dose							
1 Cohort	Unclear and moderate	-	Moderate	Moderate	Unclear	1.00	1
GMC							
@ 1 mo post 3rd dose							
1 RCT	Unclear	-	High	High	Unclear	0.34 - 0.47	1
2 Observ. prospective	Moderate	-	High	Moderate	Unclear	0.11 – 0.55	1
@ age 19 mo							
1 RCT	Unclear	-	High	High	Unclear	0.23	1
Immunogenicity Tetanus							
Percentage ≥0.01 IU/ml							
@ 1 mo post 3rd dose							
1 Cohort	Unclear and moderate	-	Low *	Moderate	Unclear	1.00	1
GMC							
@ 1 mo post 3rd dose							
1 RCT	Unclear	-	High	High	Unclear	0.19 - 0.41	1
2 Observ. prospective	Moderate	-	High	Moderate	Unclear	In both groups, all above limit	1
@ age 19 mo							
1 RCT	Unclear	-	High	High	Unclear	0.51	1
Reactogenicity							
0 studies							

RCT: Conway 1993(3,4,5 mo vs. 3,4,10 mo); included two short-schedule groups

Observational prospective: Giammanco 1998 (2,4,6 mo vs. 3,5,11 mo; cohort) and Booy 1992 (2,3,4 mo vs. 3,5,9 mo; synopsis)

* Putatively protective antitoxin concentration (neturalization test against tetanus): ≥0.01 IU/ml

Table set 6. Characteristics and criticial appraisal of studies included per protocol

Barkin R.M., 1985

Methods	Site : USA, period not specified (early 1980s?) Design: Cohort study (or RCT?) Follow-up: up to age 18 mo (relevant data age 8 mo)
Participants	Included : Healthy infant population in private practices (N=143) Excluded: not specified
Interventions	**Primary DTaP series: 2 vs. 3 doses** Vaccines : DTwP (Connaught): Dt 6.7 Lf; Tt 5 Lf Dose schedule: Group 1: 2,6 mo (N=29) Group 2: 2,4,6 mo (N=39) *Not included : Other groups that received 3 doses of DTP and DT*
Outcomes	**Immunogenicity**: Timing of assessment: age 8 mo Serological assay: **neutralization tests for diphtheria (mice; not-per-protocol) and tetanus (rabbits)** - Mean (SEM) pre- and post-immunization **Reactogenicity:** Not taken into account here, as wP considered more reactogenic than DT Clinical effectiveness not assessed.

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection bias	Unclear risk	Few information on study participants and study population
Attrition bias	Low risk	Probably low drop-out
Performance bias	Unclear risk	Information not available on the blinding of participants
Confounding	Moderate risk	Indication bias possible No correction for possible confounding variables
Detection bias	Low risk	Serological testing done in blinded fashion
Selective reporting	Unclear risk	The protocol not provided. Authors include manufacturer

Author: Bisgard KM, 2000

Methods	Site: Russia, 1991-1992 Design: Case control study
Participants	Included: - Cases: Reported cases of diphtheria in persons between 6 months to 14 years, who live in Moscow and who had illness onset during 1991 or 1992. - Controls: Persons in the age group (±3months), geographically matched by clinics Excluded: Information not provided
Intervention	Routine schedule since 1980: Three doses of DT by the age of 12 months, and a fourth dose at the age of 2 years. Minimal age at first dose: 42 days, minimal interval between doses: 28 days
Outcomes	Clinical cases of diphtheria: Cases were diagnosed based on the clinician's assessment of the signs and symptoms of diphtheria; or isolation of *Corynebacterium diphtheriae*

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection bias (with regard to case and controls)	Moderate to high risk	Controls randomly chosen from exhaustive population list Matching for age and residency Other characteristics that are different between cases and controls mainly related to socio-economic status, could induce bias Indication bias possible
Missing data on exposure	Low risk	Only 11/ 904 children excluded for missing vaccination status
Performance bias	Unclear risk	No details reported
Exposure assessment bias	Moderate risk	Similar procedure of assessment for cases and controls: investigational form vs. clinical immunization records
Selective reporting	Unclear or low risk	Probably all results reported

Author: Booy R., 1992

Methods	Site: Oxford, UK 1988 and 1990 Design: cohort analysis of two studies using two different schedules Follow-up: 1 month after 3rd dose
Participants	Included: 210 infants (method of inclusion and exclusion not explained)
Intervention	**Primary Series: accelerated (2,3,4 mo) vs. long (3,5,9 mo) schedule**

	Vaccine: DTwP (Welcome). Dose schedule: Number of doses= 3 doses Dosing interval: 1 month vs. 2 and 4 months Number vaccinated with 3 doses: 103 (2,3,4 group), and 98 (3,5,9 group)
Outcomes	Immunogenicity: Time of assessment = 1 month after 3rd dose of primary schedule Serological assay: **ELISA (diphtheria and tetanus**). - Antibody concentrations as GMT (pre- and postimmunization) Reactogenicity was not taken into account, due to possible counteraction with the more reactogenic pertussis antigen in the vaccine.

Bias	**Reviewers' judgment**	**Support for judgment**
Indication bias	Moderate risk	The two groups were observed in interval of two years; not clear how inclusion into two studies was done
Attrition bias	Moderate risk	There was about 7.6% loss to follow up.
Performance bias	Unclear risk	There is no information on how participants and assessors were blinded, or interacted. The 3,5,9-mo group may have received extra diphtheria toxoid from the combined Hb OC conjugate vaccine, while the 2,3,4-mo group may also have received extra tetanus toxoid from the combined PRP-T component of the vaccine
Confounding	Moderate risk	No reporting of characteristics per group or adjustment
Detection bias	Low risk	Serology using standardized method
Selective reporting	Unclear	The protocol is not included in the article.

Author: Carlsson RM., 1998

Methods	Site: Sweden, 1994-1995 Design: RCT Follow-up: 6 weeks after booster (age 14 mo)
Participants	Included: Healthy full term infants of at least 2500 g weight (N=236) - Excluded: non reported
Intervention	**Primary and booster vaccination DTaP, comparing 2 to 3 primary doses, before and after a booster 7 mo later.** Vaccine: Pentavalent DTaP (with IPV, Hib): Pasteur Mérieux 2-component (PT, FHA) Group 1: 2,4,6,13-mo-schedule (N=118) Group 2: 3,5,12-mo-schedule (N=113)

Outcomes	**Immunogenicity:** Timing of assessment: 4-6 weeks post primary, 7 mo post primary, 4-6 weeks post booster dose Serological assay: **ELISA and neutralization test not further specified) for diphtheria, ELISA for tetanus;** - Geometric mean (IU/ml) (pre- and postimmunization) - proportion above cut-off 0.01 and 0.10 IU/ml (pre- and postimmunization) Reactogenicity: not taken into account, as DT in combination with aP, which is considered more reactogenic Clinical efficacy: not reported

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Low risk	Inclusion criteria as usual, no exclusion criteria states
Random sequence generation (selection bias)	Low risk	Randomization in blocks of 10
Allocation concealment (selection bias)	Unclear risk	No procedures described
Blinding of participants (performance bias)	Low risk	It was an open trial on immunogenicity
Blinding of outcome assessment (detection bias)	Low risk	Analyses done in blinded fashion
Selective reporting	Unclear or moderate risk	Protocol not disclosed; authors include manufacturer

Conway S., 1993

Methods	Site: UK 1986-7 Design: parallel group double-blind RCT Follow up: 15 months after dose 3 (age 21 months)
Participants	Included: **69 preterm infants:** <32 wks (N=32); 32-35 wks (N=37) Excluded: not specified Only 1572 participants from a larger efficacy trial participated in the immunogenicity study (children whose parents consented to the collection of capillary blood)
Interventions	**Primary DTwP series: third dose early after 1 mo or late after 6 mo** **Booster DTwP: effect of booster 18 mo vs. no booster** Vaccines : DTwP (not specified) Dose schedule: A: 3,4,5 mo (N=22) : 3 doses, interval 1-1 mo B: 3,4,10 mo (N=21) : 3 doses, interval 1-6 mo

	C: 3,4,5,18 mo (N=26) : 4 doses, interval 1-1-13 mo
Outcomes	**Immunogenicity** : Timing of assessment: 1 month after 3rd dose; at 19th mo of age; at 4-5 yrs of age Serological assay: **diphtheria and tetanus ELISA** (Conway 1987) - Mean antibody titers post-immunization (IU/ml) Clinical effectiveness and reactogenicity: no data presented

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Unclear risk	Not reported
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants (performance bias)	Unclear risk	Not reported
Blinding of outcome assessment (detection bias)	Unclear risk	Not reported

Author: Dengrove J., 1986

Methods	Site: USA, 1979 Design: RCT Follow-up: until 9 months of age
Participants	Included: full-term new born infants (N=91) Excluded: subjects with insufficient sera for assay
Intervention	**Primary series DTwP: birth dose (<4 days) or not** Comparison: 0,2,4,6 mo (N=14) vs. 2,4,6 mo (N=13) Dosing interval: 2 months Vaccine: DTwP (Wyeth)
Outcomes	**Immunogenicity:** Time of assessment: 6 and 9 months of age Serological assay: **ELISA for diphtheria and tetanus** - GMT, extracted from graph (pre- and postimmunization) - By IgG subclasses; by level of maternal antibody -

Reactogenicity and clinical efficacy not reported		
Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Moderate risk	The inclusion criteria was stated and followed, but all sera were only available for 30% of enrolled participants (not reason presented).
Random sequence generation (selection bias)	Unclear risk	Participants were randomly assigned to interventions, but the method was not reported.
Allocation concealment (selection bias)	Unclear risk	Method used not stated
Blinding of participants (performance bias)	Low risk	Unblended, but low risk in serological evaluation (unless drop-out related to group)
Blinding of outcome assessment (detection bias)	Unclear risk	Information not provided
Selective reporting	Unclear risk	Protocol not reported

Giammanco G., 1998

Methods:	Site : Italy, period not specified Design: Cohort study Follow-up: until one month after the 3rd dose
Participants	Included : Healthy infants weighing ≥2000g at birth (N=565) Excluded: contradiction to vaccination
Interventions	**Primary DTaP series: accelerated vs. long schedule** Vaccines : DTaP –HepB (SKB) Dose schedule: Group 1: 2,4,6 mo (N=208) Group 2: 3,5,11 mo (N=357)
Outcomes	**Immunogenicity**: Timing of assessment: one month after 3rd dose (Group 1: 7 mo; Group 2; 12 mo) and one month after 2nd dose (Group 2: 6 mo) Serological assay: **diphtheria and tetanus toxin neutralization test (IU/ml)** with reference serum. - GMT (95% CI) at 1 mo after third dose - against tetanus, all sera were above test upper limit Seropositive "rates": cut-off 0.01 IU/ml for both toxins **Reactogenicity:**

Not taken into account here, as aP considered more reactogenic than DT		
Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection bias	Unclear risk	Few information on study participants and study population
Attrition Bias	High risk	There was about 35% loss to follow-up.
Performance Bias	Unclear risk	Information not available on the blinding of participants and assessors, or the methods the participants were monitored.
Confounding	Moderate risk	Indication bias possible (but possibly less important in serological evaluation) No correction for possible confounding variables
Detection bias	Unclear risk	Not clear whether testing done in blinded fashion
Selective reporting	Unclear risk	The protocol not provided. Authors include manufacturer

Author: Halasa NB., 2008

Methods	Site: USA, period not specified Design: RCT Follow-up: until 18 months of age
Participants	Included: healthy full term infants between 2-14 days of age (N=50) Excluded: moved from study area.
Intervention	**Primary and booster series of DTaP: birth dose vs. no birth dose** Vaccines : DTaP (Daptacel, Sanofi Pasteur), with IPV, Hib, HepB, PCV Dose schedule: Group 1: 0,2,4,6 + 17 mo (N=22) Group 2: 2,4,6 + 17 mo (N=20)
Outcomes	**Immunogenicity**: Timing of assessment: age 7 and 18 mo (one month after dose 2/3 or 3/4) Serological assay: **ELISA for diphtheria and tetanus** - GMC post-immunization - Percentage ≥0.01 IU/ml (age 7 mo) and ≥0.1 IU/ml (age 18 mo) Reactogenicity and clinical effectiveness not reported.

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Unclear or moderate risk	Study population not further specified

Random sequence generation (selection bias)	Unclear risk	Methods of sequence generation not specified.
Allocation concealment (selection bias)	Unclear risk	Procedure not specified
Blinding of participants (performance bias)	Moderate risk	Probably no blinding of parents, moderate risk (serology)
Blinding of outcome assessment (detection bias)	Unclear risk	Not clear whether serological analyses were blinded
Selective reporting	Unclear risk	Protocol not reported

Author: Kimura M., 1991

Methods	Site: Japan Design: Cohort, follow-up until one month post booster (age 16-46 mo)
Participants	Included: Infants aged 3-30 months Excluded: not reported
Intervention	**Primary series DTaP: 3 doses initiated before or after age 9 mo** Schedule: initiation at 3-8 months (N=182) vs. between 9-23 months (N=92); interval 6-10 weeks; booster at 12-18 mo post primary Vaccine: DTaP (Takeda)

Outcomes	Immunogenicity:
	- Timing of assessment: after 3rd dose
	- Serology assay:
	  o Diphtheria : microcell culture method using VERO cells
	  o Tetanus passive hemagglutination using tetanus toxoid-coated sheep erythrocytes
	- antibody reported in GMT (IU/ml) (pre-and post-immunization)

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection bias	Unclear risk	Few information on study participants and study population
Attrition Bias	High risk	There was about 25% loss to follow-up.
Performance Bias	Unclear risk	Information not available on the blinding of participants and assessors, or the methods the participants were monitored.
Confounding	Moderate risk	Indication bias likely No correction for possible confounding variables
Detection bias	Unclear risk	Not clear whether testing done in blinded fashion
Selective reporting	Unclear risk	The protocol not provided.

Li R.C., 2011 (I)

Methods	Site: China time not specified Design: RCT (no details on randomization or blinding) Follow-up: 1 month post third dose (9% and 3% drop-out)
Participants	Included: healthy infants aged 60-74 days, full-term Excluded: immunodeficiency/suppression, history of seizures, bleeding disorder, fever on day of inclusion…
Interventions	**Primary DTaP series: 3,4,5 mo vs. 2,3,4 mo** Vaccines : **Pentavalent DTaP (with IPV, Hib): Sanofi Pasteur 2-component (PT, FHA)** Dose schedule 1. Group 1: 3,4,5-mo-schedule (N=263) 2. Group 2: 2,3,4-mo-schedule (N=263)
Outcomes	**Immunogenicity** : Timing of assessment: 1 month post 3rd dose (age 6 and 5 mo, respectively) Serological assay: **ELISA against diphtheria and tetanus** - Cut-off ≥0.01 IU/ml for both antigens - Geometric mean titers (GMT) pre- and post-immunization **Reactogenicity:** not taken into account here, as aP considered more reactogenic Clinical effectiveness: no data reported

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Low risk	Usual exclusion criteria, but no details on study population
Random sequence generation (selection bias)	Unclear risk	No details reported
Allocation concealment (selection bias)	Unclear risk	No details reported
Blinding of participants (performance bias)	Low / high risk	Unblinded trial : low risk for serology
Blinding of outcome assessment (detection bias)	Unclear risk	Unblinded; potentially a problem for serology
Selective reporting	Unclear risk	Protocol not reported. Authors include vaccine manufacturer, but trial registration

Li R.C., 2011 (II)

Methods	Site: China time not specified Design: RCT (no details on randomization or blinding) Follow-up: 1 month post booster dose (9% and 3% drop-out)
Participants	Included: participants of previous trial (Li 2011, I) (N=719, 98.3%) Excluded: compliance with booster protocol
Interventions	**Booster dose DTaP at 18-20 mo, after primary series: 3,4,5 mo vs. 2,3,4 mo** Vaccines : **Pentavalent DTaP (with IPV, Hib): Sanofi Pasteur 2-component (PT, FHA)** Dose schedule 1. Group 1: 3,4,5-mo-schedule (N=251) 2. Group 2: 2,3,4-mo-schedule (N=233)
Outcomes	**Immunogenicity** : Timing of assessment: 1 month post booster dose (age 19-21mo) Serological assay: **ELISA against diphtheria and tetanus** - Cut-off ≥0.01 IU/ml for both antigens - Geometric mean titers (GMT) pre- and post-immunization **Reactogenicity:** not taken into account here, as aP considered more reactogenic Clinical effectiveness: no data reported

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Low risk	Usual exclusion criteria, but no details on study population
Random sequence generation (selection bias)	Unclear risk	No details reported
Allocation concealment (selection bias)	Unclear risk	No details reported
Blinding of participants (performance bias)	Unclear risk	No details reported (possibly low risk for serology)
Blinding of outcome assessment (detection bias)	Unclear risk	No details reported
Selective reporting	Unclear risk	Protocol not reported. Authors include vaccine manufacturer, but trial registration

Mangay-Angara A., 1978

Methods	Site : Philippines, 1970s Design: Clinical trial, participants were "allotted" to groups Follow-up: until one month after the 3rd dose
Participants	Included : Healthy infants 3-8 months of age with no symptoms of intestinal disease, respiratory infection or fever (N=522) Excluded: not specified
Interventions	**Primary series: 2 doses DTwP vs. D** Vaccines : DTwP (adsorbed; N=177) vs. D (N=174) (Alabang Institute, Manila) Dose schedule: 2 doses in 6-mo interval, starting 3-8 months A third group was vaccinated with a Dutch DTwP vaccine.
Outcomes	**Immunogenicity**: Timing of assessment: 40 days after 2nd dose Serological assay: **neutralization test for tetanus** - GMT at 40 days post 2nd dose - Tetanus GMT pre-immunization Reactogenicity: not taken into account, as wP was considered more reactogenic Clinical efficacy not evaluated.

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Low risk	Usual exclusion criteria, but no details on study population
Random sequence generation (selection bias)	Unclear risk	No details reported
Allocation concealment (selection bias)	Unclear risk	No details reported
Blinding of participants (performance bias)	Unclear risk	No details reported (possibly low risk for serology)
Blinding of outcome assessment (detection bias)	Unclear risk	No details reported
Selective reporting	Unclear risk	Protocol not available.

Scheifele D.W., 1999

Methods	Site : Canada, 1990 Design: RCT Follow-up: until 4-6 weeks after booster
Participants	Included : healthy participants of a previous trial on DPT-IPV-PRP-T vaccine (N=257) Excluded: contradiction, laboratory-confirmed target infection, blood products, immune impairment
Interventions	**Booster immunization: DTwP at differents ages** Vaccines : DTwP (PENTA™, Pasteur Mérieux) Booster at 12, 15 or 18 mo after a primary series finished before age 7 mo
Outcomes	**Immunogenicity** : Timing of assessment: 4-6 wks after booster Serological assay: diphtheria and tetanus antitoxin concentration measurements by **EIA** - GMC (95%-CI) at pre- and post-booster - Prevalence antitoxin ≥0.1 IU/ml Reactogenicity: not taken into account, as wP was considered more reactogenic. Clinical efficacy not evaluated.

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Low risk	Usual inclusion and exclusion criteria
Random sequence generation (selection bias)	Low risk	Details reported, quality procedures
Allocation concealment (selection bias)	Low risk	Details reported, quality procedures
Blinding of participants (performance bias)	Low risk	Serological evaluation, probably low risk
Blinding of outcome assessment (detection bias)	Unclear risk	Not clear whether serological assessment blinded
Selective reporting	Unclear risk	Protocol not available. Manufacturers among authors

Swartz TA., 1985

Methods	Site: Israel, 1981 Design: comparison of two infant cohorts (not clear how recruited) ; follow-up until age 8 years No report or control for confounders

Participants	Included: 2+1 group from a cohort of 7000 hildren in two health district in central Israel; serum samples only from "Jewish segment" of cohort; 3+1 group was a random sample from 700 children participating in a nutritional survey in Jerusalem (same age group and "population segment"). Total included N=548, reports only on N=132
Exposure	**Primary series DTwP 2+1 vs. 3+1 schedule** **Vaccine:** DTwP adsorbed (Mérieux) Dose schedules: Group 1: 2,3.5,10 mo (N=60) Group 2: 2,4,6,12 mo (N=72)
Outcomes	**Immunogenicity** : Timing of assessment: 1 mo post booster (age 11 or 13 mo), 3 yrs; age 6 year and 8 years (after dT booster) not taken into account Serological assay: **ELISA** and RIA (Pasteur-Mérieux in house) **against diphtheria and tetanus** - Proportion with agglutinin titer <0.01, 0.01-0.099 and ≥0.1 - GMT post-vaccination (IU/ml) Clinical effectiveness and reactogenicity: no data presented

Bias	**Reviewers' judgment**	**Support for judgment**
Selection bias	Moderate risk	Cohorts may differ, no control for confounding variables
Attrition bias	Moderate risk	High drop-out, unclear whether differential between groups
Performance bias	Low risk	No particular event reported
Detection bias	Low risk	Blinded testing
Selective reporting	Unclear or moderate risk	Protocol not available; manufacturer among authors

Tiru et al., 1999

Methods	Site: Sweden, 1993-1994 Design: Cohort analysis of trial groups Follow-up: until 19 months
Participants	Included: Children aged 2 months, not vaccinated Excluded: Not stated
Intervention	**Primary DTaP and DTwP series: 2+1 vs. 3+0 schedule** Vaccines : **DT in different concentrations, depending on aP or wP content (ranging from 15 to 32 Lf); but comparable distribution of vaccine types between schedule groups** Dose schedule:

	- Group 1: 3,5,12-mo-schedule (N=531) - Group 2: 2,4,6-mo-schedule (N=306) -
Outcomes	Timing of assessment: at 1 and 7 months after 3rd dose; **but only at age 7 mo was reported** Serological assay: **ELISA against diphtheria** - % with cut-off ≥0.10 IU/ml post-immunization - Median titer post-immunization

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection bias	Unclear risk	Inclusion and exclusion criteria no specified
Attrition Bias	Unclear risk	Information was not provided, but indicated that only participants with pre vaccination samples were analyzed.
Performance Bias	Unclear risk	No details reported
Detection bias	Unclear risk	Unclear whether serological assays were done in blinded fashion
Confounding	Moderate risk	No information on group characteristics or adjustement
Selective reporting	Unclear risk	Protocol not reported

Table set 7. Characteristics and criticial appraisal of additional not-per-protocol studies

Author: Bhandari, 1981

Methods	Site: India Design: RCT Follow up: 1 month after the last dose for the different groups.
Participants	Included: Healthy full-term infants, (Ages 3months – 5 years) Excluded: Not defined
Intervention	**Primary series: comparison between 2 doses vs. 3 doses** Vaccine: DTP vaccine (central research Institute Kasauli; 15 Lf anti-D, and 5Lf anti-T) Dose schedule: Not clear (only stated 2 doses vs. 3 doses). Group 1=2 doses (N=100), group 2= 3 doses (N=100)
Outcomes	**Immunogenicity:** Timing of assessment**:** 1 month after the last dose for the different groups. Serological assay: **Passive hemagglutination method** – converted to international Unit (IU) by comparing with standard anti toxin in each set. - Cut-off 0.1 IU/ml for both antigens Reactogenicity and clinical efficacy: not reported

Bias	**Reviewers' judgment**	**Support for judgment**
Inclusion bias	High Risk	The age gap of the study population was high, the older age group is more likely to have higher antibody based on acquired immunity.
Random sequence generation (selection bias)	Low risk	Random sampling table was used
Allocation concealment (Selection bias)	Unclear risk	Information not reported
Blinding of participant (Performance bias)	Unclear risk	Information not reported
Blinding of outcome (detection bias)	Unclear risk	Protocol not reported
Selective reporting	Unclear risk	Protocol not available

Author: Gyhrs A., 1999

Methods	Site : Denmark (general practitioners in Copenhagen), 1993-96 Design: Cohort study Follow-up: until age 24 months

Participants	Included: Healthy children with gestational age of ≥37 weeks, and birth weight of ≥2000g at birth. Enrolled at the age of 5 weeks (N=270) Excluded: Not indicated
Interventions	**Primary DTaP series: early vs. late long schedule** Vaccines : Group 1: DTaP-IPV Vaccine (Statens Serum Institute), Group 2: DT-IPV + wP (Statens Serum Institute); **the latter contained 2-fold amount of DT toxoids** Dose schedule: Group 1: 3,5,12 mo (N=186) Group 2: 5,6,15 months (N=84)
Outcomes	**Immunogenicity**: Timing of assessment: one month after 2nd and 3rd doses, and at age 24 months Serological assay: diphtheria and tetanus **toxin fluorescence immunoassay (DELFIA**) - GMT (95% CI - Percentage ≥0.01 IU/ml and ≥0.1 IU/ml Reactogenicity: not taken into account, as aP considered more reactogenic Clinical effectiveness not reported.

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection Bias \	Unclears or low risk	Probably representative of Copenhagen pediatric practices (35 study sites)
Attrition Bias	Moderate risk	High loss to follow up, greater in group 2 (42%) than in group 1 (31%)
Performance Bias	Unclear or low risk	No particular event reported
Detection bias	Unclear risk	Not clear whether serological analyses blinded.
Confounding	Moderate risk	Indication bias, as parents influenced allocation. No detailed description of group characteristics or adjustment reported. Possibly only moderate risk of bias for serological outcome.
Selective reporting	Unclear risk	The protocols for the study was not included

Hoppenbrouwers K., 1999

Methods	Site: Belgium, Turkey, 1990s Design: parallel group open RCT Follow-up: up to one month after third dose of primary vaccination (booster not evaluated between schedules)

Participants	Included: 410 healthy unvaccinated children < 2 months-old in three study groups (only two included in this report) Excluded: no details provided Loss to follow-up after complete vaccination : 7.5% in Belgium, 49.2% in Turkey
Intervention	**Primary series DTaP, comparing short to longer schedule (3 doses)** Vaccine : DTaP (Pasteur Mérieux) Dose schedule: Group 1: 3,4,5 mo (N=135) Group 2: 2,4,6 mo (N=137)
Outcomes	**Immunogenicity :** Timing of assessment: **1 month post-third dose**; high pre-vaccination maternal antibody concentration => not reported Serological assay: **RIA for diphtheria and tetanus** - Percentage seroconverted after three doses (≥0.01 and ≥0.1 IU/ml) - GMT , total and by country **Reactogenicity**: not taken into account here, as aP considered more reactogenic Clinical efficacy not reported.

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Inclusion bias	Low risk	No unusual exclusion criteria
Random sequence generation (selection bias)	Low risk	Randomization list
Allocation concealment (selection bias)	Low risk	Randomization list, no further detail
Blinding of participants (performance bias)	Low risk	Non-blinded => low risk for immunogenicity evaluation high drop-out in Turkey => potential selection for better tolerance?
Blinding of outcome assessment (detection bias)	Low risk	Serology testing was blinded
Selective reporting	Moderate risk	Protocol not available, manufacturer among authors

Laurell G., 1957

Methods	Site: Sweden 1953-56 and following Design: cohort study, vaccine assignment not explained
Participants	Included: 325 children, aged <0.5 year Inclusion and exclusion criteria not detailed
Exposure	**Primary series: DTwP vaccination in 3 doses compared to 2 doses** Vaccine schedule: Not specified, but in a 6-weekly interval, ended at about 6 months. Vaccine not specified. Number of doses: Group 1 (3 doses): 103 children, group 2 (no 2 doses): 41 children
Outcomes	Immunogenicity : - Method of Assay: **intradermal -performed test (rabbit) against diphtheria** - Timing of Assay 3-6 weeks after last dose - Immunogenicity median titer extracted from graph Reactogenicity or clinical effectiveness not assessed.

Bias	**Reviewers' judgment**	**Support for judgment**
Selection bias	Unclear or low risk	No details provided, apart from relatively good accordance of age and living conditions between groups
Attrition bias	Low risk	High follow-up
Performance bias	Unclear risk	No details reported; 24% of vaccinees received 4th dose
Detection bias	High risk	Not clear whether testing done in blinded fashion.
Selective reporting	Low risk	Probably all results reported

Miller E., 1997

Methods	Site: UK, 1988-94 Design: Synopsis of two parallel group double-blind RCT evaluating wP vs. aP, each using two different schedules The two trials are presented as using an identical protocol
Participants	Included: Children attending clinics for primary vaccination, partents accepting randomization to wP or aP (2 vaccine types can be evaluated for schedule impact) Excluded: history of pertussis, neurological disorder or serious chronic disease 4.2% drop-out
Interventions	**DTaP accelerated vs. long schedule** Vaccines : DTaP, **four different products with different D and T toxoid contents** Dose schedule: Group 1: **2,3,4 mo** (N=94 and 74 for vaccines 1 and 2)

	Group 2: **3,5,8-10 mo** (N=88 and 89 for vaccines 1 and 2)
Outcomes	**Immunogenicity** : Timing of assessment: **the two groups were not assessed at the same age:** School age 4-5 yrs (3,5,8-10 mo) and age 12-18 mo (12-18 mo) Serological assay: **ELISA against diphtheria and tetanus toxin** - GMT (95% CI) post-vaccination - Prevalence of detectable antibody (0.1 IU/ml for diphtheria, 0.01 IU/ml for tetanus) Reactogenicity: not taken into account as aP considered more reactogenic. Clinical effectiveness: no data presented

Bias	**Reviewers' judgment**	**Support for judgment**
Selection bias	Unclear or moderate risk	Probability or factors deciding whether to be included into one or the other trial not reported; bias if this probability is differential between schedules
Attrition bias	Moderate risk	4.2%, similar in both trials Follow-up serology at 12-18 mo in <50%, reason for loss not specified
Performance bias	Low or unclear risk	No event reported
Detection bias	Low risk High risk	Immunogenicity evaluation Larger intervals could have impacted reporting probability of reactions
Selective reporting	Low risk	Large range of outcomes presented
Age groups not comparable (antibody waning)		

Ramsay M.A.B., 1993 (II)

Methods	Site: UK, 1988 – 1990 Design: Synopsis of three studies: 1. cohort 1989-90 in Colchester: 2,3,4 mo (N=57); follow-up 6-8 weeks post third dose (46% drop-out for pertussis serology) 2. cohort 1989-91 in three districts with delayed schedules change: 3, 4.5-5, 8.5-11 mo (N=50); follow-up 6-8 weeks post third dose (16% drop-out for pertussis serology) 3. cohort 1988-89 in North Hertfordshire : 3, 4.5-5, 8.5-11 mo (N=32); follow-up 12 months post third dose; the timing of assessment is delayed compared to the other cohorts (18 vs. 11 months), thus this cohort was not included in the review
Participants	Included: Children schedules for primary vaccination, attending general practices or clinics Excluded: not specified

Interventions	**DTwP accelerated vs. long schedule** Vaccines : DTwP (Wellcome) Dose schedule: Group 1: **2,3,4 mo** (N=31) Group 2: **3, 4.5-5, 8.5-11 mo** (N=42)
Outcomes	**Immunogenicity** : Timing of assessment: 12+ months after 3rd dose (mean age 20 and 22 mo in two groups) And 6-8 weeks after third dose (accelerated schedule only) Serological assay: **diphtheria and tetanus (solid phase RIA)** ("good correlation with ELISA") - GMC (95% CI) post-vaccination Clinical effectiveness and reactogenicity: no data presented

Bias	**Reviewers' judgment**	**Support for judgment**
Selection bias	Unclear risk	Risk could be low as far as assignment followed national recommendations, not individual choice; but no data presented
Attrition bias	Moderate risk	Considerable drop-out, particularly in group 1; differential impact on serology may be small
Performance bias	Unclear risk	No event reported
Detection bias	Low risk	Immunogenicity evaluation
Selective reporting	Moderate risk	Secondary evaluation of existing data

Taranger J., 2000

Methods	Site: Sweden, 1992-1997 Design: Cohort study Follow-up: until age 48 mo
Participants	Inclusion: criteria not indicated, recruitment in child health centers in six districts Exclusion: health problems, loss to follow-up, pertussis infection
Intervention	**Primary series of DTaP with booster: 2+1 vs. 3+1 schedule** Vaccines : DTaP (Statens Serum Institute) with IPV, Hib; **different toxoid content between groups** **Dose schedule**: Group 1: 3,5+12-mo-schedule (N=103); 25 Lf DT and 7 Lf TT Group 2: 2,4,6+15-mo-schedule (N=118); 15 Lf DT and 6 Lf TT

Outcomes	**Immunogenicity** : Timing of assessment: 1 mo post last primary, at booster, 1 mo post booster and at 48+ mo Serological assay: Vero cell assay (diphtheria) and ELISA (tetanus) - Cut-off ≥0.01 IU/ml and ≥0.10 IU/ml for both antigens - Geometric mean titers (GMT) pre- and post-immunization Reactogenicity: not taken into account here, as aP considered more reactogenic Clinical effectiveness not reported

Risk of Bias	**Reviewer judgment**	**Support for judgment**
Selection bias	Unclear risk	No details about study population and sample
Attrition bias	Low risk	Only about 1.8% loss to follow-up
Confounding	Moderate risk	The two groups were enrolled in two groups of three districts each. No comparison of characteristics (except for child's sex) between groups or adjustment reported.
Performance bias	Unclear risk	No details reported
Detection bias	Unclear risk	Unclear whether serology done in blinded fashion
Selective reporting	Unclear risk	Protocol was not available

Author: Vahlquist B., 1949

Methods	Site : Sweden, 1940s Design: Cohort study (assignment not specified) Follow-up: until 6 months after immunization
Participants	Included: not specified (N=45): newborns, age 2-3 mo, age 6-8 mo Excluded: Not indicated
Interventions	**Primary dose D, different ages** Vaccines : **aluminium-precipitated standard diphtheria toxoid** Dose schedule: Group 1: at birth (N=15) Group 2: age 2-3 mo (N=15) Group 3: age 6-8 mo (N=15)
Outcomes	**Immunogenicity**: Timing of assessment: pre-immunization, and 1, 3, and 6 mo post immunization Serological assay: not specified - Mean antitoxin level (U/ml)

	- Percentage <0.0005, 0.0005-<0.02 and ≥ 0.02U/ml Clinical effectiveness and reactogenicity not reported.

Risk of Bias	Reviewer judgment	Support for judgment
Selection Bias \	Unclear risk	No details reported
Attrition Bias	Unclear risk	No details reported
Performance Bias	Unclear risk	No details reported
Detection bias	Unclear risk	Not clear whether serological analyses blinded.
Confounding	Moderate risk	No detailed description of group characteristics or adjustment reported. Possibly only moderate risk of bias for serological outcome.
Selective reporting	Unclear risk	The protocol for the study was not included

Wong SL., 2008

Methods	Site: Malaysia 2000-2002 Design: unblinded RCT Follow-up: until age 12 months
Participants	Included: healthy term infants in two not further specified study centers Excluded: immunosuppressive disorder or congenital defect; previous disease or vaccination against study diseases, allergy against vaccines…
Intervention	**Primary series: 3 doses starting age 1.5 mo vs 3 mo** Schedule: Group 1: 1.5,3,5 mo vs. Group 2: 3,4,5 mo Vaccines: Group 1: DTwP-HepB (Tritanrix-HepB, GSK) mixed with Hib; Group 2: DTwP (D.T.COQ, Pasteur Mérieux Connaught) with HepB and Hib in separate injections
Outcomes	Immunogenicity: - Timing of assessment: age 6 and 12 months (1 and 7 months after last dose) - Serological assay: diphtheria and tetanus ELISA with cut-off set at 0.1 IU/ml - GMC and % of participants above cut-off (0.1 IU/ml) - Diphtheria at age 12 months: testing with Vero cells (cut-off 0.016 IU/ml) if ELISA negative

Risk of Bias	Reviewer judgment	Support for judgment
Inclusion bias	Low risk	Usual inclusion/exclusion criteria
Random sequence generation (selection bias)	Unclear risk	The method of sequence generation was not indicated.
Allocation concealment (selection bias)	Unclear risk	Method is not disclosed
Blinding of participants (performance bias)	moderate risk	Non-blinded trial; immunogenicity outcome only at moderate risk of bias
Blinding of outcome assessment (detection bias)	Unclear risk	Not mentioned whether blinded serology
Selective reporting	Unclear or moderate risk	Protocol not disclosed; authors include manufacturer

Table set 8. Data from included and additional studies evaluating primary vaccination schedule impact on relevant outcomes

Table 8b-A: Included studies on primary vaccination, schedule impact on immunogenicity

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria mean antibody titer (SD) (IU/ml)	Tetanus mean antibody titer (SD) (IU/ml)
Accelerated vs. long schedule						
Conway 1993 UK	RCT Unclear risk Preterm babies 32-35 gestational week	A/B: 3,4,5 mo C: 3,4,10 mo *B: see booster*	One month after 3rd dose	A/B: last interval 1 mo (N=11 and 15) C: last interval 6 mo (N=11)	3.17 (2.1) / 4.36 (5.22) 9.2 (5.48)	1.49 (0.7) / 3.26 (3.01) 8.01 (6.75)
			Age 19 mo	A: last interval 1 mo (N=6) C: last interval 6 mo (N=10)	0.67 (0.64) 2.86 (3.91)	0.52 (0.37) 1.02 (0.91)

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria		Tetanus	
Accelerated vs. long schedule					**GMC (IU/ml)**			
Booy 1992 UK	Synopsis of two studies, Moderate risk	2,3,4 vs. 3,5,9 mo	1 month after 3rd dose	Group 1: 2,3,4 mo (N=103) Group 2: 3,5,9 mo (N = 98)	3.87 7.00		0.70 1.90	
					GMT (95% CI) (IU/ml)	**% ≥0.01 IU/ml**	**GMT (IU/ml)**	**% ≥0.01 IU/ml**
Giammanco 1998 Italy	Cohort study Unclear or moderate risk	2,4,6 vs. 3,5,11 mo	1 month after 3rd dose	Group 1: 2,4,6 mo (N=172) Group 2: 3,5,11 mo (N=196)	0.188 (0.159-0.222) 1.712 (0.509- 1.942)	100 100	All sera above upper test limit	100 100

NB: Neutralization test for both diphtheria and tetanus (cells not specified)								
3 vs. 2 primary doses								
Giammanco 1998 Italy			Age 7 or 6 mo	Group 1: 2,4,6 mo (N=172) Group 2: 3,5 mo (N=196)	0.188 (0.159-0.222) 0.108 (0.092–0.126)	-	All sera above upper test limit	-
NB: Neutralization test for both diphtheria and tetanus (cells not specified)								

Publication and country	**Design Risk of bias**	**Schedules evaluated**	**Timing of assessment**	**Comparison groups**	**Diphtheria (IU/ml)**			**Tetanus (IU/ml)**		
3 vs. 2 primary doses					**GMC**	**% ≥0.01**	**% ≥0.10**	**GMC**	**% ≥0.01**	**% ≥0.10**
Carlsson, 1998 USA	Open RCT Low to moderate risk	2,4,6,13 mo vs. 3,5,12 mo	4-6 weeks after primary vaccination	Group 1: 2,4,6 mo (N = 116) Group 2: 3,5 mo (N=111)	0.41 0.32	100 100	96.6 87.4	1.21 0.60	100 100	96.1 96.4
			7 months after primary vaccination	Group 1: 2,4,6 mo (N = 111) Group 2: 3,5 mo (N=108)	0.05 0.04	96.4 89.9	27.9 26.9	0.20 0.12	97.3 94.4	80.4 59.3
			4-6 weeks after booster	Group 1: 2,4,6,13 mo (N=114) Group 2: 3,5,12 mo (N=109)	1.19 1.25	100 100	58.8 63.3	4.67 4.36	100 100	96.3 97.3

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria	
2 vs. 3 doses					**Median concentration (EU/ml)**	**% with titers ≥0.10 EU/ml**
Tiru 1999 Sweden	Cohort Unclear or moderate risk	3,5,12 mo vs 2,4,6 mo	Age 7 mo	Group 1: 3-5 mo (N=531) Group 2: 2-4-6 mo (N=306)	0.3 – 1.5 0.6 – 4	85 – 95 97 - 100
NB: Different D toxoid content in vaccines, but comparable distribution of vaccine types between schedule groups Data extracted from graph; min-max across toxoid contents						

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria	Tetanus
2 vs. 3 doses					Mean (SEM) (U/ml)	Mean (SEM) (U/ml)
Barkin 1985 USA	Cohort (not clear whether randomized) Moderate risk	2,6 mo vs. 2,4,6 mo	Age 8 mo	Group 1: 2,6 mo (N=29) Group 2: 2,4,6 mo (N=39)	0.13 (0.07) 0.26 (0.04)	0.26 (0.06) 0.51 (0.06) *P*-value <0.05
NB: Neutralization test for tetanus (rabbits) **Not-per-protocol**: diphtheria neutralization test on mice						

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria			Tetanus		
3+1 vs. 2+1					**%≥0.01 IU/ml**	**%≥0.1 IU/ml**	**GMT (IU/ml) (95% CI)**	**%≥0.01 IU/ml**	**%≥0.1 IU/ml**	**GMT (IU/ml) (95% CI)**
Swartz, 2003 Israel	Cohort Moderate and unclear risk	2,4,6 + 12 vs. 2,3.5 + 10 mo	One month after booster	Group 1: 3+1 (N=72) Group 2: 2+1 (N=60)	100 100	100 91.7	1.043 (0.887 - 1.227) 0.403 (0.319 – 0.510)	100 100	100 100	4.87 (4.18 – 5.68) 3.45 (2.93 – 4.06)
			Age 3 yrs	Group 1: 3+1 (N=49) Group 2: 2+1 (N=61)	97.9 100	18.7 12.1	0.043 (0.034 – 0.055) 0.043 (0.037 – 0.050)	100 100	91.8 95.1	0.26 (0.20 – 0.34) 0.31 (0.27 – 0.37)
Testing by ELISA; additional RIA testing is not per protocol										

Publication and country	Design Risk of bias	Vaccines, schedules evaluated	Timing of assessment	Comparison groups	Diphtheria		Tetanus	
3,4,5 vs 2,3,4 mo					**Proportion (%, 95% CI) ≥0.01 IU/ml**	**GMT (95%-CI) post-vaccination**	**Proportion (%, 95% CI) ≥0.01 IU/ml**	**GMT (95%-CI) post-vaccination**
Li, 2011 (I) China	RCT Unclear or low risk	3,4,5 mo vs. 2,3,4 mo	One mo post 3rd dose	Group 1 : 3,4,5 mo (N=239) Group 2 : 2,3,4 mo (N=257)	100.0 (98.4-100.0) 100.0 (98.6-100.0)	0.516 (0.489-0.544) 0.431 (0.405-0.459)	100.0 (98.4-100.0) 100.0 (98.5-100.0)	3.02 (2.92-3.12) 2.88 (2.79-2.98)
Li, 2011 (II) China	RCT Unclear or low risk	3,4,5 +18-20 mo vs. 2,3,4 +18-20 mo	One mo post booster	Group 1 : 3,4,5 mo (N=232) Group 2 : 2,3,4 mo (N=250)	100.0 (98.4-100.0) 100.0 (98.5-100.0) *	1.583 (1.459-1.719) 1.399 (1.291-1.516)	100.0 (98.4-100.0) 100.0 (98.5-100.0) *	6.13 (5.79-6.19) 6.19 (5.82-6.57)
					* same proportions found at cut-off 0.10 IU/ml			

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria	Tetanus
Birth dose					**GMT (µg/ml)**	
Dengrove 1986 US	RCT Unclear and low risk	0,2,4,6 vs. 2,4,6 mo	Age 6 mo	Group 1: Birth dose (N=14) Group 2: No birth dose (N=13)	Not shown	2.5 3
			Age 9 mo	Group 1: Birth dose Group 2: No birth dose	Not shown	7 10
					GMC (IU/ml) ***(P-value for difference)***	
Halasa 2008 USA	RCT Unclear and moderate risk	0,2,4,6 + 17 vs. 2,4,6 + 17 mo	Age 7 mo	Group 1: Birth dose (N=23) Group 2: No birth dose (N=22)	1.64 3.00 *(0.002)*	4.25 3.76 *(0.584)*
			Age 18 mo, after booster	Group 1: Birth dose (N=22) Group 2: No birth dose (N=20)	3.92 5.97 *(0.078)*	7.25 6.33 *(0.602)*

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	GMT (95%-CI) (IU/ml)
Early vs late initiation of 3 doses					**Diphtheria**
Kimura 1991 Japan	Cohort Unclear or high risk	Initiation @ 3-8 months vs. @ 9-23 months 3 doses at 6-10-wk interval Booster 12-18 mo post primary	Baseline	3-8 months (N=16) 9-23months (N=22)	<0.01 <0.01

			Before 3rd dose	3-8 months (N=16) 9-23months (N=22)	0.8 (0.6-1.0) 0.5 (0.3-0.7)
			1 mo after 3rd dose	3-8 months (N=16) 9-23months (N=22)	1.60 (1.2-2.1) 1.5 (1.1-2.0)
			Before booster	3-8 months (N=45) 9-23months (N=21)	0.3 (0.2-0.4) 0.3 (0.2-0.4)
			1 mo after booster	3-8 months (N=45) 9-23months (N=21)	6.7 (4.9-9.2) 10.2 (7.2-14.5)
NB: Diphtheria: Neutralization test (Vero cells) Tetanus: not per-protocol (hemagglutination method)					

Tables 8b-B: Additional studies - Primary vaccination, schedule impact on immunogenicity

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Mean concentration (IU) (SD and P-value for difference)	
2 vs. 3 doses					**Diphtheria**	**Tetanus**
Bhandari 1981 India	RCT Unclear	2 vs. 3 doses (schedule not specified)	1 month after primary schedule	Group 1: 2 doses (N= 80) Group 2: 3 doses (N = 82)	0.7431 (0.5162) 3.9054 (0.9212) *P*<0.001	1.3359 (0.7888) 6.1372 (1.8176) *P*<0.001
Not per protocol: serological testing using passive hemagglutination method						

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria median titre
2 vs. 3 doses					
Laurel 1957, Sweden	Cohort study Unclear or moderate risk	2 vs. 3 doses at 6 weekly interval	3-6 weeks after 3rd dose	2 doses (N=41) 3 doses (N=105)	0.5 1.5
Not per protocol: method of immunoassay did not conform to the protocol requirements.					

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria (IU/ml)			Tetanus (IU/ml)		
2 vs. 3 doses					**GMC**	**% ≥0.01**	**% ≥0.10**	**GMC**	**% ≥0.01**	**% ≥0.10**
Taranger 2000 Sweden	Cohort, unclear or moderate risk	3-5 +12 vs. 2-4-6 +15 mo	1 month after primary vaccination (6 and 7 mo)	Group 1: 3,5 mo (N=103) Group 2: 2,4,6 mo (N = 116)	0.38 0.26 *P*<0.01	100 100	88 77 *P*<0.05	1.6 1.7	100 100	100 100
			At booster (12 and 15 mo)	Group 1: 3,5 mo (N=102) Group 2: 2,4,6 mo (N = 112)	0.12 0.05 *P*<0.001	97 95	54 27 *P*<0.001	0.36 0.23 *P*<0.01	100 100	89 77 *P*<0.05
2+1 vs. 3+1 doses										
			1 mo post booster (13 and 16 mo)	Group 1: 3,5 mo (N=101) Group 2: 2,4,6 mo (N=112)	2.7 1.9 *P*<0.01	99 100	98 99	3.5 3.5	100 100	100 100
			Age 48 mo +	Group 1: 3,5 mo (N=54) Group 2: 2,4,6 mo (N = 74)	0.09 0.05 *P*<0.05	100 93	46 32	0.21 0.21	100 100	74 82

Not per protocol: two schedules were given with vaccines containing different amount of D and T toxoid (25 vs. 15 Lf and 7 vs. 6 Lf, respectively).

Diphtheria: Vero cell neutralization assay. Tetanus: ELISA

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria			Tetanus		
3+1 vs. 2+1					**%≥0.01 IU/ml**	**%≥0.1 IU/ml**	**GMT (IU/ml) (95% CI)**	**%≥0.01 IU/ml**	**%≥0.1 IU/ml**	**GMT (IU/ml) (95% CI)**
Swartz, 2003 Israel	Cohort Moderate and unclear risk	2,4,6 + 12 vs. 2,3.5 + 10 mo	One month after booster	Group 1: 3+1 (N=69) Group 2: 2+1 (N=57)	100 100	100 100	2.084 (1.691 – 2.567) 0.932 (0.751 – 1.155)	100 100	100 100	7.19 (6.08 – 8.50) 5.42 (4.36 – 6.74)
			Age 3 yrs	Group 1: 3+1 (N=32) Group 2: 2+1 (N=43)	100 100	65.6 41.9	0.145 (0.114 – 0.183) 0.085 (0.072 – 0.101)	100 100	100 93.9	0.41 (0.31 – 0.53) 0.30 (0.24 – 0.37)
Not per protocol: **RIA testing** (Pasteur-Mérieux in house assay); study also reports ELISA => included										

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria		Tetanus	
Accelerated vs. long schedule				**Min-max across 4 different vaccines**	**GMT**	**% ≥0.1 IU/ml**	**GMT**	**% ≥0.01 IU/ml**
Miller 1997 UK	RCT synopsis Moderate risk	2,3,4 vs. 3,5,8-10 mo	Age 4-5 yrs vs. age 12-18 mo	Group 1: 2,3,4 mo (N=170) Group 2: 3,5,8-10 mo (N=181)	0.06 – 0.20 0.12 – 0.25	26 – 52 55 – 75	0.25 – 0.39 0.32 – 0.43	81 – 93 100
Not per protocol: Timing of assessment not comparable in the two groups. Four different vaccines used (aP, wP) with different D and T potency, comparable distribution in both schedule groups.								

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	GMC (95% confidence interval) (IU/ml)	
Accelerated vs. long schedule					**Diphtheria**	**Tetanus**
Ramsey 1993 UK	Cohort synopsis Unclear	2,3,4 vs. 3,5,10 mo	Age 20/22 mo, 12+ weeks after third dose	Group 1: 2,3,4 mo (N=31/28) Group 2: 3,5,10 mo (N=31/30)	0.100 (0.065-0.153) 0.131 (0.087-0.196)	0.197 (0.137-0.284) 0.341 (0.239-0.484)
Not per protocol: solid phase RIA for serological testing						

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria (IU/ml)			Tetanus (IU/ml)		
Comparison of two long schedules					**GMC**	**% ≥0.01**	**% ≥0.10**	**GMC**	**% ≥0.01**	**% ≥0.10**
Gyhrs, 1999 Sweden	Cohort Unclear to moderate risk	3,5,12 vs. 5,6,15 mo	One mo after 2nd dose	Group 1: 3,5 mo (N=131/69) Group 2: 5,6 mo (N=49)	0.61 (1.30-2.01) 1.67 (1.21-1.30)	100 100	96.9 100	0.87 (0.65-1.17) 0.61 (0.39-0.95)	100 100	98.6 89.8
			One mo after 3rd dose	Group 1: 3,5,12 mo (N=117/55) Group 2: 5,6,15 mo (N=26)	6.14 (5.02-7.50) 15.19 (10.78-21.39)	100 100	99.1 100	6.33 (4.55-8.83) 17.16 (12.62-23.35)	100 100	98.2 100
			Age 24 mo	Group 1: 3,5,12 mo (N=76/51) Group 2: 5,6,15 mo (N=18)	0.44 (0.32 – 0.59) 0.93 (0.61 – 1.40)	100 100	88.2 100	0.74 (0.53-1.05) 1.44 (0.81 – 2.52)	100 100	96.1 100
Not per protocol: The two schedules were delivered with DTaP-IPV vs DT-IPV+wP, the latter containing 2-fold amount of D and T toxoid.										

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Mean concentration of IgG (IU/ml) (95% confidence interval)	
Short schedules: Various age at first dose, interval between doses					**Diphtheria**	**Tetanus**
Wong, 2008 Malaysia	RCT Low and unclear risk	1.5,3,5 vs. 3,4,5 mo	Age 6 mo (1 mo after last primary dose)	Group 1: 1.5,3,5 months (N=102) Group 2: 3,4,5 months (N=103)	2.03 (1.66 – 2.50) 2.24 (1.89 – 2.65)	2.99 (2.48 – 3.61) 1.20 (1.03 – 1.41)
			Age 12 mo (7 mo after last primary dose)	Group 1: 1.5,3,5 months (N=82) Group 2: 3,4,5 months (N=82/88)	0.20 (0.16 – 0.26) 0.31 (0.25 – 0.39)	0.76 (0.60 – 0.96) 0.51 (0.43 – 0.61)
					Percentage with putatively protective titers (% with 95%-CI))	
			Age 6 mo (1 mo after last primary dose)	Group 1: 1.5,3,5 months (N=102) Group 2: 3,4,5 months (N=103)	98.0 (93.1 – 99.8) 100.0 (96.5 – 100.0)	100.0 (96.4 – 100.0) 100.0 (96.5 – 100.0)
			Age 12 mo (7 mo after last primary dose)	Group 1: 1.5,3,5 months (N=82) Group 2: 3,4,5 months (N=82/88)	89.0 (95.5 – 100.0) 93.2 (85.2 – 97.2)	96.3 (89.7 – 99.2) 95.5 (88.9 – 98.8)
Not per protocol: schedules delivered with different vaccine products (DTwP-HepB vs. DTwP)						
Cut-off for putatively protective titers: ⩾0.1 IU/ml for both diphtheria and tetanus						
Diphtheria at age 12 months: testing with Vero cells if ELISA negative (cut-off 0.016 IU/ml)						

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups						
Short schedules: Various age at first dose, interval between doses					**Diphtheria**			**Tetanus**		
					%≥0.01 EU/ml	**%≥0.1 EU/ml**	**GMT (95% CI)**	**%≥0.01 EU/ml**	**%≥0.1 EU/ml**	**GMT (95% CI)**
Hoppenbrouwers, 1999 Belgium, Turkey	RCT Low risk	3,4,5 vs 2,4,6 mo	One month after 3rd dose	Group 1: 3,4,5 mo (N=135) Group 2: 2,4,6 mo (N=137)	100 100	100 99	0.98 (0.86-1.13) 1.06 (0.93-1.21)	100 100	100 100	1.08 (0.93-1.26) 1.84 (1.59-2.13)
Not per protocol: serological assay was radio-immunoassay										

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	GMT (95%-CI) (IU/ml)
Early vs late initiation of 3 doses					**Tetanus**
Kimura 1991 Japan	Cohort Unclear or high risk	Initiation @ 3-8 months vs. @ 9-23 months 3 doses at 6-10-wk interval Booster 12-18 mo post primary	Baseline	3-8 months (N=16) 9-23months (N=31)	<0.01 <0.01
			Before 3rd dose	3-8 months (N=16) 9-23months (N=29)	1.2 (0.8-1.7) 1.1 (0.8-1.5)
			1 mo after 3rd dose	3-8 months (N=16) 9-23months (N=31)	2.0 (1.5-2.8) 2.1 (1.6-2.8)
			Before booster	3-8 months (N=45) 9-23months (N=21)	0.2 (0.1-0.2) 0.4 (0.3-0.7)
			1 mo after booster	3-8 months (N=45) 9-23months (N=21)	3.1 (2.4-4.1) 4.9 (3.3-6.7)
Not-per-protocol: Tetanus antitoxin measured by hemagglutination method					

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria		
Various ages at single dose							
					%≥0.0005 U/ml	**%≥0.02 U/ml**	**Mean level (U/ml)**
Vahlquist 1949 Sweden	Cohort Unclear risk	Single dose at birth, age 2-3 mo or 6-8 mo	1 mo post vaccination	Group 1: birth (N=15) Group 2: 2-3 mo (N=15) Group 3: 6-8 mo (N=15)	47 100 93	20 47 47	<0.0005 >0.01, <0.02 0.02
			3 mo post vaccination	Group 1: birth (N=15) Group 2: 2-3 mo (N=15) Group 3: 6-8 mo (N=15)	87 66 60	47 33 47	0.02 0.02 >0.02 < 0.05
			6 mo post vaccination	Group 1: birth (N=15) Group 2: 2-3 mo (N=15) Group 3: 6-8 mo (N=15)	60 33 40	40 27 20	>0.02 < 0.05 >0.02 < 0.05 0.02

Table set 9. Data from included and additional studies evaluating primary vaccination absolute impact on relevant outcomes

Table 9a-A: Included studies on primary vaccination, absolute vaccine effectiveness/efficacy

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	Diphtheria Vaccine Effectiveness (%) 95% CI	
Diphtheria, 1-4 doses vs. zero doses					**0-2 years (22 cases)**	**3-5 years (43 cases)**
Bisgard 2000, Russia	Matched CC Moderate risk	3 doses DT before age 12 mo, interval ≥28 days	Age 0 – 2 yrs	1vs. zero doses	93.3 (31.8 - 99.4)	85.5 (-20.3 – 98.3)
				2 vs. zero doses	100	91.3 (63.0 – 98.0)
				3 vs. zero doses	97.2 (86.3 - 99.4)	96.1 (87.4 – 98.9)
		Booster at age 2 yrs		4 vs. zero doses	100	99.1 (96.6 – 99.8)

Table 9b-A: Included studies on primary vaccination, absolute vaccine immunogenicity

Publication and country	Design Risk of bias	Schedules evaluated	Timing of assessment	Comparison groups	
Diphtheria, 1-4 doses vs. zero doses					**Tetanus (IU/ml)**
Mangay-Angara, 1978 Philippines	Clinical trial Unclear risk	2 doses in 6-mo interval Children aged 3-8 mo	40 days after 2nd dose	Group 1 : 2 doses DTwP Group 2 : 2 doses D	0.24 0.011
NB : neturalization test on mice					

Table set 10. Data from included and additional studies evaluating booster vaccination schedule impact on relevant outcomes

Table 10b-A: Included studies on booster vaccination, schedule impact on immunogenicity

Publication and country	Design Risk of Bias	Schedule used	Timing of assessment	Comparison groups	Diphtheria		Tetanus	
Age at booster					**GMC (95%-CI)**	**Proportion (%) with titer ≥0.1 U/ml**	**GMC (95%-CI)**	**Proportion (%) with titer ≥0.1 U/ml**
Scheifele 1999, Canada	RCT Low risk	DTwP booster after 3d-primary series, ages 12, 15 or 18 mo	4-6 wks after booster	Group 1: age 12 mo (N=86) Group 2: age 15 mo (N=85) Group 3: age 18 mo (N=82)	6.1 (4.7 – 7.9) 5.8 (4.4 – 7.7) 9.0 (6.6 – 12.2)	100 98.8 100	2.3 (1.9 – 2.8) 2.4 (2.0 – 2.8) 3.0 (2.5 – 3.7)	100 100 100

Table set 11. Data from included and additional studies evaluating booster vaccination absolute impact on relevant outcomes

Table 11b-A: Included studies on booster vaccination, absolute immunogenicity

Publication and country	Design Risk of bias	Schedules used	Timing of assessment	Comparison groups	Diphtheria mean antibody titer (SD) (IU/ml)	Tetanus mean antibody titer (SD) (IU/ml)
Booster vs. no booster						
Conway 1993 UK	RCT Unclear risk Preterm babies 32-35 gestat. weeks	A: 3,4,5 mo, B: 3,4,5, 18 mo	Age 19 mo	A: Booster (N=13) B: No booster (N=6)	16.07 (21.86) 0.67 (0.64)	16.79 (10.98) 0.52 (0.37)

Appendix 1. Search terms

Search queries in PubMed

	Query	Items found
#87	(#53) AND #86	1825
#86	(#84) OR #85	5500726
#85	(((#73) OR #74) OR #77) OR #81	1926019
#84	(((#68) OR #69) OR #70) OR #72	4014848
#81	"case* series"[tw]	31266
#77	Case Reports [pt]	1609362
#74	((case* AND control*)[tw])	1161
#73	cohort*[tw]	293650
#72	Case-Control Studies/	624790
#70	cohort studies/	1250927
#69	Epidemiologic Studies/	1498703
#68	Epidemiological Methods/	3981368
#65	(#53) AND #64	1544
#64	(#62) NOT #63	2676437
#63	(animals [mh] NOT humans [mh])	3759657
#62	(((((((#54) OR #55) OR #56) OR #57) OR #58) OR #59) OR #60) OR #61	3121994
#61	groups [tiab]	1265127
#60	trial [tiab]	324489
#59	randomly [tiab]	193109
#58	drug therapy [sh]	1576079
#57	placebo [tiab]	146959
#56	randomized [tiab]	282417
#55	controlled clinical trial [pt]	85155
#54	randomized controlled trial [pt]	339710
#53	(#47) AND #52	4132
#52	((#49) OR #50) OR #51	1644579
#51	booster[tw]	7248
#50	month*[tw]	1017178
#49	week*[tw]	781978
#48	(#41) OR #47	23981
#47	((((#42) OR #43) OR #44) OR #45) OR #46	17805
#46	Tetanus Vaccine/	12221
#45	Diphtheria Vaccine/	7311
#44	Tetanus Toxoid/	10663
#43	Diphtheria Toxoid/	5931
#42	Pertussis Vaccine/	8163
#41	(#40) AND #39	23195

#40	((#8) OR #15) OR #22	59292
#39	(#35) OR #38	780762
#38	(#36) OR #37	772569
#37	immun*[tw]	624684
#36	vaccin*[tw]	252474
#35	((((#26) OR #31) OR #32) OR #33) OR #34	295999
#34	Immunization, Secondary/	10149
#33	Immunization Schedule/	10140
#32	Immunization/	213756
#31	Vaccination/	108937
#26	Vaccines/	181844
#22	(#18) OR #21	18138
#21	(#19) OR #20	18094
#20	Corynebacterium diphtheriae[tw]	2485
#19	Diphtheria[tw]	16993
#18	(#16) OR #17	18138
#17	Corynebacterium diphtheriae/	2533
#16	Diphtheria/	16998
#15	(#11) OR #14	24911
#14	(#12) OR #13	24887
#13	tetanus[tw]	24614
#12	Clostridium tetani[tw]	1003
#11	(#9) OR #10	24911
#10	Clostridium tetani/	1034
#9	Tetanus/	24614
#8	(#3) OR #7	28001
#7	((#4) OR #5) OR #6	28001
#6	Bordetella pertussis[tw]	5906
#5	whoop*[tw]	7367
#4	Pertuss*[tw]	25481
#3	(#1) OR #2	16369
#2	Whooping Cough/	7244
#1	Bordetella pertussis/	11311

Search queries for EMBASE

No.	Queries	Results
#58	**#56** OR **#57**	**723,499**
#57	**#44** OR **#45** OR **#49** OR **#50** OR **#51** OR **#52** OR **#54**	**430,195**
#56	**#32** OR **#35** OR **#36** OR **#37** OR **#38** OR **#40** OR **#41**	**367,017**
#55	('**cross sectional**' NEAR/1 (**study** OR **studies**)):ab,ti AND [embase]/lim	**54,981**
#54	(**epidemiologic*** NEAR/1 (**study** OR **studies**)):ab,ti AND [embase]/lim	**53,542**
#52	(**observational** NEAR/1 (**study** OR **studies**)):ab,ti AND [embase]/lim	**44,611**
#51	('**follow up**' NEAR/1 (**study** OR **studies**)):ab,ti AND [embase]/lim	**35,146**
#50	('**case control**' NEAR/1 (**study** OR **studies**)):ab,ti AND [embase]/lim	**57,451**
#49	(**cohort** NEAR/1 (**study** OR **studies**)):ab,ti AND [embase]/lim	**86,192**
#45	'**cohort analysis**'/exp AND [embase]/lim	**111,978**
#44	**#42** NOT **#43**	**139,471**
#43	'**randomized controlled trial**'/exp AND [embase]/lim	**239,204**
#42	'**prospective study**'/exp AND [embase]/lim	**156,742**
#41	'**retrospective study**'/exp AND [embase]/lim	**191,755**
#40	'**longitudinal study**'/exp AND [embase]/lim	**35,911**
#38	'**family study**'/exp AND [embase]/lim	**9,898**
#37	'**family study**' AND [embase]/lim	**12,541**
#36	'**case control study**'/exp AND [embase]/lim	**45,046**
#35	'**case control study**'/de AND [embase]/lim	**39,525**
#32	'**clinical study**'/de AND [embase]/lim	**88,635**
#31	**#13** AND **#30**	**1,394**
#30	**#24** OR **#29**	**1,026,630**
#29	**#25** OR **#26** OR **#27** OR **#28**	**275,208**
#28	'**crossover procedure**'/exp AND [embase]/lim	**30,216**
#27	'**double blind procedure**'/exp AND [embase]/lim	**94,889**
#26	'**single blind procedure**'/exp AND [embase]/lim	**12,674**
#25	'**randomized controlled trial**'/exp AND [embase]/lim	**239,204**
#24	**#14** OR **#15** OR **#16** OR **#17** OR **#18** OR **#19** OR **#20** OR **#21** OR **#22** OR **#23**	**983,774**
#23	((**singl*** OR **doubl***) NEAR/1 **blind***):ab,ti AND [embase]/lim	**133,191**
#22	**allocat***:ab,ti AND [embase]/lim	**56,171**
#21	**assign***:ab,ti AND [embase]/lim	**166,868**
#20	**volunteer***:ab,ti AND [embase]/lim	**142,943**

#19	'**cross-over**':ab,ti AND [embase]/lim	**17,800**
#18	'**cross over**':ab,ti AND [embase]/lim	**17,800**
#17	**crossover***:ab,ti AND [embase]/lim	**39,156**
#16	**factorial***:ab,ti AND [embase]/lim	**13,970**
#15	**placebo***:ab,ti AND [embase]/lim	**165,399**
#14	**random***:ab,ti AND [embase]/lim	**641,711**
#13	**#1** OR **#9** OR **#10** OR **#11** OR **#12**	**27,311**
#12	**pertuss***:ab,ti OR **whoop***:ab,ti AND [embase]/lim	**20,948**
#11	'**pertussis**'/de AND [embase]/lim	**6,766**
#10	'**bordetella pertussis**'/de AND [embase]/lim	**4,390**
#9	**#2** OR **#3** OR **#4** OR **#5** OR **#6** OR **#7** OR **#8**	**6,095**
#8	'**diphtheria pertussis poliomyelitis tetanus haemophilus influenzae type b hepatitis b vaccine**'/de AND [embase]/lim	**315**
#7	'**diphtheria pertussis poliomyelitis tetanus hepatitis b vaccine**'/de AND [embase]/lim	**165**
#6	'**diphtheria pertussis poliomyelitis tetanus vaccine**'/de AND [embase]/lim	**391**
#5	'**diphtheria pertussis tetanus haemophilus influenzae type b hepatitis b vaccine**'/de AND [embase]/lim	**136**
#4	'**diphtheria pertussis tetanus haemophilus influenzae type b vaccine**'/de AND [embase]/lim	**475**
#3	'**diphtheria pertussis tetanus hepatitis b vaccine**'/de AND [embase]/lim	**56**
#2	'**diphtheria pertussis tetanus vaccine**'/de AND [embase]/lim	**5,329**
#1	'**pertussis vaccine**'/de AND [embase]/lim	**4,677**

Search queries in CENTRAL

ID	Search	Hits
#1	MeSH descriptor: [Whooping Cough] 1 tree(s) exploded	201
#2	MeSH descriptor: [Pertussis Vaccine] explode all trees	602
#3	MeSH descriptor: [Bordetella pertussis] explode all trees	115
#4	(#1 or #2 or #3)	651
#5	whoop* or "whooping cough":ti (Word variations have been searched)	33
#6	whoop* or "whooping cough":ab (Word variations have been searched)	35
#7	whoop* or "whooping cough":kw (Word variations have been searched)	218
#8	(#5 or #6 or #7)	245
#9	pertuss*:ti,ab,kw	912
#10	pertuss* near/2 immun*:ti,ab,kw (Word variations have been searched)	215
#11	pertuss* next vaccin*:ti,ab,kw	714
#12	pertuss* next immun*:ti,ab,kw	106
#13	(#10 or #11 or #12)	729
#14	(#9 or #13)	921
#15	(#8 or #14)	950
#16	(#4 or #15)	950

Appendix 2

Synopsis of inclusion of references in WHO tutorials on diphtheria and tetanus (The Immunological Basis for Immunization Series [WHO 2006 and WHO 2009])

Reference	Comment
Anderson 1987	**No full text not abstract found** - 17 to 21 months, and 5 to 9 months of age
Anderson 1988	aP vs. wP
Barkin 1984	reduced dose
Barkin 1985 (Ped Inf Dis)	No schedule comparison for DT (all groups 3 doses in 2-mo interval); included for wP
Barkin 1985 (Develop Biol Standard)	Included
Barr 1950	Compare cord antibody and placental IgG in new borns, and rate of loss of passive IgG. No schedule comparison
Bhandari 1981	NPP (serol. method)
Cellesi 1989a	No relevant comparison was made. Assessed persistence of IgG in infants that received same vaccination intervention, and was assessed between 6-15 years.
Chen "1956" is 1957 (II. of series)	No control group
Crossley 1979	No relevant comparison was made. Assessed protective IgG against tetanus and diphtheria in adults.
Edwards 1989	no relevant comparison
Gatchalian 2005	Compared DTP vaccine in different combinations with other EPIs, given in the same schedule, dose , interval . No relevant comparison was made.
Guerin 1988	**No full text not abstract found - Probably no control group**
Halsey & Galazka 1985	No original data (review)
Hussey 2002	Only evaluation of the safety and immunogenity of TETRActHIB vaccine, given at 6,10, and 14 weeks.
Jones 1989	Evaluated persistence of IgG after completion of 3rd dose by 8th month of life. No relevant comparison was made.
Kimura 1991	included

Krumina 2005	No control group, only D cases
Lewis 1986	no relevant comparison
Ohuabunwo 2005	D outbreak among adults (military)
Pichichero 1987	aP vs. wP
Pichichero 1986	No evaluation of DT (DT immunity comparing DT to DTP)
Scheifele 2001	No control group
Scheifele 2005	DTaP vs. Tdap
Schou 1987	No comparison group
Trollfors 2005	effect of co-administration of pertussis on ImmGen of DT
Vahlquist 1949	NPP
Aboud 2000	The study participants were aged between 1-15 years, with subgroups: 1-5 years, and 6-15 years. The comparisons were not relevant to study protocol (compared based on the defined age groups and geographical locations).
Burrage 2002	Compared different schedules for other EPIs, alternated DT and dT, but did not make relevant comparison for DT
De Melker 2000	All participants received same intervention, no relevant comparison on schedule/dose
Lin 2003	All participants received same intervention, no relevant comparison on schedule/dose
Ramsay 1993	NPP
Scheifele 1999	Included (booster DT and wP)
Simonsen 1984	Vaccinees were older than 11 years, and outcome assessed in older age. No relevant comparison
Simonsen 1987a Vaccine	Evaluatted persistence of IgG in vaccinees older ≥ 25 years. All vaccinated in same schedule , no relevant comparison
Simonsen 1987 J Trauma	Vaccinees /participants were adults who were revaccinated 17-20 years after primary vaccination. No relevant comparison.
Swartz 2003	Included for DT
Trinca 1974	Participants/vaccinees were aged 17 – 49 years, all recieved same intervention in same schedule. No relevant comparison. Measured IgG to successive booster doses.
Turner 1954	**Full text article not found - Probably no control group**
Vergara 2005	Compared IgG in response to booster doses of full strenght and

	reduced strength DT, given to adolescents aged 10- 14 year.
Volk 1962	Evaluated IgG in response to booster given to vaccinees aged 13-20years . No schedule comparison.

Synopsis of inclusion of references in Orenstein

Reference	Comment