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Protection by BCG against tuberculosis: a systematic review of randomised controlled trials

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Summary: This new systematic review and analysis suggests BCG vaccination in infancy or BCG vaccination when stringent tuberculin testing excludes those with a small degree of prior infection or sensitization to environmental mycobacteria protects against pulmonary diseases even in the tropics

Abstract

Background: Randomized trials assessing BCG vaccine protection against tuberculosis have widely varying results, for reasons that are not well understood.

Methods:

We examined associations of trial setting and design with BCG efficacy against pulmonary and miliary or meningeal tuberculosis by conducting a systematic review, meta-analyses and meta-regression.

Results:

We identified 18 trials reporting pulmonary and 6 miliary or meningeal tuberculosis. Univariable meta-regression indicated efficacy against pulmonary tuberculosis varied according to three characteristics. Protection appeared greatest in children stringently tuberculin tested, to try to exclude prior infection with *M tuberculosis* or sensitisation to environmental mycobacteria (rate ratio [RR] 0.26; 95% CI 0.18-0.37), or infants (0.41; 0.29-0.58). Protection was weaker in children not stringently tested (0.59; 0.35-1.01) and older individuals stringently or not-stringently tested (0.88; 0.59-1.31 and 0.81; 0.55-1.22 respectively). Protection was higher in trials further from the equator where environmental mycobacteria are less and with lower risk of diagnostic detection bias. These associations were attenuated in a multivariable model, but each had an independent effect. There was no evidence that efficacy was associated with BCG strain. Protection against meningeal and miliary tuberculosis was also high in infants (RR 0.1; 95% CI 0.01-0.77) and children stringently tuberculin tested (0.08; 0.03-0.25).

Conclusions:

Absence of prior *M tuberculosis* infection or sensitisation with environmental mycobacteria is associated with higher efficacy of BCG against pulmonary tuberculosis and possibly against miliary and meningeal tuberculosis. Evaluations of new tuberculosis vaccines should account for the possibility that prior infection may mask or block their effects.

Introduction

Bacillus Calmette Guérin (BCG) vaccine is included in the childhood vaccination programme of many countries. However, varying estimates of its efficacy in preventing pulmonary tuberculosis, the major burden of tuberculosis disease, have been found in controlled trials[1;2] ranging from 0% in the Chingleput Trial in South India to 80% in the UK Medical Research Council trial[3-5]. Consistently high estimates of efficacy have been reported for infant BCG vaccination against severe primary progressive disease[6;7;8] .

Previous systematic reviews noted a positive association between BCG vaccine efficacy against pulmonary disease with distance from the equator at which studies were conducted[2;9], possibly related to exposure to environmental mycobacteria, which is in general, less common distant from the equator[1]. Consistent with this hypothesis, a recent sub-analysis of the Chingleput Trial suggested some protection (efficacy 29%) among participants who had low tuberculin reactivity and no reaction to non-tuberculous mycobacterial antigen (*Mycobacterium intracellulare*) at baseline, [10]. Other possible explanations for variability in the efficacy of BCG against pulmonary disease include the role of study quality[11] and that different BCG strains induce different levels of protection[12].

An improved understanding of why BCG vaccine efficacy varies to such a great extent is important to inform assessment of the new generation of tuberculosis vaccines undergoing clinical trials[13], most of which are designed to boost protection by BCG. We conducted a systematic review of all reported BCG trials, in order to estimate the efficacy of BCG against pulmonary, miliary and meningeal tuberculosis and examine associations of study characteristics, including immunological naïvity to infection, with efficacy.

Methods

We searched for studies reporting primary data on BCG vaccination efficacy in preventing tuberculosis disease in human populations of any age, in which BCG (without re-vaccination) was compared with no vaccination (placebo or other control). We excluded non-BCG tuberculosis vaccines (e.g. vole bacillus, Savioli anti-tuberculosis vaccine or other heat-killed bacillus vaccines)

and oral BCG. We did not restrict searches by study design, language, publication date or whether fully published. Two reviewers independently screened titles and abstracts, resolving disagreement via a third reviewer. We retrieved full papers if assessment from the abstract was not possible or if one reviewer considered them potentially eligible. This paper is limited to findings from randomized or quasi-randomized trials that reported pulmonary, miliary or meningeal tuberculosis outcomes.

We searched 10 medical literature electronic databases from inception to May 2009, and other databases including Google Scholar and trial registers to October 2009. An information specialist helped combine MeSH and text word terms for disease and intervention into search strategies appropriate for the different databases. Search terms included tuberculosis, tubercle bacill*, *M. bovis*, *M. africanum*, *M. canetti*, *M. microti* and *M. tuberculosis*. Terms for the intervention included BCG Vaccine, BCG, bacillus calmette. (See supplementary appendix for sources and search strategy). We identified duplicate or multiple publications, and used the most recent available data in analyses. One person extracted data onto structured piloted forms, another checked accuracy and completeness. For non-English language publications, one person discussed and agreed upon data to be extracted with an extractor fluent in the language of publication. Disagreements were resolved through discussions with other members of the study team . As most papers were published before 1973, authors were not contacted if data were not available.

We extracted trial characteristics, case definitions, outcomes, and summary results. Trial characteristics included distance from the equator by degrees of latitude (collapsed into 20^{o} latitude groups for analysis) and whether tests for tuberculin sensitivity (a marker of prior *M tuberculosis* infection as well as some indication of sensitisation to other mycobacteria[4]) with purified protein derivative (PPD) were conducted and whether a stringent testing protocol was used. Participants vaccinated as infants were assumed tuberculin negative. A stringent tuberculin testing protocol was defined as re-testing initially tuberculin negative participants using a higher dose of tuberculin to confirm negativity before vaccination. A non-stringent tuberculin testing protocol was defined as one that did not exclude non-infant participants based on tuberculin testing prior to vaccination, or which excluded subjects based on only a single tuberculin test.

BCG strain variation was assessed in terms of attenuation lineage, the molecular basis of which was classified by Brosch *et al*[12]. We classified strains in the three groups proposed. We also tested an hypothesis that as BCG strains evolved over time there would be a loss of protection.

We assessed risk of bias in trial results based on the Cochrane Collaboration's Risk of Bias tool[14], with additional items specific to BCG trials. We did not consider placebo vaccination as blinded during follow up as BCG leaves a scar. In addition, we assessed likelihood of diagnostic detection bias specific to the mode of presentation of pulmonary tuberculosis, based on Clemens *et al*[11] who noted a substantial proportion of tuberculosis is missed if disease is identified only using passive follow-up. There is thus a potential for bias if assessors were aware of the trial hypothesis and were not blinded to presence or absence of a BCG scar. Trials in which follow-up was active with regular chest X ray or other assessments were judged as at low likelihood of such bias, whether or not assessors were blind, as were trials with passive follow-up in which outcomes were from routine surveillance and assessors were blind to BCG status. Trials using other methods of ascertainment were judged to have a greater likelihood of diagnostic detection bias.

For each trial, we estimated the rate ratio (RR) of tuberculosis, comparing vaccinated with unvaccinated participants, together with the standard error of the log rate ratio. Vaccine efficacy is defined as 1-RR. Pooled results , together with both fixed- and random-effects summary effect estimates, were obtained from fixed-effect (inverse variance weighted) and DerSimonian and Laird random-effects meta-analyses[15] of (log) rate ratios from each study. If one of the randomised groups in a trial had 0 cases, 0.5 was added to each cell of the 2x2 table. Results from both types of meta-analysis were included in forest plots: differences between them may suggest the presence of small study effects[14]. We also examined possible strain effects by plotting estimated rate ratios against the year the study started.

Differences in efficacy between sub-groups of studies were quantified using random-effects meta-regression to estimate ratios of rate ratios. Heterogeneity between studies was quantified by

estimating the between-study variance τ^2. In forest plots and meta-analyses, τ^2 was estimated using the method-of-moments estimator proposed by DerSimonian and Laird. For meta-regression analyses, τ^2 was estimated by restricted maximum-likelihood, using the **metareg** command in Stata.

Results

From 21,030 titles and abstracts we identified 847 articles for retrieval. We included 211 relevant papers, (60 not published in English). These articles reported data on 21 randomised or quasi-randomised trials (supplementary figure 1), of which 18 reported on pulmonary tuberculosis, and six on meningeal and/or miliary tuberculosis outcomes. Ten trials were conducted in the USA between 1933 and 1950[16-25]; four in India between 1950 and 1988[26-29]; one each in Canada (started in 1933) [30], the UK (1950)[31], South Africa (1965)[32] and Haiti (1965) [33] (Table 1). Supplementary table 1 provides further details of each trial.

Protection against pulmonary tuberculosis

The efficacy of BCG against pulmonary tuberculosis ranged from substantial protection, in the UK MRC trial[31] (RR 0.22; 95% CI 0.16-0.31), to absence of clinically important benefit, in the Chingleput trial[28] (1.05; 0.88-1.25)). Figure 1 shows the ratio of the rates of pulmonary tuberculosis among BCG vaccinated and controls in each trial, stratified according to age at vaccination and stringency of pre-vaccination tuberculin testing, with fixed- and random-effects summary effects estimates overall and within strata, and estimates of between-trial heterogeneity. There was less heterogeneity within strata (all estimates of τ^2 less than 0.095) than overall (τ^2=0.38). The average protection by BCG was greatest in trials of school-age vaccination with stringent tuberculin testing prior to vaccination (random-effects RR 0.26; 95% CI 0.18-0.37) and studies of neonatal vaccination (0.41; 0.29-0.58). Fixed- and random-effects estimates were similar within strata and overall. There was no consistent evidence of protection in trials including participants older than school age although some protection was found in adults in some trials.

Consistent with previous observations, there were marked differences in estimated efficacy according to latitude at which trials were conducted. The protective effect of BCG was on average greater in

trials conducted at latitudes furthest from the equator. Although estimated between-trial heterogeneity was lower within latitude strata than overall, there was evidence of heterogeneity between trials at more than 40° latitude (τ^2=0.12, figure 2). Protection was in general absent or low in trials closer to the equator (latitudes <20° and 20°-40°). Among trials in which outcome assessors were considered adequately blinded to participants' vaccination status , or if there was active surveillance, there was substantial between-study variation but the average protective effect of BCG against pulmonary tuberculosis was greater (random-effects RR 0.40; 95% CI 0.25-0.64) than in trials with higher likelihood of diagnostic detection bias (0.78; 0.64-0.95) (Figure 3).

When trials were stratified according to BCG strain lineage, there was substantial between-trial heterogeneity within each stratum, while the average effect of BCG vaccination was similar for each strain group (supplementary figure 2, Figure 3). There was no clear relationship between estimated vaccine efficacy and year the trial was started, either overall or within strain group (figure 4).

Univariable meta-regression analyses suggested that, among the trial characteristics considered, distance from the equator and age at vaccination/tuberculin testing stringency explained the majority of between-trial variation in the effect of BCG (residual τ^2 0.086 and 0.044 respectively, compared to 0.284 estimated using a meta-regression model without study characteristics) (Table 2). Average protection was lower in trials conducted at 0^o-20^o and 20^o-40^o latitude, compared with those conducted at >40^o latitude There was also good evidence that protection was lower in trials including participants older than school age than in studies of neonatal vaccination. There was some evidence that average protection was lower in studies with higher likelihood of diagnostic detection bias compared with studies with lower likelihood of such bias, although this characteristic explained only 18% of the between-trial heterogeneity. There was little evidence that protection varied according to other study design characteristics, or BCG strain.

Because latitude has previously been associated with protection by BCG, we next fitted two-variable meta-regression models including latitude and each other characteristic. These analyses indicated that latitude and age at vaccination/tuberculin testing stringency could explain all of the between-trial

heterogeneity (residual τ^2=0). The final multivariable regression model, which also explained the between-trial heterogeneity, included the variables latitude, age at vaccination/tuberculin testing stringency, and likelihood of diagnostic detection bias. Estimated ratios of rate ratios were attenuated compared with univariable analyses, but each of these characteristics was separately associated with the effect of BCG, having accounted for the other two.

Protection against meningeal or miliary tuberculosis

The six trials that reported on meningeal and miliary tuberculosis found substantial protection by BCG (RR 0.15; 95% CI 0.08, 0.31) with little evidence of between-trial heterogeneity (p=0.14, figure 5). Protection appeared greatest in the two trials of neonatal vaccination (0.10; 0.01, 0.77), and the two trials of school age vaccination with stringent tuberculin testing (0.08; 0.03; 0.25). The two trials with non-stringent tuberculin testing (one at school age and one at a range of ages) found little evidence of protection. However, ratios of rate ratios were imprecisely estimated in meta-regression analyses (supplementary table 2), and there was no strong evidence that the efficacy of BCG varied according to this or other trial characteristics.

Discussion

We found three study characteristics to be associated with estimated protection by BCG against pulmonary tuberculosis. As well as the well-known association of protection with increasing latitude at which trials were conducted, our analysis indicates that protection was greater when BCG was given in infancy or at school age, in trials that used stringent tuberculin testing to try to exclude participants already sensitised to mycobacteria, and in studies with lower likelihood of diagnostic detection bias. Together, these factors were sufficient to explain the between-study variation in the protective effect BCG against pulmonary tuberculosis. We found little evidence that other study characteristics or BCG vaccine strain were associated with protection. Protection against meningeal and miliary tuberculosis also appeared greater than for pulmonary tuberculosis and when BCG was given to infants or at school age after stringent tuberculin testing.

Randomized controlled trials provide the best evidence for the effectiveness of interventions, but many BCG trials were conducted before standard methods for trial conduct and reporting were developed. Many used alternation or other "quasi-randomized" methods of allocation to BCG or control, which do not guarantee concealment of allocation at recruitment or blinding of participants and trial personnel, and some aspects of trial design were not clearly reported. Previous systematic reviews e.g. [9] of 13 trials reporting TB disease outcomes did not assess whether several of these design characteristics or the exclusion of those with prior infection or sensitisation to environmental mycobacteria using stringent tuberculin testing, were related to BCG protection. Based on comprehensive searches we included the same 13 trials, and found five more eligible trials. We used recently developed approaches to assessing risk of bias in trial results. We also assessed additional potential biases specific to BCG vaccine trials defined a-priori based on a criterion proposed by Clemens et al[11] (blinding of study staff who assessed outcome on BCG status or active surveillance) as well as the variability between trials in stringency of pre-vaccination tuberculin testing. We used meta-regression to examine these different possible explanations for variation in the estimated effect of BCG across studies. However, meta-regression analyses have limitations[34]. They are ecological analyses with trials as units of observation, hence observed associations may result from confounding by other study design characteristics. Studies examined efficacy over varying follow-up times. An alternative of restricting to the same period would have reduced the number of studies that could be included. Our multivariable analyses included seven variables, which is large compared with the total number of studies (18). Therefore, our finding that three characteristics could explain all the between-trial variation in the effect of BCG on pulmonary tuberculosis should be interpreted with caution. Too few trials reported on miliary and meningeal tuberculosis to allow a comprehensive analysis of between-trial heterogeneity.

The effect of latitude on efficacy persisted after adjustment, perhaps because even stringent tuberculin testing does not exclude all sensitisation to environmental mycobacteria. Other proposed explanations include human genetic differences, genotypic differences between infecting mycobacteria, or a variety of proposed explanations for the association of protection with latitude: exposure to ultraviolet light (due to its mycobacterial killing effect); levels of vitamin D, helminthic infestation or the effect of

poor nutrition on immune response. Previous reviews concluded that these factors are less plausible explanations than exposure to environmental mycobacteria[35].

Previous systematic reviews found substantial variation between trials in estimated protection by BCG against pulmonary tuberculosis[2;9], and one estimated average protective efficacy to be 50%[9]. However, in the absence of explanations for heterogeneity such an average cannot be applied to the use of BCG in a particular setting or population.

It is well known there are genetic differences between BCG vaccines, e.g. based on restriction fragment length polymorphism typing that suggest BCG strains have undergone evolution since 1921[12]. Brosch *et al*. recently used genome sequencing to postulate that BCG vaccines derived before 1930 or 1940 may be immunologically superior to more recent and widely used variants [12]. We found little evidence of an association between estimated effects of BCG with the year each trial commenced or that effects varied according to the groups proposed, which include strains currently in use: Denmark (in DU2 Group III), Russia (in DU2 Group I) and Japan (also in DU2 Group I) [12]. Our findings are consistent with results from the UK MRC trial [31], which found equivalent protection by the Copenhagen strain of BCG and an M. *microti* derived vaccine (vole bacillus)[5]

A possible explanation for the low protection observed in trials in southern USA versus high protection in the UK was first proposed during the 1960s, based on guinea-pig studies[1]. The findings suggested exposure to certain non-tuberculous mycobacterial antigens could mask the observed effectiveness of BCG, by providing some protection against tuberculosis in non–vaccinated groups, which was not enhanced by BCG vaccination. The authors also noted that populations in southern USA, where the trials were carried out, have a high prevalence of sensitivity to *M. intracellulare* and other environmental mycobacteria. The hypothesis that exposure to environmental mycobacteria before or after BCG induces an immune response similar to that induced by BCG, so that BCG can add little, has been supported by animal and human population studies [2;36]. More recent immunogenicity studies suggest exposure to non-tuberculous mycobacterial antigens could also block BCG vaccination offering protection when infection precedes vaccination[37]. Our findings are

consistent with these hypotheses: perhaps more consistent with the latter, BCG being more effective in immunologically naive individuals.

Because of the evidence that BCG protects against miliary and meningeal tuberculosis, in developing countries BCG vaccination is recommended at birth (or first contact with health services), taking into account HIV status [38]. Our systematic review suggests that BCG also confers protection against pulmonary disease, the greatest burden from tuberculosis, when administered both in infancy and at school age, providing that children are not already infected with *M tuberculosis* or sensitised to other mycobacterial infections. Protection against pulmonary disease was seen in the Bombay Infants trial suggesting that, even close to the equator, if BCG is administered prior to exposure to tuberculosis and environmental mycobacteria it can provide significant protection [27]. Further evidence of protection in populations close to the equator from BCG given before infection would strengthen these findings. These possible explanations for the observed variation in protection from BCG vaccine have implications for the evaluation of new tuberculosis vaccines[39]. If given in conjunction with BCG, new vaccines must be shown to offer additional protection against pulmonary disease. New "BCG-like" vaccines may only give protection if administered prior to exposure to M *tuberculosis*[40].

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Conflict of Interest

All authors report no conflicts of interests.

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Table 1- Characteristics of included trials of BCG vaccine against pulmonary and miliary or meningeal tuberculosis.

Trial (First author)	Years (start of entry to end of follow-up)	Number BCG vaccinated/ Number unvaccinated	Latitude band (distance from equator)	Age at vaccination and tuberculin testing stringency (where applicable)	Likelihood of diagnostic detection bias	Vaccine strain
Saskatchewan Infants (Ferguson)[30]*	1933 -1948	306/303	50°+	Neonatal	lower	Frappier/Pasteur 450-S1, 468-S1
Native American (Aronson)[25]*	1935-1998	1551/1457	40°-50°	School Age, Stringent	lower	Phipps/Pasteur 317 used at US sites; Pasteur 575 used at Alaskan sites
Chicago Infants CCH (Rosenthal)[20]*	1937-1960	5426/4128	40°-50°	Neonatal	lower	Pasteur, Tice
Turtle and Rosebud Infants (Aronson)[17]	1938-1946	123/139	40°-50°	Neonatal	lower	Phipps,
Chicago Infants (TT HH) (Rosenthal)[20]	1941 -1953	311/250	40°-50°	Neonatal	lower	Pasteur Tice
Ida B. Wells Housing Project (Rosenthal)[16]	1942- 1956	699/625	40°-50°	School Age, Stringent	lower	Pasteur, Tice
US Mental Health Patients (Rosenthal)[16]	1944-1948	20/15	30°-40°	Other Age, Stringent	higher	Pasteur, Tice
Illinois Mentally Handicapped (Bettag)[22]	1947-1959	531/494	40°-50°	Other Age, Stringent	higher	Not specified
Georgia (School) (Shaw)[21]	1947-1967	2498/2341	30°-40°	School Age, Stringent	higher	Tice 811K, 811L, 812E, 812L, 813E
Puerto Rico Children (Palmer)[24]*	1949-1968	50634/27338	10°-20°	School Age, Non Stringent	higher	Phipps
Madanapelle (Frimodt-Moller)[29]	1950-1971	5069/5803	10°-20°	Other Age, Stringent	lower	Danish/Copenhagen
Georgia/Alabama (Palmer)[24]*	1950-1970	16913/17854	30°-40°	Other Age, Non Stringent	higher	Tice
MRC (MRC)[31]*	1950-1970	20800/13300	50°+	School Age, Stringent	lower	Danish/Copenhagen
African Gold Miners (Coetzee)[32]	1965-1968	8317/7997	20°-30	Other Age, Non Stringent	lower	Glaxo
Haiti (Vandivière)[33]	1965-1968	641/340	10°-20°	Other Age, Non Stringent	lower	Frappier/Montreal, 1202-
Chingleput (TBPT)[28]	1968-1983	73459/36404	10°-20°	Other Age, Non Stringent	lower	Danish/Copenhagen/1331, Paris/Pasteur- 1173 P2
Bombay Infants (Mehta)[27]	1976**	396/300	10°-20°	Neonatal	lower	Danish/Copenhagen
Agra (Mehrotra)[26]	1988**	1259/1259	20°-30°	School Age, Non Stringent	lower	Not specified

CCH: County Cook Hospital; MRC: Medical Research Council; TT HH: Tuberculous households

* Miliary and/or meningeal outcomes reported as well as pulmonary disease outcomes

** Date of study publication was used if study start date was not available.)

Table 2 - Ratios of rate ratios comparing pulmonary tuberculosis among vaccinated and unvaccinated individuals, estimated using meta-regression.

	No. of trials	Rate ratio[8] (95% CI)	Univariable model Ratio of rate ratios (95% CI)	p-value[3]	τ^2	Two-variable model Ratio of Rate Ratios[1] (95% CI)	p-value	τ^2	Multivariable model (τ^2 =0) Ratio of Rate Ratios[2] (95% CI)	p-value
Latitude										
40°+	8	0.31 (0.21- 0.46)	1.00 (ref)			Included in all models			1.00 (ref)	
20°-40°	5	0.68 (0.41- 1.13)	2.17 (1.14- 4.10)						1.17 (0.58- 2.36)	
0°-20°	5	0.77 (0.52- 1.13)	2.45 (1.42- 4.21)	0.008	0.086				1.73 (0.93 -3.25)	0.054[4]
Age at Vaccination / Tuberculin Testing Stringency										
Neonatal	5	0.39 (0.24- 0.64)	1.00 (ref)			1.00 (ref)			1.00 (ref)	
School age / stringent	4	0.26 (0.17- 0.40)	0.66 (0.35- 1.25)			0.74 (0.52- 2.67)			0.76 (0.45- 1.26)	
School age / non stringent	2	0.62 (0.38- 1.01)	1.58 (0.80- 3.13)			1.29 (0.64- 2.61)			0.80 (0.37- 1.72)	
Other age / stringent	3	0.94 (0.51- 1.73)	2.38 (1.09- 5.18)			1.83 (0.85- 3.92)			1.60 (0.82- 3.12)	
Other age / non stringent	4	0.85 (0.58- 1.24)	2.16 (1.17- 3.98)	0.003	0.044	1.90 (0.97- 3.73)	0.064[4]	0.000	1.75 (0.98- 3.15)	0.013[4]
Diagnostic Detection Bias										
Lower risk of bias	13	0.43 (0.30- 0.62)	1.00 (ref)			1.00 (ref)			1.00 (ref)	
Higher risk of bias	5	0.95 (0.50- 1.81)	2.22 (1.10- 4.60)	0.036	0.232	1.71 (0.93- 3.14)	0.077[4]	0.114	1.60 (1.01- 2.54)	0.045[4]
Was the allocation sequence adequately generated?										
Lower risk of bias	1	1.05 (0.35- 3.11)	1.00 (ref)			1.00 (ref)				
Higher risk of bias	17	0.48 (0.34- 0.68)	0.46 (0.15- 1.44)	0.169	0.253	0.64 (0.29- 1.43)	0.255[4]	0.078		
Was treatment allocation adequately concealed?										
Lower risk of bias	3	0.56 (0.22- 1.41)	1.00 (ref)			1.00 (ref)				
Higher risk of bias	15	0.51 (0.34- 0.75)	0.92 (0.34- 2.49)	0.856	0.303	0.86 (0.40- 1.83)	0.670[4]	0.091		
Was knowledge of the allocated intervention prevented during the study?										
Lower risk of bias	3	0.45 (0.20- 1.02)	1.00 (ref)			1.00 (ref)				
Higher risk of bias	15	0.53 (0.36- 0.80)	1.19 (0.48- 2.96)	0.691	0.319	1.05 (0.48- 2.05)	0.867[4]	0.128		
Are reports of the study free from the suggestion of selective outcome reporting?										
Lower risk of bias	17	0.50 (0.34- 0.72)	1.00 (ref)			1.00 (ref)				
Higher risk of bias	1	0.81 (0.23- 2.84)	1.62 (0.44- 5.98)	0.445	0.299	1.09 (0.39- 3.05)	0.860[4]	0.120		
Was ascertainment of cases complete?										
Lower risk of bias	15	0.51 (0.34- 0.74)	1.00 (ref)			1.00 (ref)				
Higher risk of bias	3	0.59 (0.23- 1.53)	1.17 (0.42- 3.24)	0.756	0.310	0.80 (0.37- 1.74)	0.551[4]	0.103		
BCG Strain[5,6]										
DU1-DU2-IV	2	0.51 (0.20- 1.32)	1.00 (ref)			1.00 (ref)				
DU2-III	5	0.59 (0.32- 1.10)	1.15 (0.37 - 3.54)			0.90 (0.48- 1.73)				
DU2-IV	11	0.42 (0.25- 0.73)	0.83 (0.28 - 2.45)			0.96 (0.51- 1.81)				
Other[7]	2	0.75 (0.25- 2.31)	1.47 (0.34 - 6.28)	0.727	0.379	1.54 (0.55- 4.28)	0.011[4]	0.089		

CI: confidence interval; ref: reference category, τ^2: estimated between-study variance; 1: Adjusted for latitude category; 2:Adjusted for all other variables in the model; 3. : Overall P-value for the model for the test of the hypothesis that none of the covariates are associated with the overall BCG efficacy 4. The p-value is for the test of the null hypothesis that there is no association between the covariate and the overall BCG efficacy.5. Categories derived from Bronsch et al (2007) [12] 6. Two trials reported results stratified according to strain; 7. Not possible to identify the strain used;.8 estimated effects displayed in Fig 2 differ from those here, because of the difference between meta-regression and stratified random-effects meta-analysis

Figure legends

Figure 1. Rate Ratios for pulmonary tuberculosis, stratified by age vaccinated and stringency of pre-vaccination tuberculin testing.

Legend: Trials included in this review, ordered by year of study start with rate ratios (RR) and 95% confidence intervals (95% CIs). The "other" age group includes studies in which older persons were vaccinated as well as those in which BCG was given at any age. (CCH: Cook County Hospital; D+L: DerSimonian and Laird method; M-H: Mantel-Haenszel method; MRC: Medical Research Council; PY: Person-Years; TB HH: Tuberculosis Households; TBPT: Tuberculosis Prevention Trial; *Date of study publication was used if study start date was not available).

Figure 2: Rate ratios (95% CIs) for pulmonary tuberculosis, stratified by latitude of study location.

Legend: Ordered by year of study start. *Date of study publication was used if study start date was not available. CCH: Cook County Hospital; D+L: DerSimonian and Laird method; M-H: Mantel-Haenszel method; MRC: Medical Research Council; PY: Person-Years; TB HH: Tuberculosis Households; TBPT: Tuberculosis Prevention Trial.

Figure 3. Pooled rate ratios for pulmonary tuberculosis, estimated using random-effects meta-analysis, according to trial characteristics.

Legend: Rate ratios (RR) and 95% confidence intervals (95% CIs)

Figure 4: Scatter plot of estimated rate ratios for pulmonary tuberculosis, according to year of study start and BCG strain category

Legend: (DU1-DU2-IV: Tandem duplication 1 and fourth form of tandem duplication 2; DU2-III: Third form of tandem duplication 2; DU2-IV: Fourth form of tandem duplication 2, according to Brosch *et al*. [53]). The efficacy data for two trials (Native American [2] and Chingleput [45]), were provided for two different strains of BCG, accounting for two extra sets of results in this graph

Figure 5: Rate ratios (95% CIs) for meningeal and/or miliary tuberculosis, stratified by age at vaccination and tuberculin testing stringency.

Legend: Pooled results from fixed effects meta-analysis only as the numbers of studies were small, ordered by year of study start. (CCH: Cook County Hospital; D+L: DerSimonian and Laird method; M-H: Mantel-Haenszel method; MRC: Medical Research Council; PY: Person-Years; TB HH: Tuberculosis Households; * the outcome is miliary tuberculosis only).