# Protection by BCG Vaccine Against Tuberculosis: A Systematic Review of Randomized Controlled Trials

**Punam Mangtani,[1] Ibrahim Abubakar,[2,3] Cono Ariti,[1] Rebecca Beynon,[4] Laura Pimpin,[2,5] Paul E. M. Fine,[1] Laura C. Rodrigues,[1] Peter G. Smith,[1] Marc Lipman,[6] Penny F. Whiting,[4] and Jonathan A. Sterne[4]**

[1]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine; [2]Centre for Infectious Disease Surveillance and Control, Public Health England, London; [3]Centre for Infectious Disease Epidemiology, University College London; [4]School of Social and Community Medicine, University of Bristol; [5]Medical Research Council Human Nutrition Research, University of Cambridge; and [6]Centre for Respiratory Medicine, Royal Free Campus, University College London, United Kingdom

**(See the Editorial Commentary by McShane on pages 481–2.)**

***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 tuberculosis and 6 reporting miliary or meningeal tuberculosis. Univariable meta-regression indicated efficacy against pulmonary tuberculosis varied according to 3 characteristics. Protection appeared greatest in children stringently tuberculin tested, to try to exclude prior infection with *Mycobacterium tuberculosis* or sensitization to environmental mycobacteria (rate ratio [RR], 0.26; 95% confidence interval [CI], .18–.37), or infants (RR, 0.41; 95% CI, .29–.58). Protection was weaker in children not stringently tested (RR, 0.59; 95% CI, .35–1.01) and older individuals stringently or not stringently tested (RR, 0.88; 95% CI, .59–1.31 and RR, 0.81; 95% CI, .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, .01–.77) and children stringently tuberculin tested (RR, 0.08; 95% CI, .03–.25).

***Conclusions.*** Absence of prior *M. tuberculosis* infection or sensitization 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.

***Keywords.*** BCG vaccine; meta-analysis; trials; meta-regression; vaccine efficacy.

The BCG vaccine is included in the childhood vaccination program 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 (MRC) trial [3–5]. Consistently high estimates of efficacy have been reported for infant BCG vaccination against severe primary progressive disease [6–8].

Previous reviews noted a positive association between BCG vaccine efficacy against pulmonary disease with distance from the equator at which studies were conducted

DOI: 10.1093/cid/cit790

[2, 9], possibly related to exposure to environmental mycobacteria, which is, in general, less common at locations distant from the equator [1]. Consistent with this hypothesis, a recent subanalysis of the Chingleput trial suggested some protection (29% efficacy) among participants who had low tuberculin reactivity and no reaction to nontuberculous 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, to estimate the efficacy of BCG against pulmonary, miliary, and meningeal tuberculosis and examine associations of study characteristics, including immunological naivety 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 revaccination) was compared with no vaccination (placebo or other control). We excluded non-BCG tuberculosis vaccines (eg, vole bacillus, Savioli antituberculosis 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 1 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 (Medical Subject Headings) 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, and 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, 1 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°-latitude groups for analysis) and whether tests for tuberculin sensitivity (a marker of prior *Mycobacterium tuberculosis* infection as well as some indication of sensitization 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 to be tuberculin negative. A stringent tuberculin testing protocol was defined as retesting initially tuberculin-negative participants using a higher dose of tuberculin to confirm negativity before vaccination. A nonstringent tuberculin testing protocol was defined as one that did not exclude noninfant participants based on tuberculin testing prior to vaccination, or which excluded subjects based only on 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 3 groups proposed. We also tested a hypothesis that there would be a loss of protection as BCG strains evolved over time.

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 vaccination 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 that 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 radiography or other assessments were judged to be at a 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 randomized groups in

a trial had 0 cases, 0.5 was added to each cell of the 2 × 2 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 RRs against the year the study started.

Differences in efficacy between subgroups 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 $\tau^2$. In forest plots and meta-analyses, $\tau^2$ was estimated using the method-of-moments estimator proposed by DerSimonian and Laird. For meta-regression analyses, $\tau^2$ was estimated by restricted maximum likelihood, using the metareg command in Stata.

**Table 1. Characteristics of Included Trials of Bacille de Calmette et Guérin Vaccine Against Pulmonary and Miliary or Meningeal Tuberculosis**

| Trial (First Author) | Years, Start of Entry to End of Follow-up | No. BCG Vaccinated/ No. 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][a] | 1933–1948 | 306/303 | >50° | Neonatal | Lower | Frappier/Pasteur 450-S1, 468-S1 |
| Native American (Aronson) [25][a] | 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][a] | 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 (TB 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][a] | 1949–1968 | 50 634/27 338 | 10°–20° | School age, nonstringent | Higher | Phipps |
| Madanapelle (Frimodt-Moller) [29] | 1950–1971 | 5069/5803 | 10°–20° | Other age, stringent | Lower | Danish/Copenhagen |
| Georgia/Alabama (Palmer) [24][a] | 1950–1970 | 16 913/17 854 | 30°–40° | Other age, nonstringent | Higher | Tice |
| MRC (MRC) [31][a] | 1950–1970 | 20 800/13 300 | >50° | School age, stringent | Lower | Danish/Copenhagen |
| African Gold Miners (Coetzee) [32] | 1965–1968 | 8317/7997 | 20°–30 | Other age, nonstringent | Lower | Glaxo |
| Haiti (Vandiviere) [33] | 1965–1968 | 641/340 | 10°–20° | Other age, nonstringent | Lower | Frappier/Montreal, 1202- |
| Chingleput (Baily) [28] | 1968–1983 | 73 459/36 404 | 10°–20° | Other age, nonstringent | Lower | Danish/Copenhagen/ 1331, Paris/Pasteur-1173 P2 |
| Bombay Infants (Mehta) [27] | 1976[b] | 396/300 | 10°–20° | Neonatal | Lower | Danish/Copenhagen |
| Agra (Mehrotra) [26] | 1988[b] | 1259/1259 | 20°–30° | School age, nonstringent | Lower | Not specified |

Abbreviations: BCG, Bacille de Calmette et Guérin; CCH, County Cook Hospital; MRC, Medical Research Council; TB HH, tuberculous households.

[a] Miliary and/or meningeal outcomes reported as well as pulmonary disease outcomes.

[b] Date of study publication was used if study start date was not available.

| Trial | RR (95% CI) | BCG cases/PY's | Unvaccinated cases/PY's |
|---|---|---|---|
| **Neonatal vaccination** | | | |
| Saskatchewan Infants (Ferguson) 1933 Canada | 0.20 (0.06, 0.69) | 3/2162 | 14/2000 |
| Chicago Infants CCH (Rosenthal) 1937 USA | 0.34 (0.19, 0.60) | 16/15618 | 48/15822 |
| Turtle and Rosebud Infants (Aronson) 1938 USA | 0.40 (0.13, 1.25) | 4/968 | 11/1060 |
| Chicago Infants TB HH (Rosenthal) 1941 USA | 0.28 (0.08, 1.02) | 3/1374 | 11/1424.5 |
| Bombay Infants (Mehta) 1976* India | 0.62 (0.35, 1.07) | 22/962.5 | 27/716.25 |
| Fixed effect Within stratum (Heterogeneity: $P$ = .387) | 0.40 (0.28, 0.56) | | |
| Random effects Within stratum ($\tau^2$ = .0065) | 0.41 (0.29, 0.58) | | |
| **School age vaccination - stringent tuberculin testing** | | | |
| Native American (Aronson) 1935 USA | 0.26 (0.20, 0.33) | 79/66716 | 267/58219 |
| Ida B. Wells Housing Project (Rosenthal) 1942 USA | 0.15 (0.01, 2.87) | 0/7770 | 3/8050 |
| Georgia School (Shaw) 1947 USA | 1.25 (0.28, 5.58) | 4/29830 | 3/27966 |
| MRC (MRC) 1950 UK | 0.22 (0.16, 0.31) | 40/210800 | 163/188800 |
| Fixed effect Within stratum (Heterogeneity: $P$ = .160) | 0.25 (0.21, 0.31) | | |
| Random effects Within stratum ($\tau^2$ = .048) | 0.26 (0.18, 0.37) | | |
| **School age vaccination - non stringent tuberculin testing** | | | |
| Puerto Rico Children (Palmer) 1949 USA | 0.72 (0.57, 0.90) | 168/944520 | 127/510872 |
| Agra (Mehrotra) 1988* India | 0.40 (0.19, 0.83) | 10/6270 | 25/6232.5 |
| Fixed effect Within stratum (Heterogeneity: $P$ = .135) | 0.67 (0.54, 0.84) | | |
| Random effects Within stratum ($\tau^2$ = .095) | 0.59 (0.35, 1.01) | | |
| **Other age vaccination - stringent tuberculin testing** | | | |
| US Mental Health Patients (Rosenthal) 1944 USA | 0.26 (0.01, 6.36) | 0/80 | 1/62 |
| Illinois Mentally Handicapped (Bettag) 1947 USA | 1.40 (0.57, 3.42) | 12/6300 | 8/5880 |
| Madanapalle (Frimodt-Moller) 1950 India | 0.81 (0.52, 1.26) | 33/61167 | 47/70200 |
| Fixed effect Within stratum (Heterogeneity: $P$ = .420) | 0.88 (0.60, 1.30) | | |
| Random effects Within stratum ($\tau^2$ = 0) | 0.88 (0.59, 1.31) | | |
| **Other age vaccination - non stringent tuberculin testing** | | | |
| Georgia/Alabama (Palmer) 1950 USA | 0.92 (0.56, 1.53) | 28/256585 | 32/270870 |
| African Gold Miners (Coetzee) 1965 South Africa | 0.63 (0.39, 1.00) | 29/7226 | 45/7036 |
| Haiti (Vandiviere) 1965 Haiti | 0.11 (0.01, 0.91) | 1/1903.5 | 5/1006.5 |
| Chingleput Madras (TBPT) 1968 India | 1.05 (0.88, 1.25) | 380/754718 | 180/373978 |
| Fixed effect Within stratum (Heterogeneity: $P$ = .043) | 0.96 (0.82, 1.12) | | |
| Random effects Within stratum ($\tau^2$ = .091) | 0.81 (0.55, 1.22) | | |
| Fixed effect Overall (Heterogeneity: $P$ < .0005) | 0.56 (0.51, 0.62) | | |
| Random effects Overall ($\tau^2$ = .38) | 0.50 (0.35, 0.72) | | |

Rate ratio — BCG reduces risk of TB / BCG increases risk of TB (axis: .01 .1 .2 .5 1 2 5 10 100)
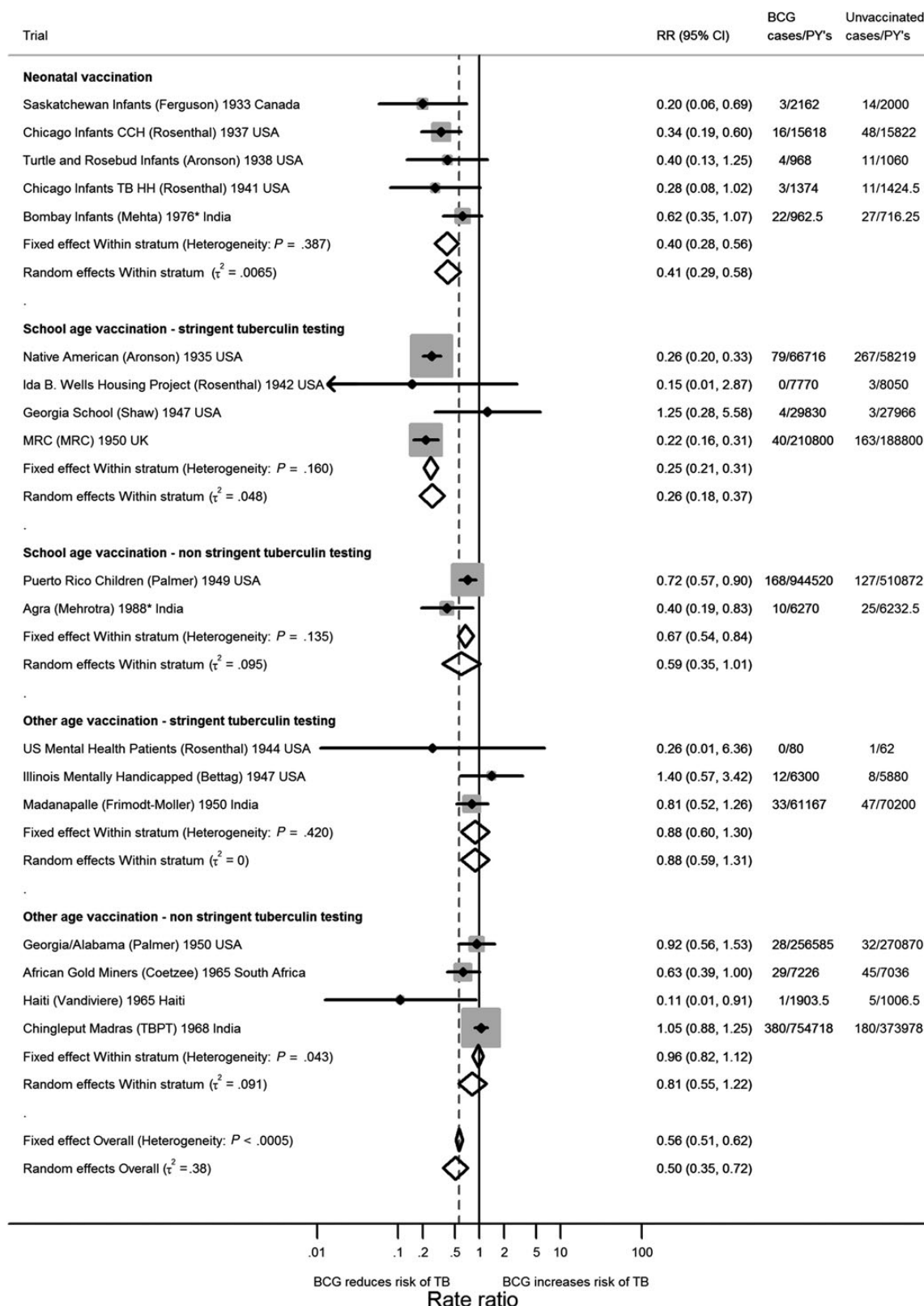
**Figure 1.** Rate ratios and 95% confidence intervals for pulmonary tuberculosis, stratified by age vaccinated and stringency of prevaccination tuberculin testing. Trials included in this review, ordered by year of study start. The "other" age group includes studies in which older persons were vaccinated as well as those in which BCG was given at any age. *Date of study publication was used if study start date was not available. Abbreviations: BCG, Bacille de Calmette et Guérin; CCH, Cook County Hospital; CI, confidence interval; D + L, DerSimonian and Laird method; M-H, Mantel-Haenszel method; MRC, Medical Research Council; PY, person-years; RR, rate ratio; TB, tuberculosis; TB HH, tuberculous households; TBPT, Tuberculosis Prevention Trial.
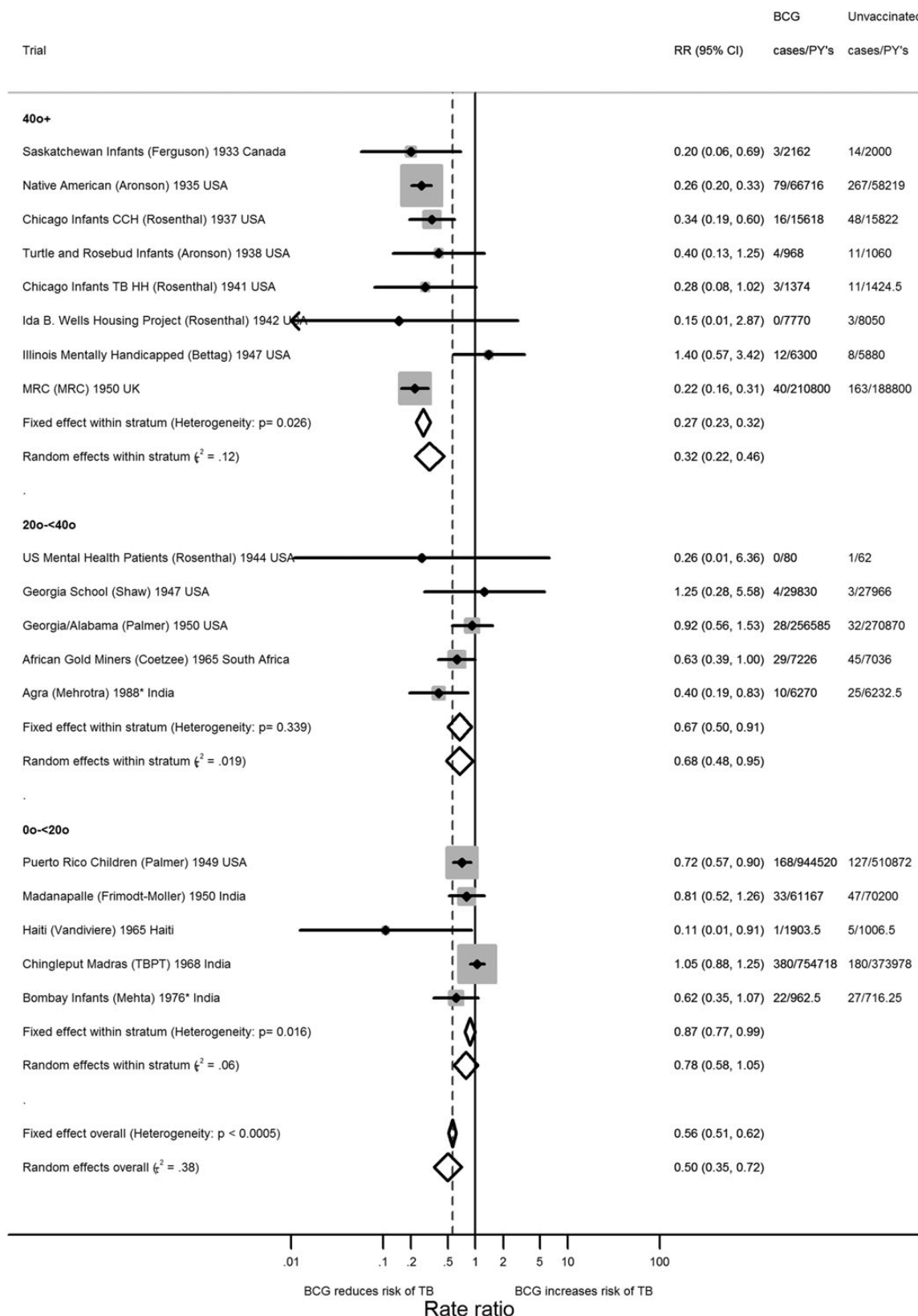
| Trial | RR (95% CI) | BCG cases/PY's | Unvaccinated cases/PY's |
|---|---|---|---|
| **40o+** | | | |
| Saskatchewan Infants (Ferguson) 1933 Canada | 0.20 (0.06, 0.69) | 3/2162 | 14/2000 |
| Native American (Aronson) 1935 USA | 0.26 (0.20, 0.33) | 79/66716 | 267/58219 |
| Chicago Infants CCH (Rosenthal) 1937 USA | 0.34 (0.19, 0.60) | 16/15618 | 48/15822 |
| Turtle and Rosebud Infants (Aronson) 1938 USA | 0.40 (0.13, 1.25) | 4/968 | 11/1060 |
| Chicago Infants TB HH (Rosenthal) 1941 USA | 0.28 (0.08, 1.02) | 3/1374 | 11/1424.5 |
| Ida B. Wells Housing Project (Rosenthal) 1942 USA | 0.15 (0.01, 2.87) | 0/7770 | 3/8050 |
| Illinois Mentally Handicapped (Bettag) 1947 USA | 1.40 (0.57, 3.42) | 12/6300 | 8/5880 |
| MRC (MRC) 1950 UK | 0.22 (0.16, 0.31) | 40/210800 | 163/188800 |
| Fixed effect within stratum (Heterogeneity: p= 0.026) | 0.27 (0.23, 0.32) | | |
| Random effects within stratum ($t^2$ = .12) | 0.32 (0.22, 0.46) | | |
| **20o-<40o** | | | |
| US Mental Health Patients (Rosenthal) 1944 USA | 0.26 (0.01, 6.36) | 0/80 | 1/62 |
| Georgia School (Shaw) 1947 USA | 1.25 (0.28, 5.58) | 4/29830 | 3/27966 |
| Georgia/Alabama (Palmer) 1950 USA | 0.92 (0.56, 1.53) | 28/256585 | 32/270870 |
| African Gold Miners (Coetzee) 1965 South Africa | 0.63 (0.39, 1.00) | 29/7226 | 45/7036 |
| Agra (Mehrotra) 1988* India | 0.40 (0.19, 0.83) | 10/6270 | 25/6232.5 |
| Fixed effect within stratum (Heterogeneity: p= 0.339) | 0.67 (0.50, 0.91) | | |
| Random effects within stratum ($t^2$ = .019) | 0.68 (0.48, 0.95) | | |
| **0o-<20o** | | | |
| Puerto Rico Children (Palmer) 1949 USA | 0.72 (0.57, 0.90) | 168/944520 | 127/510872 |
| Madanapalle (Frimodt-Moller) 1950 India | 0.81 (0.52, 1.26) | 33/61167 | 47/70200 |
| Haiti (Vandiviere) 1965 Haiti | 0.11 (0.01, 0.91) | 1/1903.5 | 5/1006.5 |
| Chingleput Madras (TBPT) 1968 India | 1.05 (0.88, 1.25) | 380/754718 | 180/373978 |
| Bombay Infants (Mehta) 1976* India | 0.62 (0.35, 1.07) | 22/962.5 | 27/716.25 |
| Fixed effect within stratum (Heterogeneity: p= 0.016) | 0.87 (0.77, 0.99) | | |
| Random effects within stratum ($t^2$ = .06) | 0.78 (0.58, 1.05) | | |
| Fixed effect overall (Heterogeneity: p < 0.0005) | 0.56 (0.51, 0.62) | | |
| Random effects overall ($t^2$ = .38) | 0.50 (0.35, 0.72) | | |

Rate ratio axis: .01  .1  .2  .5  1  2  5  10  100

BCG reduces risk of TB — BCG increases risk of TB

**Rate ratio**

**Figure 2.** Rate ratios and 95% confidence intervals for pulmonary tuberculosis, stratified by latitude of study location. Ordered by year of study start. *Date of study publication was used if study start date was not available. Abbreviations: BCG, Bacille de Calmette et Guérin; CCH, Cook County Hospital; CI, confidence interval; D + L, DerSimonian and Laird method; M-H, Mantel-Haenszel method; MRC, Medical Research Council; PY, person-years; RR, rate ratio; TB, tuberculosis; TB HH, tuberculous households; TBPT, Tuberculosis Prevention Trial.

# RESULTS

From 21 030 titles and abstracts, we identified 847 articles for retrieval. We included 211 relevant papers (60 in languages other than English). These articles reported data on 21 randomized or quasi-randomized trials (Supplementary Figure 1), of which 18 reported on pulmonary tuberculosis and 6 reported on meningeal and/or miliary tuberculosis outcomes. Ten trials were conducted in the United States between 1933 and 1950 [16–25]; 4 in India between 1950 and 1988 [26–29]; and 1 each in Canada (started in 1933) [30], the United Kingdom (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% confidence interval [CI], .16–.31), to absence of clinically important benefit, in the Chingleput trial [28] (RR, 1.05; 95% CI, .88–1.25). Figure 1 shows the ratio of the rates of pulmonary tuberculosis among BCG-vaccinated individuals and controls in each trial, stratified according to age at vaccination and stringency of prevaccination 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 $\tau^2$ <0.095) than overall ($\tau^2$ = 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, .18–.37) and studies of neonatal vaccination (RR, 0.41; 95% CI, .29–.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 farthest from the equator. Although estimated between-trial heterogeneity was lower within latitude strata than overall, there was evidence of heterogeneity between trials at >40° latitude ($\tau^2$ = 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, .25–.64) than in trials with higher likelihood of diagnostic detection bias (RR, 0.78; 95% CI, .64–.95) (Figure 3).

When trials were stratified according to BCG strain lineage, there was substantial between-trial heterogeneity within each stratum, and 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
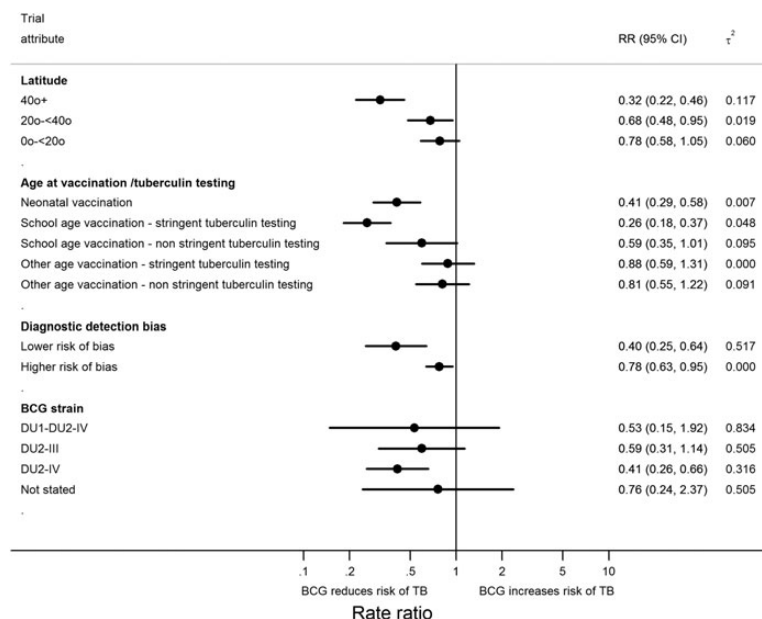


**Figure 3.** Pooled rate ratios for pulmonary tuberculosis, estimated using random-effects meta-analysis, according to trial characteristics. Rate ratios and 95% confidence intervals are shown. Abbreviations: BCG, Bacille de Calmette et Guérin; DU, duplication; TB, tuberculosis.

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 $\tau^2 = 0.086$ 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°–20° and 20°–40° latitude, compared with those conducted at >40° 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 2-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 $\tau^2 = 0$). 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 RRs were attenuated compared with univariable analyses, but each of these characteristics was separately associated with the effect of BCG, having accounted for the other 2.

### Protection Against Meningeal or Miliary Tuberculosis

The 6 trials that reported on meningeal and miliary tuberculosis found substantial protection by BCG (RR, 0.15; 95% CI, .08–.31), with little evidence of between-trial heterogeneity (P = .14, Figure 5). Protection appeared greatest in the 2 trials of neonatal vaccination (RR, 0.10; 95% CI, .01–.77) and the 2 trials of school-age vaccination with stringent tuberculin testing (RR, 0.08; 95% CI, .03–.25). The 2 trials with non-stringent tuberculin testing (1 at school age and 1 at a range of ages) found little evidence of protection. However, ratios of RRs 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 3 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 sensitized 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 of 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



**Figure 4.** Scatter plot of estimated rate ratios for pulmonary tuberculosis, according to year of study start and BCG strain category. 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 [12]). The efficacy data for 2 trials (Native American [25] and Chingleput [28]) were provided for 2 different strains of BCG, accounting for 2 extra sets of results in this graph. Abbreviations: BCG, Bacille de Calmette et Guérin; DU, duplication.

**Table 2. Ratios of Rate Ratios Comparing Pulmonary Tuberculosis Among Vaccinated and Unvaccinated Individuals, Estimated Using Meta-regression**

| Study Characteristics | No. of Trials | Rate Ratio[a] (95% CI) | Univariable Model Ratio of Rate Ratios (95% CI) | $P$ Value[b] | $\tau^2$ | Two-Variable Model Ratio of Rate Ratios[c] (95% CI) | $P$ Value | $\tau^2$ | Multivariable Model ($\tau^2=0$) Ratio of Rate Ratios[d] (95% CI) | $P$ Value |
|---|---|---|---|---|---|---|---|---|---|---|
| Latitude | | | | | | | | | | |
| >40° | 8 | 0.31 (.21–.46) | 1.00 (ref) | | | Included in all models | | | 1.00 (ref) | |
| 20°–40° | 5 | 0.68 (.41–1.13) | 2.17 (1.14–4.10) | | | | | | 1.17 (.58–2.36) | |
| 0°<20° | 5 | 0.77 (.52–1.13) | 2.45 (1.42–4.21) | .008 | 0.086 | | | | 1.73 (.93–3.25) | .054[e] |
| Age at vaccination/tuberculin/testing stringency | | | | | | | | | | |
| Neonatal | 5 | 0.39 (.24–.64) | 1.00 (ref) | | | 1.00 (ref) | | | 1.00 (ref) | |
| School age/stringent | 4 | 0.26 (.17–.40) | 0.66 (.35–1.25) | | | 0.74 (.52–2.67) | | | 0.76 (.45–1.26) | |
| School age/nonstringent | 2 | 0.62 (.38–1.01) | 1.58 (.80–3.13) | | | 1.29 (.64–2.61) | | | 0.80 (.37–1.72) | |
| Other age/stringent | 3 | 0.94 (.51–1.73) | 2.38 (1.09–5.18) | | | 1.83 (.85–3.92) | | | 1.60 (.82–3.12) | |
| Other age/nonstringent | 4 | 0.85 (.58–1.24) | 2.16 (1.17–3.98) | .003 | 0.044 | 1.90 (.97–3.73) | .064[e] | 0.000 | 1.75 (.98–3.15) | .013[e] |
| Diagnostic detection bias | | | | | | | | | | |
| Lower risk of bias | 13 | 0.43 (.30–.62) | 1.00 (ref) | | | 1.00 (ref) | | | 1.00 (ref) | |
| Higher risk of bias | 5 | 0.95 (.50–1.81) | 2.22 (1.10–4.60) | .036 | 0.232 | 1.71 (.93–3.14) | .077[e] | 0.114 | 1.60 (1.01–2.54) | .045[e] |
| Was the allocation sequence adequately generated? | | | | | | | | | | |
| Lower risk of bias | 1 | 1.05 (.35–3.11) | 1.00 (ref) | | | 1.00 (ref) | | | | |
| Higher risk of bias | 17 | 0.48 (.34–.68) | 0.46 (.15–1.44) | .169 | 0.253 | 0.64 (.29–1.43) | .255[e] | 0.078 | | |
| Was treatment allocation adequately concealed? | | | | | | | | | | |
| Lower risk of bias | 3 | 0.56 (.22–1.41) | 1.00 (ref) | | | 1.00 (ref) | | | | |
| Higher risk of bias | 15 | 0.51 (.34–.75) | 0.92 (.34–2.49) | .856 | 0.303 | 0.86 (.40–1.83) | .670[e] | 0.091 | | |
| Was knowledge of the allocated intervention prevented during the study? | | | | | | | | | | |
| Lower risk of bias | 3 | 0.45 (.20–1.02) | 1.00 (ref) | | | 1.00 (ref) | | | | |
| Higher risk of bias | 15 | 0.53 (.36–.80) | 1.19 (.48–2.96) | .691 | 0.319 | 1.05 (.48–2.05) | .867[e] | 0.128 | | |
| Are reports of the study free from the suggestion of selective outcome reporting? | | | | | | | | | | |
| Lower risk of bias | 17 | 0.50 (.34–.72) | 1.00 (ref) | | | 1.00 (ref) | | | | |
| Higher risk of bias | 1 | 0.81 (.23–2.84) | 1.62 (.44–5.98) | .445 | 0.299 | 1.09 (.39–3.05) | .860[e] | 0.120 | | |
| Was ascertainment of cases complete? | | | | | | | | | | |
| Lower risk of bias | 15 | 0.51 (.34–.74) | 1.00 (ref) | | | 1.00 (ref) | | | | |
| Higher risk of bias | 3 | 0.59 (.23–1.53) | 1.17 (.42–3.24) | .756 | 0.310 | 0.80 (.37–1.74) | .551[e] | 0.103 | | |
| BCG strain[f,g] | | | | | | | | | | |
| DU1-DU2-IV | 2 | 0.51 (.20–1.32) | 1.00 (ref) | | | 1.00 (ref) | | | | |
| DU2-III | 5 | 0.59 (.32–1.10) | 1.15 (.37–3.54) | | | 0.90 (.48–1.73) | | | | |
| DU2-IV | 11 | 0.42 (.25–.73) | 0.83 (.28–2.45) | | | 0.96 (.51–1.81) | | | | |
| Other[h] | 2 | 0.75 (.25–2.31) | 1.47 (.34–6.28) | .727 | 0.379 | 1.54 (.55–4.28) | .011[e] | 0.089 | | |

Abbreviations: BCG, Bacille de Calmette et Guérin; CI, confidence interval; DU, duplication; ref, reference category, $\tau^2$, estimated between-study variance.

[a] Estimated effects displayed in Figure 2 differ from those here, because of the difference between meta-regression and stratified random-effects meta-analysis.

[b] Overall $P$ value for the model for the test of the hypothesis that none of the covariates are associated with the overall BCG efficacy.

[c] Adjusted for latitude category.

[d] Adjusted for all other variables in the model.

[e] The $P$ value is for the test of the null hypothesis that there is no association between the covariate and the overall BCG efficacy.

[f] Categories derived from Bronsch et al [12].

[g] Two trials reported results stratified according to strain.
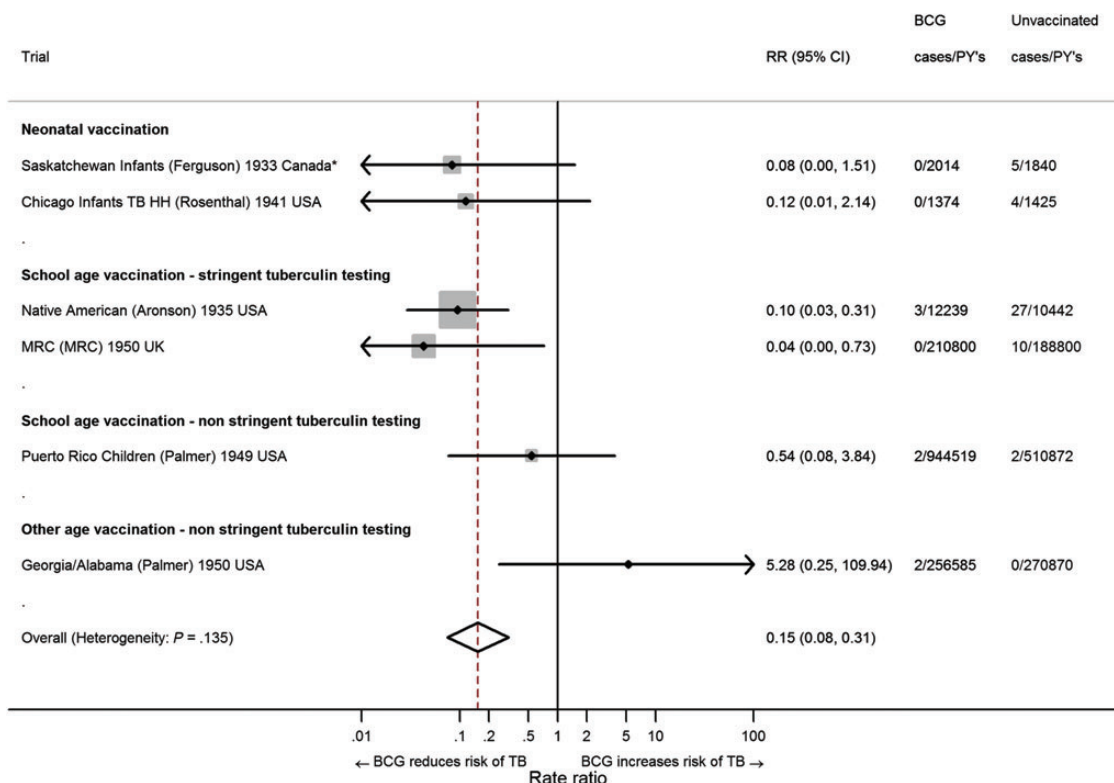
[h] Not possible to identify the strain used.

**Figure 5.** Rate ratios and 95% confidence intervals for meningeal and/or miliary tuberculosis, stratified by age at vaccination and tuberculin testing stringency. Pooled results from fixed effects meta-analysis only as the numbers of studies were small, ordered by year of study start. *Outcome is miliary tuberculosis only. Abbreviations: BCG, Bacille de Calmette et Guérin; CI, confidence interval; MRC, Medical Research Council; PY, person-years; RR, rate ratio; TB, tuberculosis; TB HH, tuberculosis households.

of trial design were not clearly reported. Previous systematic reviews (eg, [9]) of 13 trials reporting tuberculosis disease outcomes did not assess whether several of these design characteristics or the exclusion of those with prior infection or sensitization to environmental mycobacteria using stringent tuberculin testing were related to BCG protection. Based on comprehensive searches, we included the same 13 trials, and found 5 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 7 variables, which is large compared with the total number of studies (18). Therefore, our finding that 3 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 sensitization 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 1 review 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 that there are genetic differences between BCG vaccines, for example, based on restriction fragment-length polymorphism typing that suggests 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 a *Mycobacterium microti*–derived vaccine (vole bacillus) [5].

A possible explanation for the low protection observed in trials in the southern United States vs high protection in the United Kingdom was first proposed during the 1960s, based on guinea-pig studies [1]. The findings suggested that exposure to certain nontuberculous mycobacterial antigens could mask the observed effectiveness of BCG, by providing some protection against tuberculosis in nonvaccinated groups, which was not enhanced by BCG vaccination. The authors also noted that populations in the southern United States, 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 that exposure to nontuberculous mycobacterial antigens could also block BCG vaccination from 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 sensitized 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].

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

## References

1. Palmer CE, Long MW. Effects of infection with atypical mycobacteria on BCG vaccination and tuberculosis. Am Rev Respir Dis **1966**; 94: 553–68.
2. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. Lancet **1995**; 346:1339–45.
3. [No authors listed]. Trial of BCG vaccines in south India for tuberculosis prevention: First report 144. Bull World Health Organ **1979**; 57: 819–27.
4. Fine PE. BCG vaccination against tuberculosis and leprosy. Br Med Bull **1988**; 44:691–703.
5. Hart PD, Sutherland I. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life. Final report to the Medical Research Council. Br Med J **1977**; 2:293–5.
6. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. Lancet **2006**; 367:1173–80.
7. Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. Int J Epidemiol **1993**; 22:1154–8.
8. Colditz GA, Berkey CS, Mosteller F, et al. The efficacy of bacillus Calmette-Guerin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. Pediatrics **1995**; 96(1 Pt 1):29–35.
9. Colditz GA, Brewer TF, Berkey CS, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA **1994**; 271:698–702.

10. Tuberculosis Research Centre (ICMR). Influence of sex, age and nontuberculous infection at intake on the efficacy of BCG: re-analysis of 15-year data from a double-blind randomized control trial in south India. Indian J Med Res. 2006; 119–24.

11. Clemens JD, Chuong JJ, Feinstein AR. The BCG controversy. A methodological and statistical reappraisal. JAMA 1983; 249:2362–9.

12. Brosch R, Gordon SV, Garnier T, et al. Genome plasticity of BCG and impact on vaccine efficacy. Proc Natl Acad Sci U S A 2007; 104:5596–601.

13. Dye C, Fine PE. A major event for new tuberculosis vaccines. Lancet 2013; 381:972–4.

14. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011; 343:d5928.

15. Dersimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7:177–88.

16. Rosenthal SR, Leslie EI, Loewinsohn E. BCG vaccination in all age groups. Methods and results of a strictly controlled study. JAMA 1948; 136:73–9.

17. Aronson JD. Protective vaccination against tuberculosis with special reference to BCG vaccination. Am Rev Tuberc 1948; 58:255–81.

18. Rosenthal SR, Colbert MJ, Nikurs L. Tuberculin reaction trends and BCG vaccination. Special reference to young adults (medical students). Arch Envir Health 1965; 11:794–803.

19. Rosenthal SR, Afremow ML, Nikurs L, et al. BCG vaccination and tuberculosis in students of nursing. Am J Nurs 1963; 63:88–93.

20. Rosenthal SR, Blahd M, Leslif EI. Ten years' experience with BCG (experimental and clinical). J Pediatr 1945; 26:470–80.

21. Shaw LW. Field studies on immunization against tuberculosis. I. Tuberculin allergy following BCG vaccination of school children in Muscogee County, Georgia. Public Health Rep 1951; 66:1415–49.

22. Bettag OL, Kaluzny AA, Morse D, Radner DB. BCG study at a state school for mentally retarded. Chest 1964; 45:503–7.

23. Levine MI. Immunization against tuberculosis. A study of the essential factors. Am Rev Tuberc Pulm Dis 1938; 38:632–43.

24. Palmer CE. Community trials of BGG vaccination. Am Rev Tuberc Pulm Dis 1958;77:6–907.

25. Aronson NE, Santosham M, Comstock GW, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: a 60-year follow-up study. JAMA 2004; 291:2086–91.

26. Mehrotra AK, Dabral SB, Nandan D, et al. Assessment of efficacy of BCG vaccination among slum children of Agra city. Indian J Public Health 1988; 32:164–5.

27. Mehta KP, Merchant SM, Korde U. Environmental influence on immunity due to B.C.G. vaccination. Indian Pediatr 1976; 13:525–32.

28. Baily GVJ, Narain R, Mayurnath S. Tuberculosis prevention trial, Madras. Indian J Med Res 1980; 72(suppl):74.

29. Frimodt-Moller J. A community-wide tuberculosis study in a south Indian rural population, 1950–1955. Bull World Health Organ 1960; 22:61–170.

30. Ferguson RG, Simes AB. BCG vaccination of Indian infants in Saskatchewan. Tubercle 1949; 30:5–11.

31. [No authors listed]. B.C.G. and vole bacillus vaccines in the prevention of tuberculosis in adolescents; first [progress] report to the Medical Research Council by their Tuberculosis Vaccines Clinical Trials Committee. BMJ 1956; 1:413–27.

32. Coetzee AM, Berjak J. B.C.G. in the prevention of tuberculosis in an adult population. Proc Mine Med Officers Assoc 1968; 48:41–53.

33. Vandiviere HM, Dworksi M, Melvin IG. Efficacy of bacillus Calmette Guerin and isoniazid resistant bacillus Calmette Guerin with and without isoniazid chemoprophylaxis from day of vaccination. II. Field trial in man. Am Rev Respir Dis 1973; 108:301–13.

34. Thompson SG, Higgins JP. How should meta-regression analyses be undertaken and interpreted? Stat Med 2002; 21:1559–73.

35. Fine PEM, Carneiro IAM, Milstien JB, Clemens JD. Issues relating to the use of BCG in immunization programmes. Geneva, Switzerland: World Health Organization, 1999. Report No.: WHO/V&B/99.23.

36. Valadas E. Nontuberculous mycobacteria: clinical importance and relevance to bacille Calmette-Guerin vaccination. Clin Infect Dis 2004; 39:457–8.

37. Andersen P, Doherty TM. The success and failure of BCG—implications for a novel tuberculosis vaccine. Nat Rev Microbiol 2005; 3:656–62.

38. World Health Organization. Revised BCG vaccination guidelines for infants at risk for HIV infection. 82th ed. Geneva, Switzerland: World Health Organization, 2007:193.

39. Kaufmann SH, Hussey G, Lambert PH. New vaccines for tuberculosis. Lancet 2010; 375:2110–9.

40. Tameris MD, Hatherill M, Landry BS, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet 2013.