

Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial

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Summary

Background Many countries offer a second BCG vaccination to prevent tuberculosis, although there is little evidence of whether this confers additional protection. BCG vaccination is routine in Brazil but BCG revaccination procedures vary by state. We studied revaccination efficacy in two Brazilian cities with tuberculosis prevalence representative of Brazil.

Methods We did a cluster-randomised trial of the protection against tuberculosis from BCG revaccination in school-aged children who had had one BCG vaccination as infants. 767 schools in the cities of Salvador and Manaus, Brazil, participated; schools were the unit of randomisation. The study was open label with no placebo. Cases of tuberculosis were identified through record linkage to the Tuberculosis Control Programme. Revaccination status was masked during linkage and validation of cases. The incidence of tuberculosis was the primary outcome. Analysis was by intention to treat.

Findings 386 schools (176 846 children) were assigned BCG revaccination and 365 (171 293 children) no revaccination. 42 053 children in the vaccine group and 47 006 in the control group were absent from school on the day of the visit and were excluded. 31 163 and 27 146, respectively were also excluded because they had no BCG scar, two or more scars, or a doubtful scar on assessment. The crude incidence of tuberculosis in the intervention group was 29·3 per 100 000 person years and in the control group 30·2 per 100 000 person-years (crude-rate ratio 0·97; 95% CI 0·76–1·28). The efficacy of BCG revaccination was 9% (–16 to 29%).

Interpretation Revaccination given to children aged 7–14 years in this setting does not provide substantial additional protection and should not be recommended. Follow-up is ongoing and needed to assess the effect of other factors on revaccination efficacy: time since vaccination, age at vaccination, and high or low prevalence of environmental mycobacteria.

Introduction

Tuberculosis is one of the ten main causes of death in the developing world.¹ Neonatal BCG vaccination is routine in many countries; exceptions include the USA and the Netherlands. Where routinely done, neonatal BCG coverage has been high since the 1980s.² BCG protection against tuberculous meningitis is estimated to be more than 80%,³ including in Brazil.^{4–6} Efficacy of vaccination against pulmonary tuberculosis varies and in trials ranged from no protection to very high protection; variations in the BCG vaccine used or in ethnicity of the vaccinated population do not explain variations in efficacy.⁷ Efficacy tends to be low in regions where there is high prevalence of environmental mycobacteria. Because environmental mycobacteria are immunologically close to *Mycobacterium tuberculosis*, they are thought to confer protection against tuberculosis.⁷

Whether a second BCG vaccination gives additional protection is not known.⁷ In the absence of scientific evidence that revaccination confers protection, WHO global programmes on tuberculosis and on vaccines do not recommend repeated BCG vaccination.⁸ A trial of the effect of a second BCG vaccination, which was done in Malawi, reported a 50% protective effect against leprosy

but no effect against tuberculosis;⁹ this is consistent with a second dose conferring no protection in settings (such as Malawi) where a first dose confers no protection.¹⁰ BCG revaccination is routine in several countries, mostly in eastern Europe and Asia^{11,12} and in some municipalities in Brazil. In the past decade, several countries have suspended their BCG revaccination programmes. Decisions to suspend revaccination were commonly made for economic reasons, without rigorous assessment of protection. A few studies have assessed revaccination¹³ by assessing secular trends in BCG vaccination and in tuberculosis incidence.^{14–17}

Effective BCG revaccination would offer a low-cost method for controlling tuberculosis. If revaccination is not effective, it is important that the reason for ineffectiveness is identified so that effective vaccines can be developed to be given after neonatal BCG. Much attention is being given to the development and future assessment of new vaccines against tuberculosis—more than 200 possible vaccine candidates have been identified.^{18,19} Because there is no immunological marker of protection against tuberculosis, field trials with disease endpoints are needed to estimate the protective effect of a second dose of BCG or a new tuberculosis vaccine.^{20,21} In this paper we

Lancet 2005; 366: 1290–95

Published online
August 31, 2005
DOI:10.1016/S0140-6736(05)
67145-0

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report the results of a cluster-randomised trial involving more than 200 000 school-aged children which was done to estimate the efficacy of BCG revaccination against tuberculosis, and we discuss the implications for the design of trials of new tuberculosis vaccines. A full description of the study design,²² validity of scar reading,²³ parallel immunological studies,^{24,25} and the frequency of adverse events,²⁶ are presented elsewhere.

Methods

Participants

We used schools as the unit of randomisation, the study population consisting of children aged 7–14 years at study entry. The schools were government funded and located in two cities in Brazil: Manaus and Salvador. In Brazil some areas have a high prevalence of infection with environmental mycobacteria and others have a low prevalence of infection with environmental mycobacteria. Manaus was chosen for this study because a high rate of infection was expected in this city, owing to the latitude, high temperature, and high humidity. Moreover, environmental mycobacteria are commonly isolated in clinical samples from people living in Manaus.²⁷ Salvador was chosen because the prevalence of infection with environmental mycobacteria is low. The efficacy of revaccination in Manaus and Salvador combined provided an estimate of the efficacy in Brazil; a comparison of efficacy in the two cities will be possible with long-term follow-up data.

Active informed consent was not obtained because at the time of the study both the intervention (BCG revaccination) and control group procedures were in routine practice in Brazil. Consent was “opt out”—ie, parents of children in schools allocated to vaccination were given written information about the vaccine and the trial, and they were given the opportunity to withdraw their child from the study. The ethics committees of the University Hospital, Universidade Federal da Bahia, Brazil, and London School of Hygiene and Tropical Medicine approved the trial.

Procedures

Recruitment into the trial was done during 1996 and 1997 in Salvador and during 1998 in Manaus. Schools were classified according to the characteristics of their location. In Salvador, schools were classified into categories defined according to the proportion of households with a monthly income that was 0–25%, 26–50%, 51–75%, or more than 76% below the minimum wage, which is well correlated with tuberculosis incidence. In Manaus, schools were classified according to the incidence of tuberculosis and leprosy before the trial; efficacy against leprosy was a secondary objective of the trial in Manaus. Within each subgroup, pairs of schools with similar numbers of students were identified and one school of each pair was randomly allocated to the intervention group. Schools without a pair were randomly allocated by

one of the authors (SSC) to intervention or control groups by means of computer-generated random numbers. Allocation to treatment group was not concealed, nor was the intervention.

Information on children was obtained from school records and double-entered into the study database. Parents of children in schools allocated to intervention received an information pack about the trial, including a request for information on previous BCG vaccination and for any vaccination cards, instructions on what to do in the event of adverse reactions, and a form to complete if they wished to withdraw their child from the study. Parents were encouraged to withdraw their child if he or she had a severe illness.

We visited children in both treatment groups at school to confirm their identification details and to examine their arms for BCG scars. The validity of a BCG scar as an indicator of neonatal vaccination is high in this setting (sensitivity 98% and specificity 92%).²³

Children were not tested with tuberculin before BCG vaccination. Both WHO⁸ and the Brazilian Tuberculosis Programme discourage tuberculin testing before revaccination because a positive result is not easily interpretable and does not indicate protection. Children with a BCG scar attending schools that had been allocated to the intervention group were given an intradermal BCG vaccination at school. The vaccine was produced in Brazil with the Moreaux (Rio de Janeiro) strain, which is highly protective against tuberculous meningitis in Brazil.^{4–6}

Children who subsequently developed tuberculosis were identified through Brazil's federally funded Tuberculosis Control Programme, which is part of the National Health Service and integrates services through cities, states, and the country; we identified children born during the target years who were living in the metropolitan areas of Salvador and Manaus. Patients can present directly to the Tuberculosis Control Programme if they suspect they have tuberculosis; however, more commonly physicians refer patients to the programme for assessment; diagnosis and treatment is free. Only the Tuberculosis Control Programme can dispense drugs for the treatment of tuberculosis in Brazil and cases of tuberculosis are reported to the surveillance system. We collected data on tuberculosis cases not only from the surveillance programme but also through periodic visits to all health units treating tuberculosis in Salvador and Manaus.

The outpatient and hospital records of identified cases of tuberculosis were reviewed independently by two chest physicians who classified cases into confirmed (microbiological confirmation), probable (would treat given the information in the records), suspected (absence of information for a different diagnosis), and not tuberculosis (excluded from the analysis). They further classified cases into pulmonary and non-pulmonary tuberculosis. A third specialist reviewed those cases classified differently by

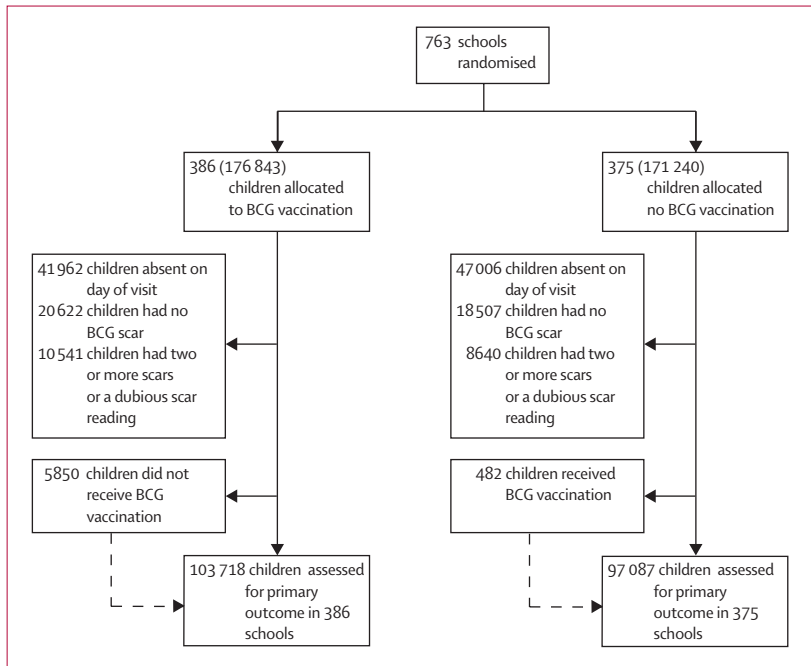


Figure: Trial profile

the two chest physicians. These physicians were unaware of vaccination status and the results of tests for *M tuberculosis* purified protein derivative (PPD). This analysis includes the first 63 months of follow-up in Salvador and 48 months in Manaus.

Cases were linked to the study population in the study database. Vaccination status was masked and linkage was done by one of the authors (SSC) according to the name of the mother, and the name, sex, and date of birth of the child. All possible matches were reviewed to assess the reliability of linkage. Most matches were unique, with complete concordance on all variables. 1% (2/144) of a sample of cases that were not linked to the database were found to belong to the database when home visits were done.

Statistical analysis

We excluded children without a BCG scar from all analyses and children absent from school on the day of the team visit (for scar reading in control schools and scar reading and vaccination in intervention schools) from the estimate of vaccine efficacy. Analysis of children included in the study was by intention to treat. The primary outcome was the incidence of tuberculosis. Vaccination efficacy was estimated for the second dose (ie, in children with one neonatal scar) to assess whether protection was at least 30%. Secondary outcomes were protection against pulmonary and non-pulmonary forms, and in each city.

We did not use traditional methods of statistical analysis, such as cluster summary statistics,^{28–30} because there were more clusters than cases. Although there was no evidence of a design effect, we analysed the data by

use of an approach suitable for overdispersed Poisson data, based on the generalised-estimating-equations (GEE) method.³¹ Our analysis was not strongly dependent on distributional assumptions.

We calculated vaccination efficacy as $(1 - [\text{rate of vaccinated/unvaccinated children}]) \times 100$. We estimated the number of children who needed to be vaccinated to prevent one case of tuberculosis in 5 years as $1 / [(5 \times \text{annual incidence of tuberculosis in the control group}) - (5 \times \text{annual incidence of tuberculosis in the intervention group})]$.

Because ascertainment of cases was passive, we did not know how many of the children included in the study stayed in the study area. We estimated person-years at risk for the children studied assuming they stayed in the study until the end of the ascertainment period. A first crude estimation of the vaccination efficacy was obtained by calculating incidence rates and rate ratios including 95% CIs. Vaccination efficacy was calculated with the Poisson regression (GEE method) adjusted for characteristics of the cluster (city, socioeconomic status, past incidence of tuberculosis and leprosy) and the individual (sex, age at vaccination, age at diagnosis). Because tuberculosis incidence in children aged 7–14 years tends to increase with age, age at diagnosis was modelled as a time-dependent variable in five categories (≤ 10 years, 11–12 years, 13–14 years, 15–16 years, ≥ 17 years). Interactions were tested to estimate the effect of age, sex, and city on vaccine efficacy. Preliminary subgroup analyses were done to estimate vaccine efficacy by city, by school year (ie, age), and by age at vaccination; to date the follow-up is too short for sufficiently powerful subgroup analyses. All statistical analyses were done with the statistical software package STATA (version 8.2, STATA Corporation, College Station, TX, USA).

Role of the funding source

Neither of the funding sources had any role in study design, data collection, data analysis, data interpretation, or writing of the report. All the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Information was collected for 348 083 children from 763 schools (figure 1). About a quarter of these children were absent from the school on the day the team visited the schools for scar reading in control schools and scar reading and BCG vaccination in intervention schools: 24% (42 053/176 846) of the children in intervention schools and 27% (47 006/171 239) in control schools. We excluded children who were absent from school on the day of the visit. Another 58 310 children were excluded (31 163 in intervention schools and 27 147 in control schools) because they had no scar, two or more scars, or because there was uncertainty about reading of the scar.

Children with no BCG scar were vaccinated in the trial, but because this was their first vaccination, results will be reported separately.

Table 1 shows the distribution of individual and cluster characteristics of the children randomly assigned to intervention and control groups. The characteristics of the two groups were similar. The proportion of children with no infant BCG scar increased with age: 11% (4413/36 201) of children who were aged 7–8 at the start of follow-up, 21% (8291/60 888) of children aged 9–10; 31% (12 200/73 953) of children aged 11–12, and 36% (14 225/68 892) of children aged 13–14.

There were 279 cases of tuberculosis in the study: 144 in the intervention and 135 in the control group. Table 2 shows the number of cases of pulmonary and non-pulmonary tuberculosis by age for each group. 184 of 279 (66%) diagnoses were classified as certain, 50 (18%) as probable, 7 (3%) as suspect; in 38 (14%) participants there was insufficient data for validation of diagnosis. These classifications were similar in intervention and controls groups. No participants were excluded after validation.

There was a total of 937 755 person years of follow up, 490 983 in the intervention group and 446 862 in the control group. The crude incidence of tuberculosis was 29.3 per 100 000 person years in the intervention group and 30.2 per 100 000 person-years in the control group (crude rate ratio 0.97; 95% CI 0.76–1.28). Comparison of parameters obtained by the naive and the GEE approaches established that there was no effect of clustering; the rate ratios and 95% CIs were similar: 0.91 (0.72–1.15) in the naive and 0.91 (0.71–1.16) in the GEE regression model. Even though the design effect was close to 1, we used GEE for all analyses. Because there was no confounding by socioeconomic factors and baseline at incidence of tuberculosis and leprosy, all estimates of vaccination efficacy were controlled for age only.

Incidence of tuberculosis in children who were excluded from the study owing to absence from school on the day of the visit was 36.1 per 100 000 person years in the intervention group (95% CI 29.2–44.1) and 36.5 per 100 000 person years in the control group (29.7–44.1). We excluded these children from subsequent analyses because this would not unbalance the comparison groups.

Table 3 shows the effectiveness of BCG revaccination against all forms of tuberculosis, pulmonary tuberculosis, and non-pulmonary tuberculosis. From these data we estimated that 3824 children need to be vaccinated to prevent one case of non-pulmonary tuberculosis over 5 years. Revaccination efficacy for children vaccinated at age 7–8 years was 62% (–44 to 90%), at 9–10 years was 1% (–44 to 77%), at 11–12 years was –40% (–119 to 10%); and at 13–14 years was 10% (–12 to 44%).

Discussion

BCG revaccination in children aged 7–14 years did not have a protective effect. The incidence of tuberculosis during the study period, and the number of pulmonary

	Allocation group	
	Vaccine	Control
Characteristics of individuals		
Number of children	103 718	97 087
Mean age, years (SD)	11.5 (2.08)	11.4 (2.10)
Number in age group at start of trial		
Aged 7–8 years at inclusion	15 622 (15%)	16 166 (17%)
Aged 9–10 years at inclusion	27 321 (26%)	25 276 (26%)
Aged 11–12 years at inclusion	32 138 (31%)	29 615 (30%)
Aged 13–14 years at inclusion	28 637 (28%)	26 030 (27%)
Number of males	50 409 (49%)	47 318 (49%)
No BCG scar	20 640 (17%)	18 495 (16%)
One BCG scar	103 700 (83%)	97 099 (84%)
Revaccinated by trial	97 868 (94%)	442 (1%)
Characteristics of clusters		
Number of schools	388 (51%)	375 (49%)
Mean number of students at schools (SD)	322 (233)	317 (215)
Range of students at schools	11–1430	10–1334
Mean proportion of boys at schools (SD)	48% (7%)	49% (6%)
Range of boys at schools	0–83%	35–99%
Number of houses with monthly income 0–25% below minimum wage	1903 (3%)	1630 (3%)
Number of houses with monthly income 26–50% below minimum wage	6413 (10%)	5652 (12%)
Number of houses with monthly income 51–75% below minimum wage	21 315 (30%)	13 682 (30%)
Number of houses with monthly income ≥76% below minimum wage	31 244 (57%)	25 579 (55%)
Mean incidence of tuberculosis per 100 000 before the trial in the school area* (SD)	121.6 (91.3)	126.3 (74.8)
Range of tuberculosis incidence per 100 000 before the trial in the school area*	14.5–618.0	14.5–618.0
Mean incidence of leprosy per 100 000 before the trial in the school area† (SD)	8.8 (9.8)	7.8 (7.2)
Range of tuberculosis incidence per 100 000 before the trial in the school area†	0.3–66.9	0–66.9

Data are mean (SD) unless otherwise stated. *Manaus only. †Salvador only.

Table 1: Characteristics of individual children and school clusters in intervention and control groups

and non-pulmonary cases by age, were similar in the intervention and control groups. BCG revaccination was ineffective against all forms of tuberculosis or against non-pulmonary tuberculosis. Revaccination efficacy was similar in certain, probable, and suspect cases, and when cases with insufficient data for validation were excluded.

Stratified randomisation and a large number of clusters, with very few cases in each cluster, resulted in a balanced trial; comparison of the naive and GEE approach showed there was no effect of clustering. Tuberculosis incidence was similar in children in intervention and control schools who were excluded from the study because they

	Pulmonary tuberculosis		Non-pulmonary tuberculosis	
	Intervention	Control	Intervention	Control
Age at diagnosis				
≤10 years	6/7	5/7	1/7	2/7
11–12 years	8/11	8/13	3/11	5/13
13–14 years	26/35	14/26	9/35	12/26
15–16 years	42/49	38/50	7/49	12/50
≥17 years	35/42	33/39	27/42	6/39
Total	117(81%)	98 (73%)	27 (19%)	37 (27%)

Table 2: Tuberculosis diagnosis by type and age in each group

	All types of tuberculosis n=279 (95% CI)	Pulmonary tuberculosis n=215	Non-pulmonary tuberculosis n=64
Both cities n=279	9% (-16 to 29)	-1% (-24 to 18)	37% (-3 to 61)
Salvador n=183	11% (-20 to 34)	10% (-45 to 29)	14% (-72 to 57)
Manaus n=96	-2% (-546 to 32)	-30% (-108 to 19)	68% (-2 to 90)

Table 3: Efficacy of revaccination against tuberculosis by tuberculosis type and city, controlled for age.

were absent on the day of the visit. Validation of earlier BCG vaccination resulted in a large proportion of certain and probable cases; protection did not change with certainty. The sample size was adequate to assess protection of at least 30%, which is the minimum amount of protection of public-health interest.

Although participants knew whether or not they were vaccinated, those in the intervention group were given information about the trial, and doctors diagnosing tuberculosis could ask whether or not the children were vaccinated (and a belief in protection of the second dose could have affected the diagnosis of borderline and mild cases because there are no clear criteria for diagnosis of childhood tuberculosis). The similarities between the intervention and control groups on certainty of diagnosis and distribution of forms of tuberculosis suggest that the absence of masking did not lead to bias. More importantly, had such bias occurred, vaccine efficacy would have increased. However, this is unlikely to have happened in this trial because we found no protective efficacy.

Cases diagnosed were ascertained through the Tuberculosis Control Programme, which could have led to underascertainment of cases. Brazil is a highly medicalised country; for example, there is more than one doctor per 1000 people living in Salvador and health care is free at the point of use. We believe that most cases of tuberculosis in young participants would have been diagnosed through the Tuberculosis Control Programme and are confident that we have ascertained most diagnosed cases. Any participants who were not diagnosed and recovered spontaneously or who might have died of tuberculosis without diagnosis would not have been included in the study. Even if there was some underdiagnosis or underascertainment, the power of the study would decrease but the results would not be biased.

In addition, participants could have been lost to follow-up owing to external migration or death. However, there is no reason why this would have been different in the intervention and control groups, especially with such large numbers. Moreover, any non-differential losses to follow-up would decrease the power of the study but would not bias the results.

As expected, the size of the study at this first analysis was too small for conclusions to be drawn about protection by city and by type of tuberculosis. Continued follow-up will provide the power to assess the secondary outcomes: whether revaccination protection increases with time since vaccination, if it varies by age at

vaccination, whether the first dose of BCG vaccination at school is protective, and if the effect of BCG is the same in cities with low and high prevalence of environmental mycobacteria.

The only other trial of the effectiveness of a second BCG vaccination against tuberculosis was done in Malawi,¹² where the first vaccination does not seem to give protection. The reason for the lack of protection is not clear, but it is possible that the high prevalence of helminth infections in Malawi and Brazil³² could have downregulated the immune response to vaccination leading to lower than maximum efficacy of BCG revaccination.³³

The results presented here suggest that revaccination in this setting and age group does not confer additional protection of public-health importance and should not be recommended. This trial provides some new evidence to support the WHO recommendation not to revaccinate with BCG. Trials of new tuberculosis vaccines should be planned with regard to the issues (ranging from duration of vaccine efficacy, potential variation by age and time since vaccination, and success of passive follow-up) this trial highlights.^{34–36}

Contributors

L C Rodrigues and M L Barreto designed the study, supervised field work, analysed data, interpreted results, and wrote the paper; S M Pereira, S S Cunha, M Y Ichihara, S C de Brito, M A Hijjar, and Ines Dourado did field work, interpreted results, and edited the paper; A A Cruz and C Sant'Anna did clinical supervision, interpreted results, and edited the paper; A L Bierrenbach interpreted results and edited the paper; B Genser led the data analysis, helped with interpretation of results, and edited the paper.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The trial was funded by grants from the Department of International Development (DFID), UK and the National Health Foundation (FUNASA), Brazil. We thank the Health and the Education Secretariat for the states of Bahia and Amazonas, and for the cities of Salvador and Manaus; the National Programme of Immunisation and the National Centre for Epidemiology in Brazil (both from FUNASA), particularly J Barbosa, M L Maia, M Carvalho, and L Pinto; E Ackerman, I Cunha, M H Rios, F Praia, and J C Goes from the field team, and the members of the vaccination and data collection teams. We are grateful to A C Lemos for reviewing discordant cases, C Struchiner, J U Braga, R Ximenes, A Ruffino-Netto for scientific advice, and C Victoria, P G Smith, and S Cousens for their scientific advice and comments on the paper.

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