BCG vaccine effectiveness in preventing tuberculosis and its interaction with human immunodeficiency virus infection

María Patricia Arbeláez,[a] Kenrad E Nelson[b] and Alvaro Muñoz[b]

Background	To explore *Bacillus Calmette-Guérin* vaccine (BCG) as a protective factor against tuberculosis (TB) and how human immunodeficiency virus (HIV) infection modifies the effect of BCG on TB.
Methods	Two matched case-control studies were conducted. One study compared TB cases and controls who were HIV positive. The second compared TB cases and controls who were HIV negative. The study population consisted of 88 TB cases and 88 controls among HIV-positive individuals and 314 TB cases and 310 controls among HIV-negative individuals. Cases were new TB diagnoses, confirmed by either bacteriology, pathology, radiology or clinical response to treatment; controls were selected from people without TB symptoms and who sought medical attention in the same institution where a case was enrolled. BCG was assessed by the presence of a typical scar.
Results	The level of protection against all clinical forms of TB was 22% among HIV positive individuals (odds ratio [OR] = 0.78, 95% CI : 0.48–1.26) and 26% among HIV negatives (OR = 0.74, 95% CI : 0.52–1.05). There was a significant difference ($P = 0.002$) in the level of protection against extrapulmonary TB (ETB) between HIV-negative (OR = 0.54, 95% CI : 0.32–0.93) and HIV-positive individuals (OR = 1.36, 95% CI : 0.72–2.57).
Conclusion	BCG has a modest protective effect against all forms of TB independent of HIV status, and BCG confers protection against extrapulmonary TB among HIV-negative individuals. However, HIV infection seems to abrogate the protective effect of BCG against extrapulmonary TB. Our data support the public health importance of BCG vaccine in the prevention of extrapulmonary TB among immunocompetent individuals.
Keywords	BCG effectiveness, tuberculosis, extrapulmonary tuberculosis, HIV infection
Accepted	8 May 2000

Tuberculosis (TB) continues to be a major public health problem around the world and, according to WHO estimates, the incidence continues to increase. Some of the reasons for the increasing incidence are: inadequate access to health care, migration, deterioration of TB control programmes, low compliance with TB treatment, multidrug-resistant strains, and the acquired immunodeficiency syndrome (AIDS) epidemic.

The means used to prevent and control TB are improvement of socioeconomic conditions, case finding and treatment, chemoprophylaxis, and vaccination.[1] Improving socioeconomic conditions has proven to be slow and difficult in a world of social and political instability. Case finding and treatment, and chemoprophylaxis require an organized control programme, which many countries do not yet have. *Bacillus Calmette-Guérin* vaccine (BCG) is an alternative preventive measure that can be achieved in newborns in a single visit. It is an attenuated strain of *Mycobacterium bovis*, applied in 1921 in France by Albert Calmette and Camille Guérin as a vaccine against TB.[2] The mechanism of protection from BCG vaccination involves a reduction of the haematogenous spread of bacilli from the site of primary infection. It protects against the acute manifestations of the disease, and reduces the lifelong risk of endogenous reactivation and dissemination associated with foci acquired from prior infection.[3,4]

[a] Universidad de Antioquia, Facultad Nacional de Salud Pública, Medellín, Colombia.

[b] The Johns Hopkins University, School of Hygiene and Public Health, Department of Epidemiology, USA.

Reprint requests: María Patricia Arbeláez, Facultad Nacional de Salud Pública, Universidad de Antioquia, AA 51922, Medellín, Colombia. E-mail: mpam@guajiros.udea.edu.co

Multiple studies have been conducted to evaluate BCG vaccine efficacy as a preventive measure against TB. Conflicting results from those studies show protective effects that range from 80% to a negative effect.[1,5] The factors underlying the differences in degree of protection include: methodological differences in the studies; variations of the BCG strain used; high prevalence of non-tuberculous *Mycobacterium* infection in the population; high risk of re-infection; and differences of host response to vaccination.[6–10] Nevertheless, there is some consensus about the protective effect of BCG vaccine against disseminated forms of TB disease in children.[11,12]

Even though it is known that immunosuppression produced by human immunodeficiency virus (HIV) infection could alter the response to vaccines and increase the risk of mycobacterial infections, the efficacy of BCG vaccine against TB in HIV-infected people has not been reported.[13] This study focuses on determining the effectiveness of early BCG vaccination in preventing TB among adults, and how that effect is modified by the presence of HIV infection. The wide use of BCG vaccination in Colombia since the early 1960s, a lower coverage with this vaccine among those aged 18–45 years in comparison with children, the endemicity of TB, and the spread of the HIV epidemic in this country offered an appropriate scenario to conduct this study.

Materials and Methods

Study design

A matched case-control study, consisting of two groups of TB cases differentiated by their HIV infection status, was conducted. Controls without TB were also selected by their HIV status. In addition, cases and controls were matched by the health institution in which they were diagnosed, age (±5 years), and gender. Based on clinical manifestations in the HIV-positive group, controls were also selected at similar stages of HIV infection.

Matching by HIV status precludes quantification of association between HIV and TB but does not preclude the investigation of interactions between BCG vaccination and HIV status on the risk of TB, which was the primary scientific aim of our study. Matching by HIV status made this study feasible.

Study area and population

Cali and Medellín are the second and third largest cities in Colombia, with a 1995 population estimated at 2 million inhabitants in each city. In 1995, the annual incidence rates (per 100 000 inhabitants) of TB in these cities were 39.8 and 30.5, respectively, based on passive reporting.

In Colombia, BCG vaccine has been administered according to the WHO policies since the early 1960s. Initially, the vaccine was introduced through mass vaccination campaigns for people under 15 years. After the 1970s, the policy was to administer the vaccine during childhood, in the newborn period or during the first 4 years of life.

In Colombia, the sources of BCG vaccine prior to 1978 were England and Japan. Thereafter, the National Institute of Health of Colombia prepared lyophilized vaccine with the French strain—Pasteur Institute 1173-P2. The same strain is still used throughout the country.[14] The population under study was comprised incident TB cases diagnosed between September 1994 and February 1996 among 18–45-year-old men and women in the metropolitan areas of Medellín and Cali, Colombia.

Notification of each diagnosed case of TB is mandatory. The sensitivity of this surveillance system is probably fairly high, since the treatment is always provided by the government free of charge. We recruited the cases diagnosed from government sponsored clinics, and social security health institutions. For this study, 42 different institutions were involved: 22 from Medellín and 20 from Cali. A physician and a nurse in each city visited each health institution weekly, to detect every new TB case diagnosed, obtain their informed consent to participate in the study, and collect data in a standardized way.

Definition of cases and controls

A case was defined as a person with newly diagnosed TB, confirmed by either bacteriology, pathology, radiology, or clinical response to specific treatment. A control was a person with no history of TB disease, and no current symptoms compatible with TB, who sought medical attention in the same institution where a case was enrolled. The matching criteria for HIV-negative controls included age (±5 years) and gender. The HIV-positive controls were also matched with the HIV-positive cases by age and gender. In addition, HIV-positive cases and controls were in the same HIV infection stage according to the 1987 CDC clinical classification.[15]

Exposure assessment

To determine BCG vaccination status, participants were examined for the presence of a round scar, of about 4–8 mm diameter, on the deltoid area of either arm on the back shoulder. The result was coded as 'present' or 'absent'. An independent reading was performed by a nurse or laboratory technician. Each patient, identified only by a unique code, had a laboratory test request which had an evaluation form attached. Before drawing the required blood sample, the nurse or laboratory technician looked for the BCG scar. The people who performed the second reading did not know the results of the questionnaire administered to the patient, nor did they know the study hypothesis, or the case or control status of the patient. When there was disagreement between the two readers, the first reading was coded. There was disagreement between observers in nine readings, the observed agreement was 98% and the Kappa Statistic was 0.97.

Laboratory tests

HIV serology

Enzyme-linked immunoabsorbent assays (ELISA) were performed, using the Organon Teknika® and New LAV Blot of Sanofi diagnostic Pasteur® test kits. If a specimen was ELISA positive, the ELISA test was repeated a second time. If the second ELISA test was negative, a third one was performed. If the duplicate ELISA test was negative, the patient was considered negative. All repeatedly ELISA-positive samples were confirmed with the Western blot technique. Western blotting was also performed using Organon Teknika blot kits; these tests were conducted in the reference laboratory in each city.

T-cells subsets count

Tests to determine the number of CD4+ and CD8+ cells per mm^3 were performed in all HIV-positive patients using flow cytometry. Samples were processed for immunophenotyping and haematology at each city in a specialized laboratory according to the manufacturer's recommended procedures (FACSort®, Becton Dickinson, San Jose, CA and FACScan XL®, Coulter Corporation, Miami, FL).

Data analysis

Bivariate and stratified analyses included determination of crude and matched odds ratios (OR) and their 95% CI.[16] Adjusted OR were determined using logistic regression models. A logistic regression model was developed with the odds of TB defined as the dependent variable. Independent variables included: BCG status and an interaction term for BCG vaccination and HIV status (BCG*HIV), adjusted by educational level; employment; contact with a TB case; place of birth; crowding; and socio-economic status. The study design meant that the effect of HIV on TB incidence could not be determined; however, the coefficient of BCG status and the interaction of BCG and HIV represent the effect of BCG on HIV-negative individuals and the modification that HIV exerts on the effect of BCG on TB, respectively. The regression coefficients were fitted by the maximum likelihood method. The significance at the 5% level of the effects was also established by the likelihood ratio statistic (LRS).[16] Vaccine effectiveness was measured as $(1 - OR) \times 100$.[17]

Results

In all, 800 participants were enrolled into this study, 176 HIV positives and 624 HIV negatives. In the HIV-positive group, 88 cases and 88 controls were included. In the HIV-negative group, 314 cases and 310 controls were included (Table 1).

Description of cases

Pulmonary TB was the most frequent form of TB, accounting for 50 (57%) cases in the HIV-positive group and 248 (79%) in the HIV-negative group. Overall, 93% of the 402 cases were confirmed bacteriologically (culture or AFB-smear) or histo-pathologically (caseating granulomata). The diagnosis of TB using only clinical manifestations, radiology and/or improvement with treatment was more frequent in the HIV-positive group (16%) than in the HIV-negative group (4%).

Among the 38 cases of extrapulmonary TB in the HIV-positive group, 23 cases (60%) were lymphatic TB, and 8 cases (21%) were pleural. The other cases had other forms of disseminated infection, including miliary, meningeal, intestinal, peritoneal or pericardial disease. In contrast, among the 66 patients in the HIV-negative group, who were diagnosed with extrapulmonary TB, 38 cases (58%) had pleural TB and 11 cases (17%) had lymphatic TB.

Extrapulmonary TB was confirmed by culture in 21% (8 cases) of HIV-positive patients and 26% (17 cases) of HIV negatives. Diagnosis was made by histopathology (granulomata) or staining in 71% and 64% of HIV positives and negatives, respectively. The other extrapulmonary cases were diagnosed by clinical manifestations, radiology and improvement with specific treatment.

Table 1 Characteristics of study participants by human immunodeficiency virus (HIV) and tuberculosis (TB) status

| | HIV | |
	Positive	Negative
TB cases	88	314
Non-TB controls	88	310

Case-control analysis according to risk factors for TB by HIV status

Matching by age and gender was successfully implemented for cases and controls in this study. The mean age (and standard deviation) was 32.4 (6.8) and 33.0 (6.6) among HIV-positive cases and controls, respectively, and 30.0 (7.5) and 30.3 (7.5) among HIV-negative cases and controls, respectively; 87% were males in the HIV-positive group, and 47% were males in the HIV-negative group. Among HIV-positive patients, the matching process also included HIV stages by clinical manifestations and, after they were enrolled in the study, lymphocyte counts were measured. The T-cell subsets were measured in 79 (90%) of the HIV-positive cases and in 76 (86%) of the controls. No differences were found in CD4+ and CD8+ cells counts between cases and controls. Specifically, the mean CD4+ cell count was 193 per mm^3 among cases and 179 among controls, and the mean for CD8+ cell counts were 954 and 890, respectively.

Odds ratios for TB according to sociodemographic characteristics by HIV status are shown in Table 2. Birth in an urban area (cities of Medellín or Cali) was associated with a lower risk of TB. Not being currently employed was associated with TB. Among the HIV negatives, participants of high socioeconomic status (SES) had an OR of 0.41 compared to those with low SES (χ^2 for trend = 4.88, $P = 0.027$). Both HIV-positive and HIV-negative groups with a higher educational level had a lower odds of TB. Individuals with more than a high school education had approximately one-quarter the odds of TB compared to subjects with only an elementary school education (χ^2 for trend = 10.9 and 43.3, $P < 0.001$). Also, TB was positively associated with crowding and a history of living with someone with TB, and negatively associated with the receipt of any kind of vaccine in childhood.

Case-control analysis of TB according to BCG vaccination by HIV status

Among 800 participants involved in the study, 73.5% (588) had evidence of a BCG scar. The proportion was 77% (405) among participants under 35, and 67% (183) for subjects aged over 35. The crude (unmatched) odds of any form of TB after receiving BCG vaccination was 0.69 (95% CI : 0.32–1.47) in the HIV-positive group, and 0.65 (95% CI : 0.44–0.94) in the HIV-negative group (Table 3). There were no differences in the level of protection by age group; the OR was 0.65 in participants aged 18–24 years, 0.64 for participants 25–34 years, and 0.68 for participants 35–45 years old (χ^2 for interaction = 0.05, $P = 0.83$).

The inferences were practically identical when we preserved the matching by age and gender in the analysis. Namely, the matched OR for TB based on BCG vaccination status by HIV infection were: 0.70 (95% CI : 0.34–1.48) in the HIV-positive group, and 0.68 (95% CI : 0.48–0.96) in the HIV-negative group.

Multivariate analysis

We used a logistic regression model to adjust the OR of TB for BCG vaccination status by educational level and other variables associated with TB, such as current employment, contact with a TB case, place of birth, crowding, and socioeconomic status. Receipt of any kind of vaccine during childhood was excluded because of collinearity with BCG vaccination. Table 3 also shows the adjusted OR for TB, according to BCG vaccination by HIV status. The OR were 0.78 (95% CI : 0.48–1.26) among HIV-positive

Table 2 Odds ratios (OR) for tuberculosis (TB) according to sociodemographic characteristics by human immunodeficiency virus (HIV) status. Medellín and Cali, Colombia, 1996

	HIV+			HIV−		
	Cases (n = 88)	Controls (n = 88)	OR (95% CI)	Cases (n = 314)	Control (n = 310)	OR (95% CI)
Place of birth						
Rural	49%	40%	1	53%	41%	1
Urban	51%	60%	0.69 (0.36–1.32)	47%	59%	0.61 (0.44–0.85)
Employment						
Employed	69%	78%	1	82%	91%	1
Unemployed	31%	22%	1.61 (0.77–3.36)	18%	9%	2.37 (1.41–3.99)
Socioeconomic status						
Low	36%	35%	1	47%	41%	1
Middle	48%	48%	0.97 (0.48–1.96)	48%	49%	0.83 (0.59–1.17)
High	16%	17%	0.90 (0.34–2.39)	5%	10%	0.41* (0.20–0.83)
Education						
Elementary	21%	10%	1	35%	19%	1
Some high school	46%	31%	0.74 (0.26–2.07)	38%	29%	0.76 (0.49–1.18)
Completed high school	15%	25%	0.30 (0.09–0.96)	15%	24%	0.36 (0.22–0.60)
More than high school	18%	34%	0.27* (0.09–0.81)	12%	28%	0.23* (0.13–0.39)
Crowding						
No	81%	88%	1	68%	76%	1
Yes	19%	12%	1.70 (0.69–4.24)	32%	24%	1.51 (1.04–2.18)
Ever contact with a TB case						
Never	71%	81%	1	72%	78%	1
Yes but not living with	15%	11%	1.61 (0.59–4.45)	13%	15%	0.94 (0.57–1.52)
Yes and lived with	14%	8%	1.91 (0.65–5.81)	15%	8%	2.08 (1.19–3.66)
Reported any vaccination at childhood						
No	17%	10%	1	10%	4%	1
Yes	83%	90%	0.55 (0.21–1.43)	90%	96%	0.33 (0.15–0.70)

*χ^2 for trend, P-value < 0.05.

Table 3 Crude (OR) and adjusted (AOR) odds ratios for tuberculosis (TB) according to BCG vaccination stratified by human immunodeficiency virus (HIV) status. Medellín and Cali, Colombia, 1996

	HIV+		HIV−	
	Cases	Controls	Cases	Controls
BCG				
Yes	64	70	215	239
No	24	18	99	71
OR (95% CI)	0.69 (0.32–1.47)		0.65 (0.44–0.94)	
AOR (95% CI)[a]	0.78 (0.48–1.26)		0.74 (0.52–1.05)	

[a] Adjusted by: place of birth, current employment, socioeconomic status, educational level, crowding, and contact with a TB case. Interaction term BCG*HIV P-value = 0.615.

individuals and 0.74 (95% CI : 0.52–1.05) among the HIV negatives. These OR correspond to a level of protection of BCG vaccination against TB of 22% among HIV-positive individuals and 26% for the HIV negatives, ranging from 50% protection to no protection. Those values were not statistically significant, nor were they different by HIV status.

Because of the documented protective effect of BCG against severe forms of TB, including extrapulmonary forms, additional analysis was carried out in order to evaluate the protective effect of BCG vaccination against extrapulmonary forms of TB by HIV status (Table 4). Cases with pulmonary TB were excluded from the analysis and patients with both pulmonary

and extrapulmonary TB (4% of total cases) were considered in the extrapulmonary group. A non-significant crude OR was found for extrapulmonary TB, according to BCG vaccination among HIV-positive individuals (OR = 0.96, 95% CI : 0.34–2.76). In contrast, when the same association was explored among HIV-negative individuals, the OR for extrapulmonary TB decreased by half (OR = 0.52, 95% CI : 0.28–0.96). Similarly, the adjusted OR showed no effect of BCG vaccination in HIV-positive patients (OR = 1.36, 95% CI : 0.72–2.57), but a statistically significant protective effect of 46% was found in HIV negatives (OR = 0.54, 95% CI : 0.32–0.93). There was a significant modification of the effect of BCG vaccination on

Table 4 Crude (OR) and adjusted (AOR) odds ratios for extrapulmonary tuberculosis (ETB) according to BCG vaccination stratified by human immunodeficiency virus (HIV) status. Medellín and Cali, Colombia, 1996

	HIV+		HIV−	
	ETB	Controls	ETB	Controls
BCG				
Yes	30	70	42	239
No	8	18	24	71
OR (95% CI)	0.96 (0.34–2.76)		0.52 (0.28–0.96)	
AOR (95% CI)[a]	1.36 (0.72–2.57)		0.54 (0.32–0.93)	

[a] Adjusted by: place of birth, current employment, socioeconomic status, educational level, crowding, and contact with a TB case. Interaction term BCG*HIV P-value = 0.002.

extrapulmonary TB due to HIV infection ($P = 0.002$) (Figure 1). This Figure shows the different effects of BCG against all forms of TB and the protective effect conferred against extrapulmonary TB among HIV-negative individuals; an effect that was abrogated by the presence of HIV infection. The modification by HIV infection on the effect of BCG in preventing extrapulmonary TB is clearly evident in this Figure.

Discussion

Our study found a low protective effect of BCG vaccine against all forms of TB. Several reasons for this modest efficacy can be hypothesized. First, limitations of the present study related to the potential misclassification of BCG vaccination status based on the presence of a scar. Some vaccinated people might not have any scar,[18] or in contrast, the presence of a smallpox vaccine scar could have been erroneously classified as a scar form. These classification biases could make BCG appear to be less effective that it was.

Second, variations in the protective efficacy of BCG against all forms of TB have been widely documented.[19–21] Among young adults recent studies have reported similar findings.[22–24] Different mechanisms are involved in the pathogenesis of TB during adulthood, such as reactivation of latent infection, rapid progression of primary infection, or re-infection. These are difficult

Figure 1 Odds ratios (OR) for all forms of tuberculosis (TB) and for extrapulmonary TB according to BCG vaccination stratified by human immunodeficiency virus (HIV) status. Medellín and Cali, Colombia, 1996

to differentiate in this study. However, these pathogenetic mechanisms imply different immune responses, so the efficacy of the BCG vaccine could vary related to each of these mechanisms. A study conducted in subtropical Australia provided some support for the hypothesis that BCG vaccine can offer a higher level of protection against newly acquired disease than against disease due to late endogenous reactivation.[22]

In a recent study conducted in Brazil of the transmission of TB to close contacts of patients with multidrug-resistant TB, researchers documented that BCG vaccine conferred 69% protection against TB among these contacts.[25] Studies conducted in children that showed a high protective effect of BCG against tuberculous meningitis[26,27] could also support this hypothesis, considering that tuberculous meningitis occurs frequently as a progressive primary disease. In adults, endogenous reactivation is a common mechanism for developing TB disease, thus the protection of BCG may be lower in those situations.

The third reason is a high prevalence of infection with other environmental mycobacteria. The prevalence of environmental mycobacterial infections is unknown in the population under study, but may be high given the tropical conditions in Colombia. A high prevalence (65%) of atypical mycobacterial infection was found, through skin tests, among schoolchildren in a city near Cali.[14] Environmental mycobacterial infections can reach the alveoli through inhalation of bacilli in air or dust, or by the oral route in water or food. This could produce a local immunological effect on pulmonary tissues, masking the protective effect of BCG against pulmonary forms of TB.[28]

A fourth reason is the biological variability of strains of BCG vaccine.[7] Almost all individuals enrolled in this study received the BCG vaccine before 1978 when vaccines from England and Japan were used in Colombia. Those strains have been reported as more efficacious than the French strain, Pasteur Institute 1173-P2, that was used in Colombia after 1978.[7,14]

Another possible explanation for our finding of low protection offered by BCG against TB is the duration of the protective effect of BCG vaccine. Some studies have shown that the protective effect decreases with age.[1,21,22,28,29] There are two possible reasons for this decrease: a waning in the efficacy of the vaccine, which reduces the level of resistance among those vaccinated or a gradual increase in the level of resistance among the unvaccinated as a result of a natural infection by tubercle bacilli or environmental mycobacteria.[21] In this study, age was a matching variable, but there was no modification in the effect of BCG vaccine by age.

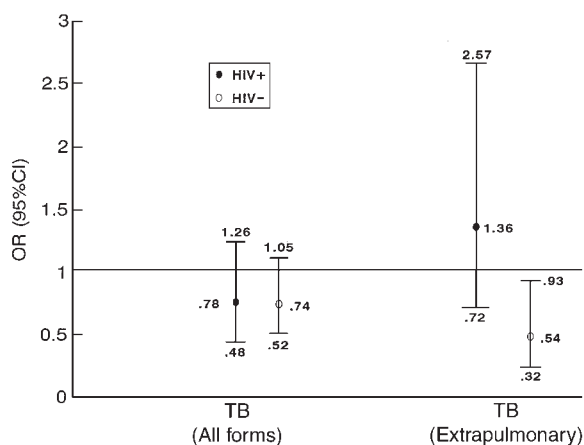In the present study, BCG vaccine reduced extrapulmonary forms of TB by 46% (95% CI : 7–68%) among HIV-negative

individuals. This finding suggests that the protective effect of BCG against extrapulmonary and disseminated forms of the disease, among immunocompetent people may persist until adulthood.

BCG vaccine's protective effect has been more consistently documented against systemic mycobacterial infections than against local pulmonary disease.[11] The protective effect of BCG vaccine against extrapulmonary TB has been documented in childhood, but few studies have been conducted to explore this effect among adults.

A controlled trial conducted in Malawi[20] reported a lower incidence of glandular TB among recipients of a second dose of BCG, than among placebo recipients (0.57, 95% CI : 0.17–1.93, 11 cases).[20] This finding suggests that, even though cases were few and the protective effect of BCG vaccination against pulmonary TB was nil, a lasting protective effect against extra-pulmonary forms, such as glandular TB, is still likely.

There were differences in the response to BCG vaccination in the HIV-positive group enrolled in this study. They showed a similar low level of BCG protection against all forms of TB, as seen in the HIV-negative group. This finding is in agreement with a study conducted in HIV-positive children in Lusaka, Zambia.[30] However, there was no efficacy of BCG vaccine against extrapulmonary TB in the HIV-positive patients. When a specific cellular-mediated immune response is required for control of haematogenous spread of the tubercle bacilli, the suppression T-cell mediated immunity produced by HIV infection may render this defence inadequate. In contrast to our results, in a population in Trinidad, childhood BCG vaccination was associated with protection against bacteraemia with *M. tuberculosis* in adult AIDS patients. That study also found that most of the strains in bacteraemic patients were clonal suggesting recent acquisition of TB rather than reactivation infection.[31] Other studies addressing the relationship between BCG and TB in HIV-positive individuals have not included control groups to explore the effectiveness of BCG in these settings.[32,33]

Finally, our data support the public health importance of BCG vaccine, especially for the prevention of extrapulmonary TB among immunocompetent individuals, although the apparent effectiveness of BCG in preventing extrapulmonary TB seems to be abrogated by the presence of HIV infection.

Acknowledgements

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