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BCG vaccination reduces risk of infection with *Mycobacterium tuberculosis* as detected by gamma interferon release assay

Michael Eisenhut[a,f,*], Shantini Paranjothy[b,f], Ibrahim Abubakar[e,f], Sam Bracebridge[d,f], Mike Lilley[c,f], Rohinton Mulla[a,f], Kay Lack[c,f], Denise Chalkley[f,g], Marian McEvoy[c,f]

[a] *Luton and Dunstable Hospitals NHS Foundation Trust, Luton LU4 0DZ, United Kingdom*
[b] *Department of Primary Care and Public Health, Clinical Epidemiology Interdisciplinary Research Group, School of Medicine, Cardiff University, Cardiff CF14 4YS, United Kingdom*
[c] *Bedfordshire and Hertfordshire Health Protection Unit, Letchworth, Hertfordshire SG6 1BE, United Kingdom*
[d] *Health Protection Agency (East of England), Regional Epidemiology Unit, Institute of Public Health, University Forvie Site, Cambridge CB2 2SR, United Kingdom*
[e] *Tuberculosis Section, Respiratory and Systemic Infections Department, Centre for Infections, Health Protection Agency, London NW9 5EQ, United Kingdom*
[f] *School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, United Kingdom*
[g] *Luton Training PCT, Liverpool Chest Clinic, Luton LU1 1HH, United Kingdom*

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ABSTRACT

Aims: To investigate whether BCG vaccination, in addition to a reduction of active tuberculosis, leads to a reduction of *Mycobacterium tuberculosis* infection during an outbreak of tuberculosis.
Methods: Pupils ($n = 199$) of a Junior School exposed to a pupil with active pulmonary tuberculosis were screened using a gamma interferon release assay for detection of *M. tuberculosis* infection (*ex vivo* ELISPOT assay). Relative risk of *M. tuberculosis* infection and pulmonary tuberculosis associated with BCG vaccination were calculated and adjusted for exposure risk.
Results: Twenty-nine percent of children with previous BCG vaccination had a reactive gamma interferon release assay compared with 47% of unvaccinated children (unadjusted RR 0.61, 95%CI 0.39, 0.96). The protective effect of BCG vaccination persisted following adjustment for other risk factors for infection like ethnicity and proximity to the source case reflected in membership of class and activity groups (corrected relative risk 0.26, 95%CI 0.09, 0.69 and risk reduction of 74%, 95%CI 31%, 91%). A higher proportion of unvaccinated children (11%) were diagnosed with active pulmonary tuberculosis compared with 5% of vaccinated children (RR 0.51 95%CI 0.15, 1.70).
Conclusion: BCG vaccination was associated with a reduction of *M. tuberculosis* infection diagnosed by gamma interferon release assay testing in school children during a point source outbreak.

1. Introduction

Systematic reviews of randomised controlled trials and retrospective case–control studies showed that *Bacillus Calmette Guerin* (BCG) immunisation is beneficial and cost-effective in reducing risk of meningitis and miliary tuberculosis in childhood [1]. Globally the indication for use of this vaccine is based on evidence of its effectiveness in reduction of these severe disease manifestations. BCG has so far not been used to reduce the rate of infection with *Mycobacterium tuberculosis*. The effect of BCG vaccination on infection with *M. tuberculosis* has not been easy to determine because traditional methods of testing for infection such as tuberculin skin testing does not clearly distinguish between the effect of BCG vaccination on reaction to the tuberculin injected intra-dermally and the effect of *M. tuberculosis* infection. The gamma interferon release assay measures gamma interferon release of T-lymphocytes stimulated by *M. tuberculosis* antigens not present in the BCG strain of *Mycobacterium bovis*. It can therefore detect an immune response to *M. tuberculosis* infection without false positive results induced by a prior BCG vaccination. This establishes a unique role for the gamma interferon release assay in detection of an effect of BCG vaccination on *M. tuberculosis* infection. Results of gamma interferon release assays performed in contacts of patients with contagious pulmonary tuberculosis have been shown to correlate significantly better with risk of exposure to the infective patient compared to results of skin testing with tuberculin [2]. There is emerging evidence of the protective effect of BCG vaccination not solely against severe disease but also against infection among children exposed to tuberculosis [3,4].

This is an important issue particularly as there has been a shift in policy towards cessation of universal BCG immunisation in

* Corresponding author at: Luton & Dunstable Hospital NHS Foundation Trust, Lewsey Road, Luton LU4ODZ, United Kingdom. Tel.: +44 8451270127.
E-mail address: michael_eisenhut@yahoo.com (M. Eisenhut).

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developed countries with low prevalence of tuberculosis, as recommended by the International Union against Tuberculosis and Lung Disease [5].

We previously reported a large point source outbreak at a Junior School in the United Kingdom, where the source case was a child [6].

In this paper we examine the effect of previous BCG immunisation on risk of infection with *M. tuberculosis* as defined by a positive gamma interferon release assay (ELISPOT assay), within this cohort of children.

2. Methods

In March 2007, a 9-year old boy was diagnosed with smear negative pulmonary tuberculosis. Clinical and public health investigation of this case led to the identification and management of an outbreak of tuberculosis in this school [6]. During this outbreak investigation, all the children who attended this school were screened using a gamma interferon release assay (T-spot-test®). This method of screening was chosen to avoid false positive skin test results due to previous BCG immunisation.

T-spot-test® (Oxford Immunotec, Abingdon, UK) is a variant of the *ex vivo* ELISPOT method validated with international quality standards (ISO13485:2003, GMP). It uses the region of difference-1 antigens early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP 10) to stimulate T-effector cells specific for *M. tuberculosis* to produce gamma interferon. Gamma interferon production by cells is visualized by developing with a conjugated anti-interferon antibody and an enzyme substrate. Wells were read with an automated ELISPOT reader to determine positive (reactive) and negative (not reactive) results as described previously [7]. The sensitivity and specificity of the T-spot® test in patients with microbiologically confirmed tuberculosis was 90% (95%CI, 86–93%) and 93% (95%CI, 86–100%) for *M. tuberculosis* infection in a systematic review [8].

During screening we collected information on symptoms, risk factors (including family history) and BCG status using a standardised questionnaire that was completed by all parents of children attending the school. Parents were asked to provide information on BCG immunisation status regardless of presence of a scar. Children's BCG status was verified using a local electronic database of vaccination records (a part of the national child health system which records information on live births and children in England and Wales that includes data on immunisation status). We defined an infected child as 'an individual with at least 8 h cumulative contact with the school since September 2006, diagnosed with *M. tuberculosis* infection detected by screening as described above (reactive T-spot test)'. Infected contacts were further defined as 'active tuberculosis' if there was clinical and/or radiological (chest X-ray) evidence of tuberculosis requiring chemotherapy and 'latent tuberculosis' if there was evidence of infection with *M. tuberculosis* but no clinical or radiological evidence of tuberculosis and the patient received chemoprophylaxis.

2.1. Statistical analysis

Statistical analysis was performed using STATA version 9.0. Infection rates were calculated as the proportion of infected children according to BCG vaccination status. The relative risk was calculated to assess the risk of infection with *M. tuberculosis* among vaccinated children compared with unvaccinated children. Adjusted odds ratios were obtained using multivariable logistic regression, to investigate whether there was an association between previous BCG immunisation and risk of infection with *M. tuberculosis*, which was independent of other risk factors that may be associated with infection, such as class group (as a proxy measure of exposure), after school activities or clubs and being a close friend of the index case. We calculated the relative risk reduction as a measure of the effect of vaccination on reducing infection, using the adjusted odds ratio. The rationale for conversion of the adjusted odds ratio to a corrected relative risk was that with common outcomes like infection with *M. tuberculosis* in our study the adjusted odds ratio from a logistic regression may exaggerate a risk association. The corrected relative risk thus obtained approximates the true relative risk better for common outcomes [9].

3. Results

The index case attended a Junior School with 199 other pupils, all of whom were screened during the outbreak investigation. Forty-two percent (83/199) (42%) pupils of the Junior School had a reactive gamma interferon release assay indicating infection with *M. tuberculosis*. Fig. 1 shows infection rates relating to year and activity groups within the school. A higher proportion of children without previous BCG vaccination (47%) were infected compared with 29% of children who were vaccinated (unadjusted RR 0.61, 95%CI 0.39, 0.96; relative risk reduction 38%). Children vaccinated with BCG had been vaccinated with BCG vaccine containing a live attenuated strain derived from *M. bovis* of Statens Serum Institut, Danish strain 1331 at birth once only.

A higher proportion of unvaccinated children (11%) were diagnosed with active tuberculosis disease compared with 5% of vaccinated children (unadjusted RR 0.51 95%CI 0.15, 1.70) (see Table 1). Following adjustment for ethnicity, class and activity groups (see Table 2), BCG vaccination had a protective effect against infection (OR 0.16 (95%CI 0.05, 0.54)). This corresponds to a corrected relative risk of 0.26 (95%CI 0.09, 0.69) corresponding to a relative risk reduction of 74% (95%CI 31%, 91%).

4. Discussion

BCG vaccination is known to prevent progression to disease among those who are infected. The role of vaccination in preventing infection has not been documented in animal models. However, the findings from our study together with previous studies [3,4] form an emerging body of evidence that suggests vaccination may reduce risk of infection.

The findings from our investigation are consistent with the result of a previous study [3] that reported a reduced risk of infection with *M. tuberculosis* in BCG immunised individuals. The magnitude of unadjusted risk reduction reported in this previous study was with 24% (95%CI 12–35) slightly lower compared to the 38% that we report here. Odds ratios reported in our study and presented in Table 2 were likely to exaggerate a risk association because *M. tuberculosis* infection was a common event in our cohort. We therefore converted the adjusted odds ratio into a corrected relative risk following the procedure recommended by Zhang et al. [9] to obtain an estimate closer to the true relative risk reduction. Based on the unadjusted risk difference reported in our study, the number needed to vaccinate in order to prevent 1 case of infection within an outbreak situation would be 5.

There are some differences between our report and the study by Soysal et al. [3]. Firstly, Soysal et al. [3] relied on scar formation as the indicator of BCG immunisation and could therefore not distinguish whether the protective effect only applied to the subgroup whose immunological response was associated with scar formation. We used the national child health system to verify the history of BCG immunisation provided by parents. Our investigation therefore included both individuals with and without a BCG

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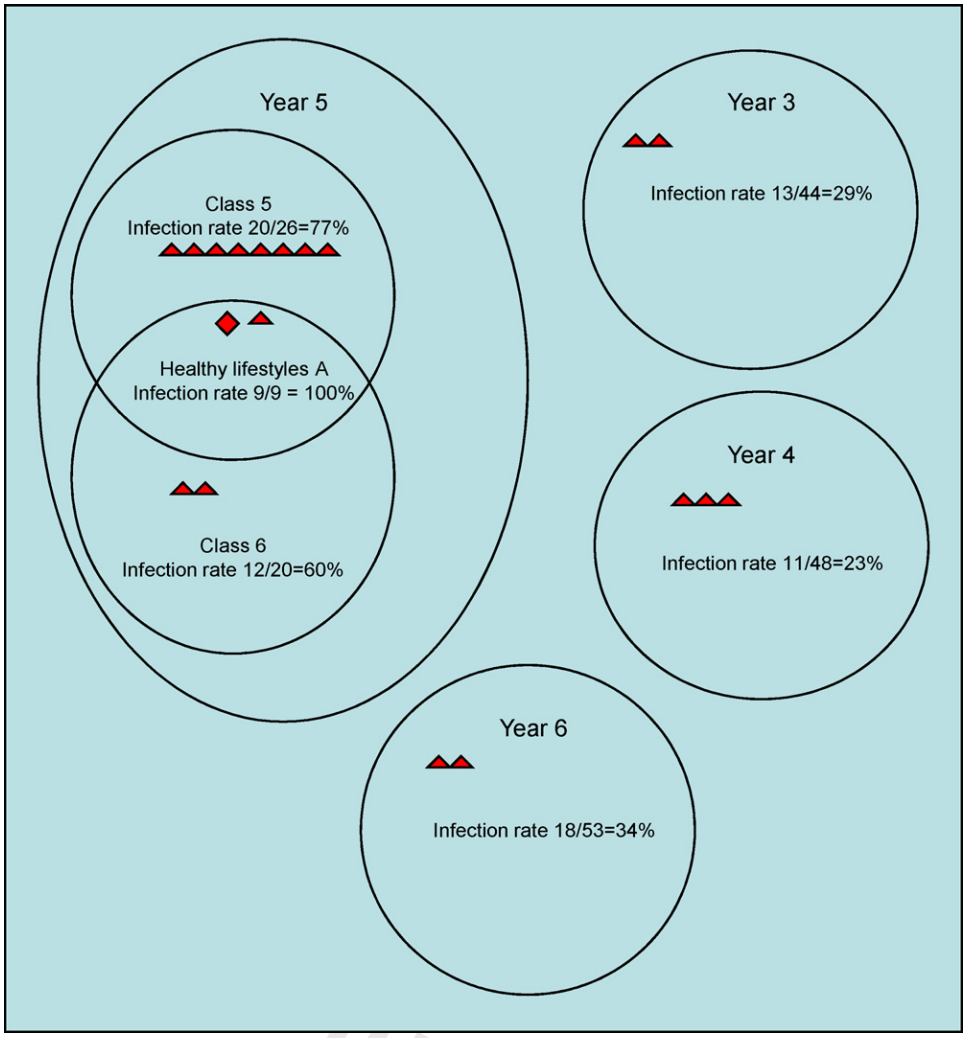

Fig. 1. (▲) Active TB detected at screening and (◆) index case. Infection rate = (number of tuberculosis infections detected/number of children screened) × 100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

scar. Secondly, Soysal et al. investigated and showed an association between BCG vaccination and infection among children in many different families exposed to tuberculosis through being a contact of adults diagnosed with smear positive sputum. We report here on a point source outbreak within a cohort of children exposed to a single source of infection. Therefore we were able to provide univariate relative risks of infection and active disease, comparing vaccinated and unvaccinated groups of children. Thirdly, the strength of our study is that we were able to adjust for proximity to the source of infection in estimating the effect of BCG vaccination in the analysis of our data. Although the study by Soysal et al. reported a protective effect of BCG vaccination, they were unable to account for differences in exposure to *M. tuberculosis* infection, which was considered an alternative explanation for the apparent protective effect attributed to BCG vaccination [3,10].

However, the protective effect of BCG vaccination that we report here is in contrast to findings from another school outbreak investigation, that reported no difference in the rate of infection with *M. tuberculosis* (measured by ELISPOT gamma interferon release assay) between vaccinated and unvaccinated children (Leicester cohort) [11]. Potential reason for this discrepancy could be the lack of control for confounding factors. The authors of this study compared ELISPOT gamma interferon release assay results for vaccinated and unvaccinated children using the Chi-squared test and did not control for confounding factors such as proximity to the index case by adjusting for class or activity group, ethnic origin or birth in high prevalence country, and history of house-hold contact by multivariable logistic regression analysis as we have done in our study. Further, the children in this study were up to 3 years older (11–15 years) than our study population (8–12 years). It is possible that the

Table 1
Association of *M. tuberculosis* infection and history of BCG vaccination. Numbers in table are *n* (%).

BCG vaccination	Not infected	Infected contacts		Unadjusted relative risk (95%CI)	
		IGRA[a] reactive[2]	Active tuberculosis	IGRA[a] reactive[b]	Active tuberculosis
No (*n* = 143)	76 (53.2)	67 (46.8)	15 (10.5)	1.0	1.0
Yes (*n* = 56)	40 (71.4)	16 (28.6)	3 (5.4)	0.61 (0.39, 0.96)	0.51 (0.15, 1.70)

[a] Interferon gamma release assay.
[b] Includes those who had active tuberculosis.

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Table 2
Association between infection with *M. tuberculosis* and risk factors for all children. Univariate odds ratios relate the odds of being an infected contact (either latent or active TB) for each risk factor variable. Adjusted odds ratios are adjusted for variables shown in the table.

Variable	Not infected	Infected contacts		Unadjusted odds ratio (95%CI)	Adjusted odds ratio (95%CI)
		Latent TB	Active TB		
BCG vaccination					
No (n = 143)	76 (53.2%)	52 (36.4%)	15 (10.5%)	1.0	1.0
Yes (n = 56)	40 (71.4%)	13 (23.2%)	3 (5.4%)	0.45 (0.23, 0.88)	0.16 (0.05, 0.54)
Ethnicity					
White-British (n = 84)	51 (60.7%)	28 (33.3%)	5 (5.9%)	1.00	1.00
Black African/Black Caribbean (n = 27)	15 (55.6%)	10 (37.1%)	2 (7.4%)	1.24 (0.51, 2.97)	1.67 (0.54, 5.18)
Bangladeshi/Indian/Pakistani (n = 43)	26 (60.5%)	11 (25.6%)	6 (13.9%)	1.01 (0.48, 2.14)	3.19 (0.91, 11.26)
Mixed (n = 27)	13 (48.1%)	11 (40.7%)	3 (11.1%)	1.66 (0.69, 3.98)	1.29 (0.42, 3.91)
Other (n = 7)	2 (28.6%)	4 (57.1%)	1 (14.3%)	3.86 (0.71, 21.1)	31.49 (3.50, 283.28)
Not known (n = 11)	9 (81.8%)	1 (9.1%)	1 (9.1%)	0.34 (0.07, 1.69)	0.38 (0.05, 3.04)

Year group (age)	Class	Not infected	Infected contacts		Unadjusted odds ratio (95%CI)	Adjusted odds ratio (95%CI)
			Latent TB	Active TB		
Three (7 years)	1 (n = 22)	18 (81.8%)	3 (13.6%)	1 (4.5%)	1.00	1.00
	2 (n = 22)	13 (59.1%)	8 (36.4%)	1 (4.5%)	3.11 (0.79, 12.3)	7.12 (1.34, 37.96)
Four (8 years)	3 (n = 25)	18 (72.0%)	4 (16.0%)	3 (12.0%)	1.75 (0.43, 7.03)	2.51 (0.53, 11.98)
	4 (n = 22)	18 (81.8%)	4 (18.2%)	0	1.00 (0.22, 4.62)	1.10 (0.20, 6.05)
Five (9 years)	5 (n = 29)	6 (20.7%)	15 (51.7%)	8 (27.6%)	17.25 (4.22, 70.48)	25.83 (4.25, 156.80)
	6 (n = 26)	8 (30.8%)	15 (57.7%)	3 (11.5%)	10.12 (2.58, 39.71)	8.50 (1.54, 46.89)
Six (10 years)	7 (n = 27)	16 (59.3%)	9 (33.3%)	2 (7.4%)	3.09 (0.82, 11.67)	3.82 (0.84, 17.42)
	8 (n = 26)	19 (73.1%)	7 (26.9%)	0	1.66 (0.41, 6.64)	2.29 (0.46, 11.44)

Variable	Not infected	Infected contacts		Unadjusted odds ratio (95%CI)	Adjusted odds ratio (95%CI)
		Latent TB	Active TB		
Healthy lifestyles A					
No (n = 190)	116 (61.1%)	57 (30.0%)	17 (8.9%)	No OR as all who attended are cases	
Yes (n = 9)	0	8 (88.9%)	1 (11.1%)		
Healthy lifestyles B					
No (n = 189)	111 (58.7%)	61 (32.3%)	17 (9.0%)	1.00	1.00
Yes (n = 10)	5 (50.0%)	4 (40.0%)	1 (10.0%)	1.42 (0.40, 5.08)	0.57 (0.11, 3.00)
Football 5					
No (n = 191)	114 (59.7%)	60 (31.4%)	17 (8.9%)	1.00	1.00
Yes (n = 8)	2 (25.0%)	5 (62.5%)	1 (12.5%)	4.44 (0.87, 22.58)	1.13 (0.12, 10.30)
Football 6					
No (n = 184)	107 (58.1%)	59 (32.1%)	18 (9.8%)	1.00	1.00
Yes (n = 15)	9 (60.0%)	6 (40.0%)	0	0.93 (0.32, 2.71)	0.61 (0.14, 2.69)
Year 3 homework					
No (n = 191)	109 (57.1%)	64 (33.5%)	18 (9.4%)	1.00	1.00
Yes (n = 8)	7 (87.5%)	1 (12.5%)	0	0.19 (0.02, 1.57)	0.11 (0.01, 1.39)
Year 4 homework					
No (n = 194)	112 (57.7%)	64 (33.0%)	18 (9.3%)	1.00	1.00
Yes (n = 5)	4 (80.0%)	1 (20.0%)	0	0.34 (0.04, 3.11)	0.42 (0.03, 6.13)
Year 5 homework					
No (n = 189)	113 (59.8%)	60 (31.7%)	16 (8.5%)	1.00	1.00
Yes (n = 10)	3 (30.0%)	5 (50.0%)	2 (20.0%)	3.47 (0.87, 13.84)	0.74 (0.13, 4.32)
Year 6 homework					
No (n = 191)	111 (58.1%)	62 (32.5%)	18 (9.4%)	1.00	1.00
Yes (n = 8)	5 (62.5%)	3 (37.5%)	0	0.83 (0.19, 3.58)	0.95 (0.17, 5.40)
Close contact of first case					
No (n = 178)	112 (69.9%)	52 (29.2%)	14 (7.9%)	1.00	1.00
Yes (n = 21)	4 (19.0%)	13 (61.9%)	4 (19.0%)	7.21 (2.32, 22.34)	1.30 (0.23, 7.28)

Notes: Percentages relate to the total number of students given for each row in the first column. All 10 children who attended healthy lifestyles A (also attended by the index case) were infected contacts: 8 on chemoprophylaxis and 2 on chemotherapy

protective effect of the neonatal BCG vaccination may have waned in this Leicester cohort, given their older average age [12]. In the study by Soysal et al. [3], which showed a protective effect of the BCG vaccination, the median age of children assessed with ELISPOT gamma interferon release assay was 7 years.

It has been suggested that the apparent protective effect of BCG vaccination reflected in a reduced reactivity in the ELISPOT assay may be due to an anergy to antigens not present in BCG (ESAT-6 and CFP10) induced by the BCG immunisation [13]. This proposed explanation, however, contradicts experimental findings showing that BCG immunisation induced an increase in T-helper cell-1 mediated reactivity to heterologous antigens like diphtheria and tetanus and tuberculin thus reducing anergy and not increasing it [14]. Another suggested explanation was that lack of reactiv-

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ity in an ELISPOT in BCG immunised exposed individuals may be due to an immune mediated reduction of the intensity of infection by *M. tuberculosis* below the threshold of detection by this assay and not a reduction of the rate of infection itself [15]. However, as has been acknowledged [15] only long term studies can answer the question whether non-reactive ELISPOT results following BCG immunisation in exposed individuals exclude infection. Such studies could investigate whether exposed individuals who are initially ELISPOT-negative show a conversion to reactive gamma interferon release assay results (e.g. on annual testing) proportional to exposure risk. Future studies investigating vaccines against tuberculosis could build on this potential ability of BCG to protect against infection and use animal models to characterize subcomponents of the BCG strain in their protective effect.

5. Conclusions

BCG vaccination at birth was associated with a significantly lower rate of reactive gamma interferon release assay using *M. tuberculosis* specific antigens ESAT-6 and CP10 (not contained in BCG) in children aged 8–12 years during a point source outbreak of tuberculosis at a Junior School.

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