
Peer reviewed version

Link to published version (if available): 10.1093/infdis/jiy207

Link to publication record in Explore Bristol Research

PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Oxford University Press at https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiy207/4964710. Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: http://www.bristol.ac.uk/pure/about/ebr-terms
Does BCG vaccination protect against non-tuberculous mycobacterial infection?

A systematic review and meta-analysis

Petra Zimmermann1,2,3, MD, Adam Finn5,6,7, FRCPCH, PhD, Nigel Curtis1,2,3, FRCPCH, PhD

Affiliations:

1 Department of Paediatrics, The University of Melbourne, Parkville, Australia

2 Infectious Diseases Unit, The Royal Children’s Hospital Melbourne, Parkville, Australia

3 Infectious Diseases & Microbiology Research Group, Murdoch Children’s Research Institute, Parkville, Australia

4 Infectious Diseases Unit, University of Basel Children’s Hospital, Basel, Switzerland

5 School of Population Health Sciences and School of Cellular & Molecular Medicine, University of Bristol, Bristol, UK

6 Bristol Children’s Vaccine Centre, Bristol, UK

7 University Hospitals Bristol NHS Foundation Trust, Bristol, UK

Address correspondence to: Dr Petra Zimmermann, Department of Paediatrics, The University of Melbourne, Royal Children’s Hospital Melbourne, 50 Flemington Road, Parkville, 3052, Australia, petra.zimmermann@rch.org.au, +61 3 9345 5522

Alternate corresponding author: Prof Nigel Curtis, Department of Paediatrics, The University of Melbourne, Royal Children’s Hospital Melbourne, 50 Flemington Road, Parkville, 3052, Australia, nigel.curtis@rch.org.au, +61 3 9345 6366

© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.
**Summary:** The incidence of non-tuberculous mycobacterial (NTM) infections is increasing worldwide. Our systematic review and meta-analysis suggest that BCG vaccination has a protective effect against NTM lymphadenitis and Buruli ulcer. This has important implications, in particular when deciding on recommendations for discontinuation of universal BCG vaccination programmes.

**Abstract**

The incidence of non-tuberculous mycobacterial (NTM) infections is increasing worldwide, particularly NTM lymphadenitis and skin infections (Buruli ulcer). This review summarises the evidence for the protective effectiveness of Bacillus Calmette–Guérin (BCG) vaccination against NTM disease. A systematic search using PRISMA guidelines was done for controlled studies investigating the protective effectiveness of BCG vaccination against NTM disease in immunocompetent individuals. This revealed ten studies, including almost 12 million participants. Three cohort studies in industrialised countries suggest that the incidence of NTM lymphadenitis is greatly reduced among BCG-vaccinated children compared to BCG-unvaccinated children, with a risk ratio (RR) of 0.04 (95% confidence interval (CI) 0.01 to 0.21). In two randomised trials in low-income countries, BCG protected against Buruli ulcer for the first 12 months following vaccination, RR 0.50 (95% CI 0.37 to 0.69). Four case control studies had conflicting results. One cohort study found that individuals with Buruli ulcer are less likely to develop osteomyelitis if they have a BCG scar, RR 0.36 (95% CI 0.22 to 0.58). No studies have compared different BCG vaccine strains or the effect of revaccination in this setting.

The protective effect of BCG vaccination against NTM should be taken into consideration when deciding on recommendations for discontinuation of universal BCG vaccination programs and in assessing new vaccines designed to replace BCG.

**Keywords:** NTM, nontuberculous, atypical, mycobacteria, lymphadenitis, epidemiology, prevention, Buruli ulcer, *M. ulcerans, M. avium*, MAC
Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous, being found in water, soil and animals. Although more than 170 species have been identified, the majority of human NTM disease is caused by fewer than 20 species [1]. In immunocompetent children, NTM most frequently cause cervicofacial lymphadenitis or skin and soft tissue infections. The commonest NTM skin infection worldwide is Buruli ulcer, a chronic, progressive skin lesion, caused by *Mycobacterium ulcerans*. Untreated, the ulcer can progress to osteomyelitis and lead to permanent bone destruction.

Although not a notifiable disease, the incidence of NTM lymphadenitis in industrialised countries is reported to be between 0.6 and 2.2 cases per 100,000 children per year [2-4], with the highest incidence in children below 4 years of age. Epidemiological studies in developing countries are lacking. Buruli ulcer has been reported in 33 countries and 15 countries regularly provide data to the World Health Organization (WHO) [5]. The incidence in Africa is estimated to be between 21 and 320 cases per 100,000 per year [6, 7] in Australia, at 1 case per 100,000 per year [5, 8], and in Japan at 0.005 cases per 100,000 per year. In Africa, about half of the cases occur in children under 15 years, whereas in Australia and Japan approximately 15% of cases occur in this age group [5].

Over the past few decades, the reported incidence of NTM lymphadenitis, as well as Buruli ulcer, has been increasing [6, 7, 9-12]. This might be attributable partly to improved awareness, enhanced reporting and better diagnostic methods, but it is also possible that the apparent increase is related to the discontinuation of Bacillus Calmette-Guérin (BCG) vaccination programmes in industrialised countries. As BCG vaccine is a live attenuated strain of *M. bovis* that shares epitopes with NTM, it is plausible that it provides specific cross-protection against NTM disease. This review and meta-
analysis summarises all studies that have investigated the protective effectiveness of BCG vaccination against NTM disease in immunocompetent children and adults.

Search strategy

A systematic search was done according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [13] for studies investigating the protective effectiveness of Bacillus Calmette–Guérin (BCG) vaccination against NTM disease. In April 2017, MEDLINE (1946 to present) and Embase (1947 to present) were searched using the Ovid interface with the following search terms: (nontuberculous OR non-tuberculous OR NTM OR atypical mycobacteria OR environmental mycobacteria OR Buruli ulcer OR Mycobacterium avium OR Mycobacterium ulcerans OR Mycobacterium avium-intracellulare) AND (BCG vaccin* OR Mycobacterium bovis) without language limitations. The references of identified articles were hand-searched for further studies. The following variables were extracted from the included studies: year of study, country, study design, number of participants, age of participants, BCG vaccination status, BCG vaccine strain, NTM disease, diagnostic methods and key findings. Review Manager (version 5.3) was used for calculation of relative risks, odds ratios and the meta-analyses. Diversity in study design and reporting, which might result in selection and reporting bias, precluded quality evaluation according to the PRISMA guidelines. The ROBINS-1 tool [14] was used to assess risk of bias (table 4).

Results

The literature searches yielded 812 articles relating to NTM and 1543 articles relating to Buruli ulcer. Of these, 10 fulfilled the inclusion criteria of controlled studies investigating the protective effectiveness of BCG vaccination against NTM disease in immunocompetent individuals. One study was excluded because it included the same patients as one of the other identified studies [15].
NTM lymphadenitis in industrialised countries

Three studies from industrialised countries, all population-based cohort studies, compared the incidence of NTM lymphadenitis in a total of 9,888,719 BCG-vaccinated children with 1,960,572 non-BCG vaccinated children. Of these children, 445 were diagnosed with NTM disease. All three studies reported a greatly reduced incidence of NTM lymphadenitis in BCG-vaccinated compared to BCG-unvaccinated children: the overall risk ratio (RR) was 0.04 (95% confidence interval (CI) 0.01 to 0.21) (table 1 and figure 1). The number needed to treat (NNT) calculated from the three cohort studies was 4835 (95% CI 4403 to 5362).

A nationwide surveillance study in Sweden, done after discontinuation of routine neonatal BCG vaccination, reported 387 children with confirmed extrapulmonary NTM disease (83% with *Mycobacterium-avium-intracellulare* complex (MAC), 97% presenting with lymphadenitis) over a period of 22 years. Only 9 of the 390 children had received BCG vaccine (0.02%). The cumulative incidence rate of NTM infection was 5.9 per 100,000 in BCG-vaccinated children below the age of 5 years and 26.8 per 100,000 in BCG-unvaccinated children [16]. Similarly, a study from the Czech Republic after discontinuation of routine BCG vaccination, in which children were screened for NTM disease by skin test, reported 27 cases of MAC lymphadenitis over a period of 6 years. All the cases occurred in BCG-unvaccinated children with an incidence of NTM lymphadenitis of 3.6 per year per 100,000 [17]. In Finland, during the period when BCG vaccine was routinely administered to newborns, the incidence of NTM lymphadenitis between 1 and 4 years of age was 0.3 per 100,000 per year in BCG-vaccinated children and 1.5 to 2.5 per year in BCG-unvaccinated children [18].
**Buruli ulcer**

Six studies investigated the protective effectiveness of BCG vaccination against Buruli ulcer, comparing the incidence in 6,475 BCG-vaccinated adults and children with 13,612 BCG-unvaccinated adults and children. The strongest evidence comes from two randomised controlled trials (RCT) done in Uganda (table 2a and figure 2a). These reported a considerably lower incidence of Buruli ulcer in BCG-vaccinated participants compared to BCG-unvaccinated with a RR of 0.50 (95% CI 0.37 to 0.69). The number needed to treat (NNT) calculated from the three cohort studies was 4835 (95% CI 4403 to 5362). Protection following BCG vaccination was higher in low-incidence than in high-incidence settings (74% vs 18%, p=0.03) [19] and was only short-term (within the first year after vaccination), with an overall reduction of Buruli ulcer of 47% (p=0.007, p<0.01).[19, 20] In one of these studies, BCG-vaccinated individuals had smaller skin lesions compared with unvaccinated individuals [20].

Four case control studies (two from Benin, one from Ghana, and one from the Congo, Ghana and Togo) investigated the protective effectiveness of BCG against Buruli ulcer (table 2b). Two studies suggest a reduced risk of Buruli ulcer in BCG-vaccinated individuals [21, 22], and two suggest no benefit [26, 27]; when the results of all four case control studies are combined there is no evidence of a protective effect of BCG, odds ratio OR 1.34 (95% CI 0.19 to 1.51) (figure 2b) [21-25].

**Osteomyelitis**

One cohort study from Benin compared the incidence of osteomyelitis in patients with Buruli ulcer in 304 BCG-vaccinated adults and children with the incidence in 68 BCG-unvaccinated adults and children (table 3 and figure 3). This showed that BCG vaccination protected against the development of osteomyelitis in patients with Buruli ulcer (RR 0.36 (95% CI %
0.22 to 0.58) [26]. However, the study did not specify how many cases were laboratory confirmed and therefore inclusion of osteomyelitis caused by pathogens other than NTM might have led to an overestimate of the rate of protection.

Discussion
The protective effectiveness of BCG vaccination against *Mycobacterium tuberculosis* and *Mycobacterium leprae* is well recognised [27, 28]. There is also evidence that infection with NTM might confer protection against *M. tuberculosis* infection or interact with the effectiveness of BCG vaccination [29-31]. In contrast, whether BCG vaccination protects against NTM infections has been controversial.

Our review found strong evidence from large European surveillance studies that BCG vaccination protects against NTM lymphadenitis in children. The rate of NTM infections in Finland, when there was universal neonatal BCG vaccination, was 30 times lower than the rate in Sweden, which did not have universal neonatal BCG vaccination, despite both countries having similar environmental and epidemiological characteristics [18]. In addition, in the Czech Republic and in Sweden, a sharp increase in NTM infection in children was observed after stopping universal neonatal BCG vaccination [16, 17].

For Buruli ulcer, there is strong evidence from two RCTs for a protective effect of BCG vaccination in the first year after the vaccination [19, 20]. The results of the case control studies are difficult to interpret given their disparate findings. Furthermore, it is important to consider that the RCTs estimated the effectiveness of BCG vaccine under the optimal storage, handling and administration conditions of a clinical trial [19, 20], whilst this was not necessarily the case in the case control studies [21-23, 25]. In addition to the study included in our review which reports smaller skin lesions
in patients with Buruli ulcer who have previously received a BCG vaccine [20], another study (not included in this review because the BCG vaccination status was not reported in the control group) reported a shorter duration to healing [24]. A further study (not included due to incomplete data) suggested that BCG vaccination protects against severe forms of Buruli ulcer with multiple skin lesions.[32] As well as the evidence from the study included in our review [26], another study (not included as there was no control group), also indicates that BCG vaccination might protect patients with Buruli ulcer from progression to NTM osteomyelitis.

Notably, all but one of the studies reporting on the protective effect of BCG vaccination against Buruli ulcer assessed BCG vaccinations status only by the presence of scar. Determining BCG vaccination status by the presence of a scar has a sensitivity of between 55% and 97% [33-35] and therefore its use may underestimate BCG vaccine effectiveness in comparative studies. However, the presence of a scar does not predict protection against tuberculosis [36, 37], and failure to develop a BCG scar might be an indication of poor vaccination technique [38]. As this might also be the case for NTM disease, using the presence of a scar rather than administration of BCG could, on the contrary, also over-estimate protection.

There is some evidence to suggest that vaccine strain and genotype influences the protective effectiveness of BCG against *M. tuberculosis* [39-41]. It is therefore plausible that there is variation between different BCG strains in their protective effectiveness against NTM disease. The vaccine strains used in the studies included in this review varied considerably, precluding meaningful analysis.
A trial that included 121,020 people in Malawi showed that revaccination with BCG approximately halved the risk of leprosy compared with a single BCG vaccination, even though it did not protect against pulmonary tuberculosis [42]. It would be of interest to determine whether revaccination with BCG increases the strength or duration of protection against non-tuberculous mycobacteria.

A number of animal studies support the notion that BCG vaccination protects against NTM infection. Mice, rabbits and guinea pigs vaccinated intracutaneously with BCG Dubos II are protected against *M. avium* administered intravenously [43]. Mice vaccinated with BCG Pasteur or Glaxo subcutaneously, intravenously or through the aerogenic route are protected against aerogenic infection with *M. avium* and *M. kansasii*, but not against *M. simiae* or *M. intracellulare* [44, 45]. One study in mice found that the effectiveness of BCG vaccination against NTM infection varies according to differences in host conditions and different strains of *M. ulcerans* [46].

Recent trials have investigated the possibility of developing vaccines with greater effectiveness against NTM. The mycobacterial antigen 85A has 85% amino acid sequence similarity in *M. ulcerans* and *M. bovis*. A DNA vaccine encoding this antigen protects mice against Buruli ulcer [47]. This vaccine has been further developed, combining antigen 85A from *M. smegmatis* with BCG in a live-recombinant vaccine, and protects mice against Buruli ulcer [48]. A single immunisation with a plasmid expressing the BCG antigen DNA-35 protects mice against infection with *M. avium* [49].

The strengths of this review are the comprehensive literature search, the clearly defined inclusion criteria and the use of meta-analysis to assess results from multiple studies. The main limitations are the heterogeneity between studies in design, including the use of different BCG strains. Further
limitations are potential differences between the groups who received and did not receive BCG vaccine, such as epidemiological factors, access to healthcare and intensity of surveillance. Additionally, the use of BCG scar to assess vaccination status in retrospective studies and the inclusion of non-laboratory confirmed cases of NTM infection probably introduces bias. The risk of bias in the studies is summarised in table 4.

Overall, our review and meta-analysis indicates that BCG vaccination protects against NTM. It is likely that effectiveness of BCG vaccination varies between different NTM diseases, populations, age groups and the BCG strain used to vaccinate. The increase in incidence of NTM lymphadenitis in industrialised countries that have discontinued universal BCG vaccination might therefore be related to the loss of protection afforded by this vaccine.

Our review suggests that the protective effect of BCG vaccination against NTM should be taken into consideration when deciding on recommendations for discontinuation of universal BCG vaccination programmes and in assessing new vaccines designed to replace BCG. In deciding vaccine policy, the incidence and the severity of the disease, as well as the NNT are important considerations. The NNT with BCG vaccine to prevent one case of NTM lymphadenitis is probably unjustifiably high when considered in isolation, as NTM lymphadenitis is relatively rare and usually has a favourable outcome, despite a frequently long and troublesome course. In contrast, Buruli ulcer is a serious condition with crippling sequelae, and has been identified by the WHO as an emerging public health problem. The potential importance of BCG vaccination for preventing Buruli ulcer has been recognised in a recent WHO position paper [50].
Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declare no conflict of interest.

Authors’ contributions

PZ drafted the initial manuscript, did the systematic review and meta-analysis. NC and AF critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted.

Funding

PZ is supported by a Fellowship from the European Society of Paediatric Infectious Diseases and an International Research Scholarship from the University of Melbourne.


Table 1 Studies reporting on the protective effect of BCG vaccination against non-tuberculous mycobacterial lymphadenitis in industrialised countries

<table>
<thead>
<tr>
<th>Author</th>
<th>Study period</th>
<th>Study type (level of evidence)</th>
<th>Study location</th>
<th>Age of participants</th>
<th>Outcome</th>
<th>Vaccine strain</th>
<th>No. of cases</th>
<th>Relative risk (95% CI)</th>
<th>Key findings, comments including NTM species cultured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katila et al [18]</td>
<td>1977-1986</td>
<td>Retrospective population-based cohort study (2C)</td>
<td>Finland</td>
<td>Children</td>
<td>Lymphadenitis</td>
<td>1977 BCG Sweden</td>
<td>25/8,333,333</td>
<td>0.15 (0.06 to 0.37)</td>
<td>BCG reduces the risk of NTM infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1978-1986 BCG Glaxo</td>
<td></td>
<td>6/300,000</td>
<td></td>
<td>• highest protection at 1-4 years of age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 35% of cases were laboratory confirmed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• MAC 9, M. malmoense 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• vaccine status determined by vaccination record</td>
</tr>
<tr>
<td>Trnka et al [17]</td>
<td>1986-1993</td>
<td>Prospective population-based cohort study (2C)</td>
<td>Czech Republic</td>
<td>Children</td>
<td>Lymphadenitis</td>
<td>BCG Russia</td>
<td>0/746,087</td>
<td>0.00 (0.00 to 0.08)</td>
<td>BCG vaccination reduces the risk of MAC lymphadenitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27/190,874</td>
<td></td>
<td></td>
<td></td>
<td>• 15% of cases were laboratory confirmed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• cervical 24, mediastinal 2, cervical plus mediastinal 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• vaccine status determined by vaccination record</td>
</tr>
<tr>
<td>Romanus et al [16]</td>
<td>1969-1990</td>
<td>Retrospective and prospective population-based cohort study (2C)</td>
<td>Sweden</td>
<td>Children &lt;15y</td>
<td>Extrapulmonary NTM infection</td>
<td>1969-1978 BCG Sweden</td>
<td>8/809,299</td>
<td>0.04 (0.02 to 0.08)</td>
<td>BCG vaccination reduces the risk of NTM infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1978-1990 BCG Denmark</td>
<td></td>
<td>379/1,469,698</td>
<td></td>
<td>• lymphadenitis/soft tissue infection 379, skin infection 5, osteo-articular infection 2, otitis media 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• 100% of cases were laboratory confirmed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• MAC 321, M. malmoense 43, M. marinum 4, M. scrofulaceum 4, Runyon III², non typable 4, M. chelonae 3, M. fortuitum 2, M. xenopi 2, M. avium 1, M. kansasii 1, M. terrae 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• vaccine status determined by vaccination record</td>
</tr>
</tbody>
</table>

1 includes 2-6 possible infections with *M. tuberculosis*

2 by culture or PCR

3 non-typed, slow growing, non-chromogenic mycobacteria

MAC - *Mycobacterium-avium-intracellulare* complex

y – year
### Table 2a Randomised controlled trials reporting on the protective effect of BCG vaccination against Buruli ulcer

<table>
<thead>
<tr>
<th>Author</th>
<th>Study period</th>
<th>Study location</th>
<th>Age of participants</th>
<th>Study type</th>
<th>Outcome</th>
<th>Vaccine strain</th>
<th>No. of cases</th>
<th>Relative risk (95% CI)</th>
<th>Key findings and comments</th>
</tr>
</thead>
</table>
| **Bradley et al [19]** | 1967-1968    | Uganda         | Children and adults (31% <15y) | Randomised controlled trial (1B) | Buruli ulcer clinical 65 histology 63 culture 31 | BCG Glaxo      | 21/606 (3%) | 44/624 (7%) | 0.49 (0.30 to 0.82) | BCG vaccination reduced the risk of Buruli ulcer  
  • overall protection rate reported as 47% (p=0.007)  
  • protection was only in the first year after vaccination (72% protective in first 6m)  
  • protection 18% in high-incidence settings, 74% in low-incidence areas (p=0.03)  
  • onset of symptoms was delayed by 2-3m in those BCG vaccinated  
  • 48% of cases were laboratory confirmed¹ |
| **Smith et al [20]** | 1970-1974    | Uganda         | Children and adults (48% <15y) | Randomised controlled trial (1B) | Buruli ulcer clinical 100 histology 48 | BCG Glaxo      | 34/2775 (1%) | 66/2764 (2%) | 0.51 (0.34 to 0.77) | BCG vaccination reduced the risk of Buruli ulcer  
  • overall protection rate reported as 47% (p<0.01)  
  • protection was only in the first year after vaccination (63% protective in first 12m)  
  • protective only in participants with tuberculin reactions of <4mm before vaccination (p<0.05)  
  • BCG vaccinated individuals had smaller skin lesions (p<0.01)  
  • no cases were laboratory confirmed²  
  • retrospective case-control part of study: RR 0.78 (0.50 to 1.21) |

¹ by culture or PCR  
² m - month  
³ y - year
### Table 2b: Case control studies reporting on the protective effect of BCG vaccination against Buruli ulcer

<table>
<thead>
<tr>
<th>Author</th>
<th>Study period</th>
<th>Study location</th>
<th>Age of participants</th>
<th>No. of participants</th>
<th>Study type (level of evidence)</th>
<th>Outcome Diagnostic methods</th>
<th>Vaccine strain</th>
<th>No. of cases</th>
<th>Odds ratio (95% CI)</th>
<th>Key findings and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raghunathan et al [23]</td>
<td>2000 Ghana</td>
<td>Children and adults (62% &lt; 15y)</td>
<td>119</td>
<td>113</td>
<td>Retrospective case control study (3B)</td>
<td>Buruli ulcer clinical 116 histology 79 stain 13 culture 54 PCR 106</td>
<td>Various strains</td>
<td>63/116 (54%)</td>
<td>56/116 (48%)</td>
<td>1.27 (0.76 to 2.13)</td>
</tr>
<tr>
<td>Debacker et al [25]</td>
<td>1997-2003 Benin</td>
<td>Children and adults (38% &lt; 15y)</td>
<td>1907</td>
<td>817</td>
<td>Retrospective case control study (3B)</td>
<td>Buruli ulcer clinical 1453</td>
<td>Various strains</td>
<td>1127/1453 (78%)</td>
<td>780/1271 (61%)</td>
<td>2.18 (1.84 to 2.57)</td>
</tr>
<tr>
<td>Nackers et al [22]</td>
<td>2002-2003 Benin</td>
<td>Children and adults (48% &lt; 13y)</td>
<td>279</td>
<td>988</td>
<td>Retrospective case control study (3B)</td>
<td>Buruli ulcer clinical 844 stain or histology or culture or PCR 134</td>
<td>Various strains</td>
<td>180/844 (21%)</td>
<td>99/423 (23%)</td>
<td>0.89 (0.67 to 1.17)</td>
</tr>
</tbody>
</table>
| Phillips et al. [21] | Children and adults (54% < 19y) | 775 | 452 | Retrospective case control study (3B) | Buruli ulcer | Congo: 2010-2011 BCG Japan 2012 BCG Japan or Russia 2013 BCG Russia Ghana: BCG Japan Togo: BCG Russia | 226/401 (56%) | 549/826 (66%) | 0.65 (0.51 to 0.83) | BCG vaccination reduces the risk of Buruli ulcer (but authors stated not after stratifying by country and age) 
- BCG vaccination does not influence duration or time to healing of skin lesions 
- approximately 95% of cases were laboratory confirmed¹ 
- vaccine status determined by presence of scar

¹ by culture or PCR

PCR - polymerase chain reaction
y - year
Table 3  Studies reporting on the protective effect of BCG vaccination against *M. ulcerans* osteomyelitis in patients with Buruli ulcer

<table>
<thead>
<tr>
<th>Author</th>
<th>Publication Year</th>
<th>Study location</th>
<th>Age of participants</th>
<th>Study type (level of evidence)</th>
<th>Outcome</th>
<th>Vaccine strain</th>
<th>No. cases</th>
<th>Relative risk (95% CI)</th>
<th>Key findings and comments</th>
</tr>
</thead>
</table>
| Portaels et al  | 2004             | Benin         | Children and adults (60% < 15y) | Cohort study (2B)               | Osteomyelitis in patients with Buruli ulcer clinical 55 stain or culture or PCR 55 | Not specified | 34/304 (11%) | 21/68 (31%)           | BCG vaccination protects against *M. ulcerans* osteomyelitis in children and adults with Buruli ulcer  
  *vaccine status determined by presence of scar*  
  *not specified how many cases were laboratory confirmed*  |

1 by culture or PCR

PCR - polymerase chain reaction

y - year
Table 4 Risk of bias summary of studies included in the review (1 = very low, 2 = low, 3 = moderate, 4 = high, 5 = very high)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Publication year</th>
<th>Study type</th>
<th>Confounding Bias</th>
<th>Selection Bias</th>
<th>Misclassification Bias</th>
<th>Performance Bias</th>
<th>Attrition Bias</th>
<th>Detection Bias</th>
<th>Reporting Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphadenitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katila[18]</td>
<td>1987</td>
<td>CS</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Trnka[17]</td>
<td>1994</td>
<td>CS</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Romanus[16]</td>
<td>1995</td>
<td>CS</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Buruli ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bradley[19]</td>
<td>1969</td>
<td>RCT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smith[20]</td>
<td>1976</td>
<td>RCT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raghunathan[23]</td>
<td>2005</td>
<td>CCS</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Debacker[25]</td>
<td>2006</td>
<td>CCS</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Nackers[22]</td>
<td>2006</td>
<td>CCS</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Phillips[21]</td>
<td>2015</td>
<td>CCS</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>M. ulcerans osteomyelitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portales[26]</td>
<td>2004</td>
<td>CS</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

CS – cohort study
CCS – case control study
RCT – randomised controlled trial
**Figure 1** Comparison of incidence of non-tuberculous lymphadenitis infection in BCG-vaccinated and BCG-unvaccinated children in industrialised countries

**Figure 2a** Comparison of incidence of Buruli ulcer in BCG-vaccinated and BCG-unvaccinated participants in randomised controlled

**Figure 2b** Comparison of incidence of Buruli ulcer in BCG-vaccinated and BCG-unvaccinated participants in case-control studies

**Figure 3** Comparison of incidence of osteomyelitis in BCG-vaccinated and BCG-unvaccinated participants with Buruli ulcer
<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>BCG Events</th>
<th>BCG Total</th>
<th>No BCG Events</th>
<th>No BCG Total</th>
<th>Total Weight</th>
<th>M-H, Random, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katila et al, Finland</td>
<td>25</td>
<td>833333</td>
<td>6</td>
<td>300000</td>
<td>39.7%</td>
<td>0.15 [0.06, 0.37]</td>
<td>1987</td>
</tr>
<tr>
<td>Trnka et al, Czech Republic</td>
<td>0</td>
<td>746087</td>
<td>27</td>
<td>190874</td>
<td>18.5%</td>
<td>0.00 [0.00, 0.08]</td>
<td>1994</td>
</tr>
<tr>
<td>Romanus et al, Sweden</td>
<td>8</td>
<td>809299</td>
<td>379</td>
<td>1469698</td>
<td>41.8%</td>
<td>0.04 [0.02, 0.08]</td>
<td>1995</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>988871</td>
<td>1960572</td>
<td></td>
<td></td>
<td>100.0%</td>
<td>0.04 [0.01, 0.21]</td>
<td></td>
</tr>
</tbody>
</table>

Total events: 33, 412

Heterogeneity: $I^2 = 82$

Test for overall effect: $Z = 3.90$ ($P < 0.0001$)
<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>BCG Events</th>
<th>No BCG Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradley et al, Uganda</td>
<td>21</td>
<td>606</td>
<td>624</td>
<td>39.5%</td>
<td>0.49 [0.30, 0.82]</td>
<td>1969</td>
</tr>
<tr>
<td>Smith et al, Uganda</td>
<td>34</td>
<td>2775</td>
<td>66</td>
<td>60.5%</td>
<td>0.51 [0.34, 0.77]</td>
<td>1976</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>3381</td>
<td>3388</td>
<td>100.0%</td>
<td></td>
<td>0.50 [0.37, 0.69]</td>
<td></td>
</tr>
</tbody>
</table>

Total events: 55 110

Heterogeneity: Tau² = 0.00; Chi² = 0.02, df = 1 (P = 0.90); I² = 0%

Test for overall effect: Z = 4.20 (P < 0.0001)
<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>BCG Events</th>
<th>No BCG Events</th>
<th>Total Weight</th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragunathan et al, Ghana</td>
<td>63</td>
<td>119</td>
<td>53</td>
<td>1.27 [0.76, 2.13]</td>
<td>2005</td>
</tr>
<tr>
<td>Nackers et al, Benin</td>
<td>180</td>
<td>279</td>
<td>664</td>
<td>0.89 [0.67, 1.17]</td>
<td>2006</td>
</tr>
<tr>
<td>Debacker et al, Benin</td>
<td>1127</td>
<td>1907</td>
<td>326</td>
<td>2.18 [1.84, 2.57]</td>
<td>2006</td>
</tr>
<tr>
<td>Phillips et al, Congo, Ghana, Togo</td>
<td>226</td>
<td>775</td>
<td>175</td>
<td>0.65 [0.51, 0.83]</td>
<td>2015</td>
</tr>
</tbody>
</table>

Total (95% CI) 3080 2370 100.0% 1.34 [1.19, 1.51]

Total events 1596 1218

Heterogeneity: $\chi^2 = 74.15$, df = 3 ($P < 0.00001$); $I^2 = 96$

Test for overall effect: $Z = 4.81$ ($P < 0.00001$)
<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>BCG Events</th>
<th>BCG Total</th>
<th>No BCG Events</th>
<th>No BCG Total</th>
<th>Weight</th>
<th>Risk Ratio M–H, Fixed, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portaels et al, Benin</td>
<td>34</td>
<td>304</td>
<td>21</td>
<td>68</td>
<td>100.0%</td>
<td>0.36 [0.22, 0.58]</td>
<td>2004</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>304</td>
<td>68</td>
<td>100.0%</td>
<td></td>
<td></td>
<td>0.36 [0.22, 0.58]</td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>34</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Not applicable
Test for overall effect: Z = 4.18 (P < 0.0001)