BCG vaccination policy in Guinea-Bissau – cost and impact on mortality

PhD dissertation

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Title

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Front-page illustration

Pedro Ivo Carvalho

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Summary in English

Bacillus Calmette-Guérin (BCG) vaccination is recommended at birth in countries with high TB burden. Increasing evidence supports that BCG may have beneficial non-specific effects in addition to the protection against TB. Despite BCG being recommended at birth, local practices of not opening a 20-dose vial of BCG unless a sufficient number of children are present for BCG vaccination have arisen to reduce vaccine wastage. However, this may cause delays in BCG vaccination. In this PhD thesis, the impact and consequences of the current BCG vaccination policy in Guinea-Bissau were evaluated.

Paper I demonstrated that the current BCG vaccination policy was associated with delayed BCG vaccination; at 1 month of age, only 38% of children had received BCG. Factors associated with delayed BCG vaccination included socio-economic status. When BCG vaccine was provided at monthly visits, socio-economic factors were no longer associated with delayed BCG and BCG coverage at 1 month of age was 88%.

Paper II showed that mothers on average sought BCG vaccination 1.26 times before getting their child vaccinated. The average household cost of seeking BCG vaccination was USD 1.89, equivalent to the UNICEF prices of a BCG vial. Thus, some of the costs saved by reducing vaccine wastage are transferred to mothers seeking BCG in vain.

In paper III, the effect of BCG on neonatal mortality and morbidity was evaluated in a natural experiment. Contrary to our hypothesis, there was no beneficial effect of BCG on neonatal mortality. The hazard ratio comparing BCG-vaccinated children with BCG-unvaccinated children was 1.26. The results were robust to sensitivity analyses.

Paper IV evaluated the effect of neonatal BCG on child mortality between 1 month and 3 years of age in TB-exposed and TB-unexposed children. Neonatal BCG was associated with 65% (95% CI: 29-83%) reduced mortality in TB-exposed children, and 45% (95% CI: 33-54%) reduced mortality in TB-unexposed children. The results were robust to sensitivity analyses.

In paper V, the impact and cost-effectiveness of disregarding the restrictive vial-opening policy was estimated to reduce TB mortality by 11.0% (95% UR: 0.5-28.8%), equivalent to 4 (UR: 0-15) averted TB deaths per annual birth cohort in Guinea-Bissau during the first 5 years of life. The estimated reduction in all-cause mortality was 8.1% (UR: 3.3-12.7%), corresponding to 392 (UR: 158-624) averted deaths per birth cohort.

In conclusion, this thesis presents important consequences of the current BCG vaccination practice in Guinea-Bissau: current BCG policy is associated with delays in BCG vaccination at increased costs for mothers seeking BCG. There was no beneficial effect of BCG on neonatal mortality, but neonatal BCG was associated with lower mortality in both TB-exposed and TB-unexposed children. Finally, disregarding the restrictive vial-opening policy was estimated to reduce both TB mortality and all-cause mortality. Based on this thesis and existing evidence, delays in BCG should be removed as it is highly cost-effective to open a vial of BCG for every child and no mother should seek BCG in vain.

Dansk resumé

Bacillus Calmette-Guérin (BCG) vaccination er anbefalet ved fødslen i lande med høj tuberkulose (TB) prævalens. Talrige studier har vist, at BCG kan have gavnlige uspecifikke effekter i tillæg til beskyttelsen mod tuberkulose. En BCG-vaccine indeholder 20-børnedoser og åbne vacciner skal kasseres efter 6 timer. Mange steder, inklusiv i Guinea-Bissau, åbnes en BCG vaccine ikke medmindre et tilstrækkeligt antal børn er tilstede for at undgå vaccinespild. I denne ph.d.-afhandling undersøges effekten og konsekvenserne af den nuværende BCG-vaccinationspraksis i Guinea-Bissau.

Artikel I viste, at kun 38% af børn var BCG-vaccinerede ved 1-månedsalderen. Socioøkonomiske faktorer var forbundet med øget risiko for forsinket BCG-vaccination. Når BCG-vaccinen blev tilbudt på månedlige besøg i landsbyen var socioøkonomiske faktorer ikke længere forbundet med forsinket BCG, og BCG-dækningen ved 1-månedsalderen var 88%.

Artikel 2 viste, at mødre i gennemsnit opsøgte BCG-vaccination til deres barn 1.26 gange før barnet blev vaccineret. Den gennemsnitlige omkostning for forældrene forbundet med at opsøge BCG-vaccination var 1.89 USD, hvilket svarer til UNICEFs listepris for en flaske BCG. Omkostningerne sparet af sundhedssystemet blev således delvist overført til forældrene.

Artikel III undersøgte effekten af BCG på neonatal dødelighed og sygelighed i et naturligt eksperiment. I modsætning til vores hypotese, var BCG ikke forbundet med reduceret neonatal dødelighed. Sammenligning af BCG-vaccinerede børn med BCG-uvaccinerede børn resulterede i en hazard ratio på 1.26 (95% CI: 0.60-2.64). Konklusionerne blev ikke ændret i sensitivitetsanalyser.

Artikel IV undersøgte effekten af neonatal BCG (<28 dage) på dødelighed i perioden mellem 28 dage og 3 år blandt børn, som var udsatte for TB-eksponering og børn, der ikke var. Neonatal BCG var forbundet med 65% (95% CI: 29-83%) lavere dødelighed i TB-eksponerede børn og med 45% (95% CI: 33-54%) laver dødelighed i TB-ueksponerede børn. Konklusionerne blev ikke ændret i sensitivitetsanalyser.

Artikel V beregnede effekten og omkostningseffektiviteten af at tilsidesætte den restriktive BCG-vaccinationspraksis. At give BCG-vaccinen ved første kontakt med sundhedsvæsenet i Guinea-Bissau blev beregnet til at medføre 11.0% (95% UR: 0.5-28.8%) lavere tuberkulosedødelighed, svarende til 4 (UR: 0-15) forhindrede tuberkulosedødsfald per fødselskohorte. Det tilsvarende fald i overordnet dødelighed var 8.1% (UR: 3.3-12.7%), svarende til 392 (UR: 158-624) færre dødsfald per fødselskohorte.

I ph.d.-afhandlingen præsentes vigtige konsekvenser af den aktuelle BCG-vaccinationspraksis i Guinea-Bissau: Den aktuelle vaccinationspraksis er forbundet med forsinket BCG-vaccination, som medfører, at forældre har øgede udgifter forbundet med at søge vaccination. Vi fandt ingen gavnlige effekter af BCG på neonatal dødelighed, men neonatal BCG-vaccination var forbundet med lavere dødelighed blandt både TB-eksponerede og TB-ueksponerede børn efter den neonatale periode. Vaccination af børn ved den første registrerede kontakt med sundhedssystemet blev estimeret til at sænke både TB-dødelighed og den overordnede dødelighed. Baseret på denne afhandling og den øvrige evidens, bør forsinkelse af BCG vaccination forhindres, da det er meget omkostningseffektivt at åbne en BCG vaccine for hvert barn og ingen mor bør opsøge BCG-vaccination forgæves.

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Tak!

Abbreviations

AEFI	Adverse event following immunisation
aHR	Adjusted hazard ratio
BCG	Bacillus Calmette-Guérin vaccine
BHP	Bandim Health Project
CI	Confidence Interval (Throughout reported as 95% confidence intervals)
GBD	Global Burden of Disease estimates
HDSS	Health and Demographic Surveillance System
HIV	Human immunodeficiency virus
HR	Hazard ratio
ICER	Incremental cost-effectiveness ratio
IGRA	Interferon-Gamma Release Assays
IR	Incidence rate
LBW	Low birth weight
LYG	Life year gained
MDR-TB	Multidrug-resistant tuberculosis
MR	Mortality rate
NBW	Normal birth weight
NSE	Non-specific effects
PYRS	Person years
TB	Tuberculosis
UNICEF	United Nation's Children's Fund
UR	Uncertainty range (Throughout reported as 95% uncertainty range)
USD	US Dollar
WHO	World Health Organisation

Introduction

WHO recommends Bacillus Calmette-Guérin (BCG) vaccination at birth to protect against tuberculosis (TB) in countries with high TB burden[1]. Increasing evidence suggests that BCG, aside from the protective effects against TB, has beneficial non-specific effects (NSE) protecting also against disease unrelated to TB[2].

Official BCG coverage estimates evaluates the BCG coverage at 12 months of age based on number of provided doses, and the estimated target population. However, the 12 months coverage of BCG do not reveal whether BCG is given timely or with delay. To reduce vaccine wastage, local practices of not opening a vial of BCG unless a sufficient number of children are present for BCG vaccination have arisen in Guinea-Bissau and other countries[3]. In the thesis, this is referred to as the restrictive vial-opening policy, although it is not an official policy.

Through the present thesis effects and consequences of the current BCG-vaccination policy on timeliness of BCG vaccination, household costs associated with seeking BCG vaccination, the effect of BCG on neonatal mortality and morbidity, the effect of neonatal BCG on post-neonatal mortality, and finally the potential impact and cost-effectiveness of disregarding the restrictive vial-opening policy are evaluated.

List of papers

Paper I:

BCG coverage and barriers to BCG vaccination in Guinea-Bissau: an observational study
Sanne Marie Thysen[1,2], Stine Byberg[1,2], Marie Pedersen[1,2], Amabelia Rodrigues[1], Henrik Ravn[2,3], Cesario Martins[1], Christine Stabell Benn[2,3], Peter Aaby[1,2,3], Ane Bærent Fisker[1,2]. *BMC Public Health* 2014, 14:1037.

Paper II:

Household costs of seeking BCG vaccination in rural Guinea-Bissau
Sanne M. Thysen[1,2,4], Stine Byberg[1,2], Justiano S. D. Martins[1], Per Kallestrup[4], Ulla K. Griffiths[7], Ane B. Fisker[1,2,3] (submitted)

Paper III:

The effect of early BCG vaccination on neonatal mortality and morbidity – a natural experiment
Sanne M. Thysen[1,2,4] , Clara Clipet-Jensen[1,2], Stine Byberg[1,2], Katarina M. Funch[1,2], Claudino Correia[1], Igualdino da Silva Borges[1], Peter Aaby[1,2], Ane B. Fisker[1,2,3] (manuscript)

Paper IV:

Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children
Sanne M. Thysen[1,2,4], Christine S. Benn[1,2,3], Victor F. Gomes[1], Frauke Rudolf[1,4], Christian Wejse[1,4], Adam Roth[5,6], Per Kallestrup[4], Peter Aaby[1,2], Ane B. Fisker[1,2,3] (manuscript)

Paper V:

Disregarding the restrictive vial-opening policy for BCG vaccine in Guinea-Bissau: impact and cost-effectiveness for tuberculosis mortality and all-cause mortality in children aged 0-4 years
Sanne M. Thysen[1,2,4], Ane B. Fisker[1,2,3], Stine Byberg [1,2,3], Peter Aaby[1,2], Ulla K. Griffiths[7], Rebecca C. Harris[8] (manuscript)

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Background

Tuberculosis

Childhood tuberculosis

Compared with adults, TB in children is much more likely to present with atypical symptoms[4,5]. Non-remitting cough, failure to gain weight or weight loss, wheezing or chronic fever are typical symptoms of TB in children[4]. However, many children with TB present with no or few symptoms, with young infants more likely to have symptoms compared with older children[5]. Most childhood TB cases are primary TB cases, which means that the primary TB infection develops to active TB disease, often within a year after contracting the infection[1,5]. Reactivation tuberculosis, which is more common in adults, is rare in infants and young children[5]. TB is usually classified as pulmonary TB or extra-pulmonary TB. Miliary TB and TB meningitis are the most severe types of extra-pulmonary TB, while the most common location in children is in the lymph nodes at the neck[5].

Childhood TB cases are usually discovered either when children present with symptoms consistent with TB and a history of TB exposure, through contact tracing of an adult TB case, or through screening programs[5]. Childhood TB can be difficult to diagnose, and unfortunately, countries with the highest burden of TB often have few diagnostic possibilities, as resources are scarce, and hence, there is less chance of identifying childhood TB.

Sputum smears is a common tool for diagnosis of TB in adults[6]. Sputum smears are however not as useful in children for a number of reasons: First, not all children are capable of producing a sputum sample. Second, children develop more extra-pulmonary TB (25-35% of childhood TB cases are extra pulmonary[5]). And finally, even in children with pulmonary TB, and where the child is able to produce a sputum sample, the sample is likely to contain few or no bacilli[4]. Hence, in children, diagnosis relies on a combination of the history of exposure, evaluation of risk factors, clinical presentation, and results of diagnostic tests such as chest x-rays, tuberculin skin test (TST), interferon-γ release assays (IGRA) or GeneXpert assay[5]. Each of the diagnostic tools have their advantages and disadvantages. Importantly, TST and IGRA are not able to distinguish between TB infection and TB disease, and TST also show reactions, albeit smaller, in BCG-vaccinated individuals[5].

HIV infection is a significant risk factor for development of TB disease[5]. In adults, progression from asymptomatic TB infection to TB disease is greatly increased in patients with HIV, even in patients who receive anti-retroviral medicine[5]. In children with HIV, TB disease often presents with severe clinical symptoms, and progresses more rapidly compared with HIV-negative children with TB[5].

Importantly, TB is a curable disease if treated, and it is therefore important to identify TB cases.

Tuberculosis burden

In 1882, Dr Robert Koch discovered *Mycobacterium Tuberculosis*, which was one of the leading causes of death in some European countries in the late 1800s[4,6]. In 1993, WHO declared TB as a global emergency[6], and in 1997 WHO published the first data on global TB incidence and mortality, but these data did not include estimates of childhood TB until 2012[4].

As part of the yearly Global Tuberculosis Report, WHO publishes estimates of childhood TB incidence and childhood TB mortality[6]. In countries like Guinea-Bissau with missing or poor quality of data, WHO estimates the burden of TB by combining case-notification data with expert opinions about case detection gaps[7]. This method is considered unreliable, and is only used when other methods are unavailable[7]. In recent years, The Global Burden of Disease (GBD) study at the Institute of Health Metrics and Evaluation at the University of Washington has also provided estimates on the global burden of TB[8]. However, as it is generally accepted, there are many undiagnosed TB cases, and thus, the global estimates carry much uncertainty. In 2015, the global estimates of TB mortality provided by WHO and GBD differed by close to half a million deaths, corresponding to half of the GBD estimate. The differences were explored; excluding countries with recent prevalence surveys resulted in a good overall correlation between the estimates[9], and the estimates differed most where the data was weakest[10], as in Guinea-Bissau.

Within recent years, probably due to both increased interest in childhood TB and increased interest in modelling of data, several modelling studies have been conducted to provide estimates of different aspects of the TB burden. Houben and Dodd estimated that 1.7 billion people were infected with latent TB in 2014[11], corresponding roughly to 23% of the World Bank estimate of the global population. Modelling studies have also provided estimates of the global childhood TB burden: In 2016, Dodd and colleagues estimated that 850,000 children developed TB in 2014, hereof 58,000 children with isoniazid-monoresistant TB, 25,000 with multidrug-resistant TB and 1,200 children with extensive multidrug-resistant TB[12]. In 2017, Dodd and colleagues estimated that 191,000 children below 5 years of age died from TB in 2015; hereof 31,000 were children with HIV infection. Most of the estimated deaths were in children not receiving TB treatment[13]. Recently, Dodd and colleagues estimated that full implementation of household contact management could prevent 159,500 TB cases in children below 15 years of age[14]. Modelled global estimates can be useful to provide a picture of the global burden of TB. However, no model provide better estimates than the data and assumptions used[15]. A good example of this is the discrepancy between the GBD and the WHO estimates: both sets of estimates are provided based on high quality models and the main differences are in countries with weak data[10]. Thus, it is important to bear in mind that the estimates regarding childhood TB, although they can be useful, carry much uncertainty, especially when available data inputs are of low quality.

TB vaccines

Currently, Bacillus Calmette-Guérin (BCG) vaccine is the only approved vaccine against TB[1]. However, several new TB vaccine candidates are in the pipeline. Different strategies are pursued in the development of a new TB vaccine; some vaccine candidates aim at preventing TB infection, some at preventing progression from TB infection to TB disease, some at preventing re-infection and some to be given in combination with TB treatment as therapeutic vaccines[4]. The focus of this thesis is not TB vaccines in general, but the BCG vaccine. However, in the chase for a new TB vaccine, it might be worthwhile to take a second look at the BCG vaccine. In a recent phase II trial evaluating the effect of H4:IC31 vaccine or BCG vaccine on TB infection, BCG-revaccination reduced rate of sustained quantiferon conversion by 45.4%, whereas the H4:IC31 vaccine reduced rate of sustained quantiferon conversion by 30.5%[16], suggesting that revaccination with BCG may have unexploited potential.

BCG vaccine against tuberculosis

BCG vaccine was first used in humans in 1921 to protect against TB[1]. Aside from TB, BCG also provides some degree of protection against leprosy and some non-tuberculous mycobacterial infections[1]. BCG is usually given in the deltoid area of the left arm as a 0.05mL dose for infants and 0.1mL dose for older children or adults. The vaccine is administered intradermally and a successful BCG vaccination usually leaves a small scar.

WHO recommendations

As per the latest WHO recommendations from February 2018[1], WHO recommends BCG vaccination at birth for all healthy neonates in settings with high TB burden and/or high leprosy burden. As an update to the previous recommendations[17,18], it is now clearly stated that BCG should be provided at the earliest opportunity after birth if not provided at birth, and specifically stated that a vial should be opened for every child despite wastage of unused vaccine in order not to cause delay in BCG vaccination[1]. Revaccination is not recommended, also not in the absence of a BCG scar. In countries with high HIV prevalence and high TB prevalence, it is recommended that neonates born to women of unknown HIV status and neonates of unknown HIV status born to HIV-infected women are vaccinated at birth. For neonates with clinical symptoms of HIV or HIV-infected neonates, BCG vaccination is recommended to be delayed until anti-retroviral therapy is started and the infant confirmed to be clinically well and immunologically stable[1]. It was also added that low-birth-weight children who are clinically stable should be BCG-vaccinated at birth[1].

BCG vaccination coverage

Despite BCG being recommended at birth, no official coverage estimates evaluate BCG coverage in early life. Official WHO/UNICEF coverage estimates are 12-month BCG coverage estimates. Hence, they carry little information about timeliness. According to official WHO/UNICEF estimates of global BCG coverage, 88 percent of children scheduled to receive BCG are vaccinated[19], and for Guinea-Bissau BCG coverage was 87 percent in 2017[20]. However, BCG is often given with delay[3,21].

WHO policy recommendation and vaccine donors' performance indicators are major motivating factors for the countries, as WHO sets the political aims and policies to strive for[1], and vaccine donors ask for performance indicators that are part of the evaluation of the vaccination programme and resource prioritisation[22]. The vaccination programmes are amongst others evaluated by the 12-month coverage estimates and the vaccine wastage estimates, leaving countries with little incentive to strive for timeliness of vaccines. BCG vaccine comes in 20-dose vials, which, once reconstituted, have to be used within 6 hours. In Guinea-Bissau, such a vial is usually not opened unless at least 10 children are present for vaccination to prevent vaccine wastage. This is not an official policy, but a local practice that occurs many places. Throughout the thesis (and papers) this local practice is coined the restrictive vial-opening policy. We assessed the impact of this restrictive vial-opening policy on BCG coverage in paper I, on household costs and mothers experience in paper II and evaluated the potential impact and cost-effectiveness of disregarding this policy in paper V.

BCG vaccine efficacy against tuberculosis

The efficacy of the BCG vaccine has varied widely between different studies. In 2014, Mangtani and colleagues published an extensive review of BCG's protection against pulmonary and miliary or meningeal TB[23]. Protection against pulmonary TB was highest in studies of neonatal BCG vaccination or

in studies enrolling school-age children based on stringent TST testing prior to vaccination, whereas there was no consistent evidence of BCG protecting against pulmonary TB in individuals older than school age[23]. Furthermore, the protection was greater in trials conducted further away from the equator, and lower in trials conducted closer to equator[23]. Interestingly, it seems like the protection of BCG is higher in areas with less environmental TB and that the protection is highest in neonates and TST-negative school age children, suggesting that the effect of BCG against pulmonary TB is highest when provided to TB-naïve individuals. A similar pattern was observed for protection against meningeal or miliary TB, where the efficacy of BCG was greatest in trials of neonates or stringently tested school-age children[23]. Importantly, trials of neonatal BCG vaccination have consistently found beneficial effects against TB[23].

The same group reviewed the effect of BCG on latent TB infection in children[24] and found that BCG was associated with 19% protective efficacy of latent TB infection. Furthermore, they found a 58% vaccine efficacy against progression from TB infection to TB disease[24].

BCG history and strains

In 1900, Albert Calmette and Camille Guérin began their research on the *M. Bovis* strain that they later, until 1921, continued to sub-culture in vitro on three-weekly intervals at the Pasteur Institute of Lille in France[25] until it in 1921 was first used in a human[1]. The vaccine was provided orally to an infant, whose mother had died of TB a few hours after childbirth. The child developed no serious side effects, and no signs of TB. This led to the vaccine being used in other infants, and already in 1924, the original culture of BCG was sub-cultured and distributed to several laboratories around the world[25]. The sub-cultures were then propagated on different culture media and following different schedules, which led to the development of genetically distinct BCG strains[25].

Currently, the WHO-prequalified BCG vaccines consists of the Danish strain (by Green Signal Bio Pharma Limited and AJ Vaccines companies), the Japanese strain (by Japan BCG Laboratory), the Bulgarian strain (by Bul Bio-National Center of Infectious and Parasitic Diseases Ltd.), and the Russian strain (by Serum Institute of India)[26]. It is not yet clear which BCG strain confer the best protection against TB, but studies have suggested that the effect of BCG on TB varies by BCG strain[27]. In a large randomised trial in Hong Kong in 1978-1991, BCG Pasteur (currently not on WHO's list of prequalified vaccines[26]) was associated with 45% (95% CI: 22-61%) less TB than BCG Glaxo[28] (also not on WHO's list of prequalified vaccines[26]). A cohort study from Kazakhstan also found varying protection of BCG according to strain[29]. However, in the review by Mangtani and colleagues, BCG strain did not explain the variations of BCG efficacy between trials[23].

Adverse effects of BCG

BCG vaccination has been associated with adverse events following immunization (AEFI). These include local reactions such as injection site abscess, severe ulceration, and suppurative lymphadenitis[1]. However, the adverse events following BCG vaccination are rare: In a recent randomised trial of neonates in Denmark, BCG-Denmark was associated with a 6.1/1000 (95% CI: 3.3/1000-1/100) risk of regional lymphadenitis and 4.7/1000 (2.3/1000-8.7/1000) risk of suppurative lymphadenitis[30]. This was higher than the manufacturer estimate at the time[30]. A possible explanation for the higher than expected frequency, is that the effect of BCG differs by strain and batch[31]. Therefore, the risk of AEFIs is also likely to differ by strain and batch.

In addition to self-limiting adverse events, a more severe AEFI is that BCG vaccination of immunocompromised children can cause disseminated BCG infection[32,33]. The risk of disseminated BCG disease has been estimated to 1.56 to 4.29 cases per million doses[1]. The risk is highest among HIV-infected children[1]. A study from South Africa identified 8 cases of disseminated BCG disease over a 3-year period, all among immunocompromised children, and 6 of these were HIV-infected[32]. Six of the children died, corresponding to a case fatality of 75%[32]. BCG can also cause BCG osteitis and BCG osteomyelitis, and when these events occur, it is often more than 12 months after vaccination[1].

Non-specific effects of vaccines

General principles of non-specific effects of vaccines

BCG is recommended to protect against TB[1]. However, several studies suggest that BCG has beneficial non-specific effects[2,34] (NSEs) and reduces mortality by more than can be explained by the protection against tuberculosis. Prior to approval, vaccines are tested for their disease-specific effects, but vaccines are usually not tested for their effect on overall mortality and morbidity. BCG is not the only vaccine with NSEs. NSEs have been assessed for four live vaccines: BCG[34], measles vaccine[35,36], oral polio vaccine[37] and smallpox vaccine[38,39], and for six non-live vaccines: diphtheria-tetanus-pertussis vaccine[40,41], pentavalent vaccine[42], inactivated polio vaccine[43], RTS,S/AS01 malaria vaccine[44], hepatitis B vaccine[45], and H1N1 influenza vaccine[46]. In 2014, WHO commissioned a review on the NSEs of the BCG vaccine, the diphtheria-tetanus-pertussis vaccine and the measles vaccine, and for BCG and measles they concluded that the existing evidence at the time suggested that the vaccines may have beneficial NSEs[2].

Based on the current evidence, the general pattern is that live vaccines have beneficial NSEs[2,47,48], and non-live vaccines have negative NSEs[49,50]. Fortunately, it seems like the negative NSEs can be neutralised or even reversed by a subsequent live vaccine. However, reversely, a non-live vaccine after a live vaccine can also neutralise or reverse the beneficial NSEs of a live vaccine. Several studies have now found that sequence of vaccines are important for all-cause child mortality[51-55]. Also in high-income countries with low child mortality, sequence of vaccines seem to matter, and fewer children are admitted to hospital when the most recent vaccine is a live vaccine than when the most recent vaccine is a non-live vaccine[56-58].

Vaccination programmes worldwide are planned for children, and no differentiation is made between boys and girls. However, several studies have found sex-differential NSEs, suggesting that NSEs of most vaccines are stronger for girls than boys[50,59-62]. It has also been demonstrated that boys' and girls' immune system differ, and that their immunological response to vaccines differ[63-65]. Thus, it may be necessary to assess effects of vaccines and other interventions by sex.

BCG vaccine

In figure 1, I provide an overview and a meta-analysis of published studies without survival bias assessing the effect of BCG on all-cause mortality. Trials and studies marked with * were included in the WHO-commissioned review conducted in 2014[2]. We have added subsequent trials of BCG on all-cause mortality to the meta-analysis. I have not included any additional observational studies, since the only published observational study assessing the effect of BCG on all-cause mortality is a cohort study from Uganda[66]. We have already commented that this study likely has survival bias[67], and the study is therefore excluded.

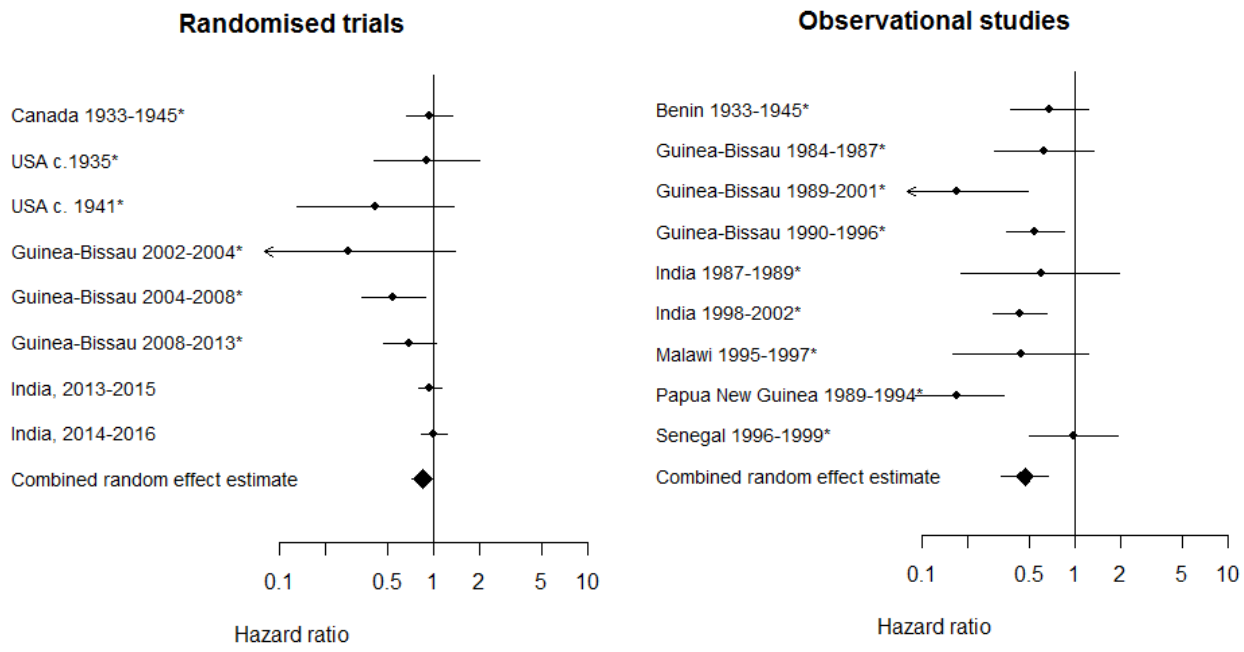

Figure 1. Metaanalyses of published randomised trials[34,68-73] and observational studies[74-82], respectively, of the effect of BCG on mortality. Studies marked with * were included in the WHO-commisioned review from 2014[2].

Currently, we are awaiting results of some randomised trials assessing the effect of BCG versus no BCG on all-cause mortality listed in Table 1 below.

Country	Trial registration	Children eligible	Trial start	Expected end of trial	Sample size	Mortality outcome
Guinea-Bissau	NCT01989026	Neonates admitted to the neonatal intensive care unit	October 2013	October 2017	3361 neonates enrolled	Primary outcome: In-hospital mortality
Uganda[83]	ISRCTN59683017	Neonates (of HIV-negative mothers) who can be discharged directly from the hospital	March 2014	July 2015	560 neonates, data collection completed	Secondary outcome: death between birth and 10 weeks of age
Uganda[84]	NCT02606526	HIV-1-exposed neonates	July 2016	December 2020	2200 neonates	Secondary outcome: infant mortality
Guinea-Bissau[85]	NCT02504203	Neonates less than 72 hours at time of home visit	July 2016	December 2020	6666 neonates	Primary outcome: early infant mortality day 1-42
India	CTRI/2017/01/007676	Neonates weighing less than 2000 g when admitted to the neonatal intensive care unit.	February 2017	January 2020	1998 neonates	Primary outcome: Neonatal mortality of children who are discharged

Table 1: Registered trials assessing the effect of BCG with mortality as a registered primary or secondary outcome.

Strain of BCG

As mentioned previously, there are several different BCG strains. As for the effect of BCG on TB, it has been suggested that different strains of BCG may have different effects on all-cause mortality and morbidity[27]. Recently, two randomised trials from India found no effect of BCG at birth on neonatal mortality[73]. In both India and Guinea-Bissau, all trials were conducted among LBW children and primary outcome was neonatal mortality. In Guinea-Bissau, BCG-Denmark was used in all trials[34], whereas BCG-Russia was used in the trials from India[73]. Strain of BCG has been suggested as a possible reason for the discrepancy between the trials in India, and the trials from Guinea-Bissau[73,86]. However, the present evidence is not sufficient to conclude on the effect of BCG strain on all-cause mortality. We are currently awaiting results of further two randomised trials from the national hospital in Guinea-Bissau. In trial 1, children were randomised to receive either the Danish strain or the Russian strain. In the still enrolling trial 2, children are randomised to receive either the Japanese strain or the Russian strain. The results of these trials will provide a better idea of whether the strain of BCG affects all-cause mortality in neonates. Furthermore, the ongoing trial in India can add clarity of whether the absence of effect of BCG on neonatal mortality was due to BCG strain or other differences between the settings.

BCG scar

A successful BCG vaccination usually causes a small scar at the injection site. However, scarring rates vary widely between study cohorts: In urban Guinea-Bissau, studies have found scarring rates between 70-95%[87,88], whereas a study in rural Guinea-Bissau found that only 52% of BCG-vaccinated children had developed a BCG scar in 2010[89]. Recently, when BCG was provided by trained BHP nurses in rural Guinea-Bissau, 98% of children developed a BCG scar[90], whereas only 79% of children vaccinated elsewhere developed a BCG scar[90], thus supporting the previous observation that vaccination technique is important[91]. Importantly, studies assessing the effect of having a BCG scar among BCG-vaccinated children, consistently find that a BCG scar is associated with lower mortality. In a meta-analysis of the five published studies and a recent accepted study among BCG-vaccinated children in Guinea-Bissau assessing the effect of having a BCG scar on mortality, having a BCG scar is associated with 45% (95% CI: 36-59%) compared with not having a BCG scar (Figure 2). Two recent studies assessing determinants for developing a BCG scar also find that strain of BCG is important[90,92].

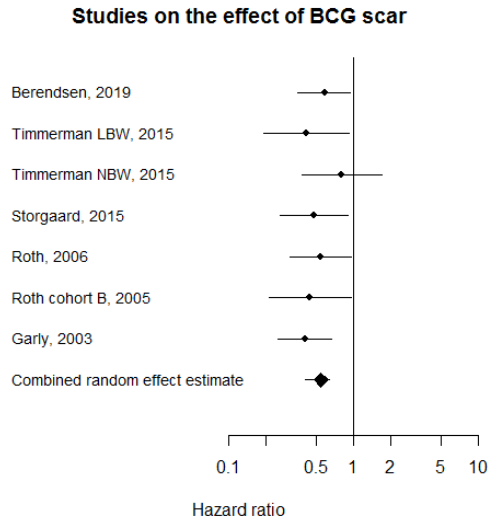

Figure 2: Meta-analysis of studies[87-89,93-95] assessing the effect on mortality of having a BCG scar among BCG-vaccinated children.

Maternal BCG vaccination

A meta-analysis of two trials from Guinea-Bissau suggested that children who are measles vaccinated in the presence of maternal measles antibodies have beneficial NSEs[96], suggesting that maternal priming can affect the NSEs of vaccines. This led to the hypothesis that maternal BCG vaccination status prior to pregnancy might affect the NSEs of BCG on all-cause mortality and morbidity. In a randomised controlled trial in Denmark, children were randomised to BCG or no BCG at birth. The main out-come of the trial was hospital admissions at 15 months of age, and they found no effect (HR: 1.05 (95% CI: 0.92-1.18) of BCG vaccination on all-cause hospital admissions in the main analysis[97]. Nevertheless, in a pre-defined secondary analysis stratified by maternal BCG status: BCG-vaccinated children of BCG-vaccinated mothers had 35% (95% CI: 6-55%) lower risk of an infectious hospital admission compared with not BCG-vaccinated children of BCG-vaccinated mothers[98]. Thus, also BCG maternal priming may play a role. The hypothesis was tested in Guinea-Bissau in an observational study within a randomised trial. Among children aged 4.5 to 36 months, having a BCG scar was associated with 41% (95% CI: 5-64%) lower mortality. Stratifying by maternal BCG status, among children of mothers with a BCG scar, having a BCG scar was associated with 66% (95% CI: 33-83%) lower mortality, whereas among children of mothers without a BCG scar, having a BCG scar was associated with 8% (-83-53%) lower mortality[93]. This is currently being evaluated further as secondary outcomes in the trials in Guinea-Bissau and in observational studies. In paper III, we also evaluated the effect of BCG separately by maternal BCG-scar status.

Aims and hypotheses

The main objective of this PhD project was to describe the present BCG vaccination practice in Guinea-Bissau. To reduce vaccine wastage, a 20-dose vial of BCG is not opened unless at least 10 children are present for BCG vaccination, thus causing a locally implemented "restricted vial-opening policy". The consequences of the policy were assessed as part of this PhD project, and the potential impact of disregarding this restrictive vial-opening policy for BCG was estimated, including the cost-effectiveness of such a policy change.

The hypotheses tested were:

1) The current BCG vaccination is associated with delays in BCG vaccination. Disregarding the restrictive vial-opening policy would reduce delays in BCG vaccination and provide a more equitable coverage.
2) BCG vaccination is associated with 45% reduced neonatal mortality.
3) Neonatal BCG vaccination is associated with lower mortality compared with later BCG vaccination for both TB-exposed and TB-unexposed children.
4) Disregarding the restrictive vial-opening policy is a cost-effective intervention.

We tested the four hypotheses in five papers:

In paper I, we assessed the current BCG coverage in Guinea-Bissau, risk factors associated with delay, and estimated the potential BCG coverage if the restrictive vial-opening policy was disregarded and children were vaccinated at the first health-facility contact.

In paper II, we assessed the economic consequences of the current BCG policy for mothers of infants by estimating the average number of times mothers sought BCG for their infant, and estimated the household-costs of seeking BCG vaccination.

In paper III, we exploited the HDSS setup with monthly visits and disregarded the restrictive vial-opening policy, which created a natural experiment in which we could assess the effect of BCG on neonatal mortality and morbidity.

In paper IV, we took advantage of the long-term registration of TB cases in the BHP urban study area to assess the potential effect of neonatal BCG on mortality between 28 days and 3 years of age for TB-exposed children and TB-unexposed children.

In paper V, we developed a static mathematical model to estimate the impact of disregarding the restrictive vial-opening policy on TB-specific mortality and all-cause mortality in Guinea-Bissau, and to estimate the cost-effectiveness of disregarding the restrictive vial-opening policy.

Methods

Setting

Guinea-Bissau and Bandim Health Project

All of the studies were conducted using data from the Bandim Health Project (BHP) in Guinea-Bissau. Guinea-Bissau is a small West-African country at the Atlantic Ocean coast, bordering Senegal in the north and Guinea to the east and south. BHP established the urban Health and Demographic Surveillance System (HDSS) in 1978 and the rural HDSS was established in 1990. The urban HDSS now covers six suburban districts and approximately 102,000 inhabitants are followed: Children are followed through three-monthly household visits until 3 years of age, pregnancies are followed through monthly household visits, and the remaining population is followed through censuses every 2-5 years. Data from the urban HDSS were used in paper IV and paper V.

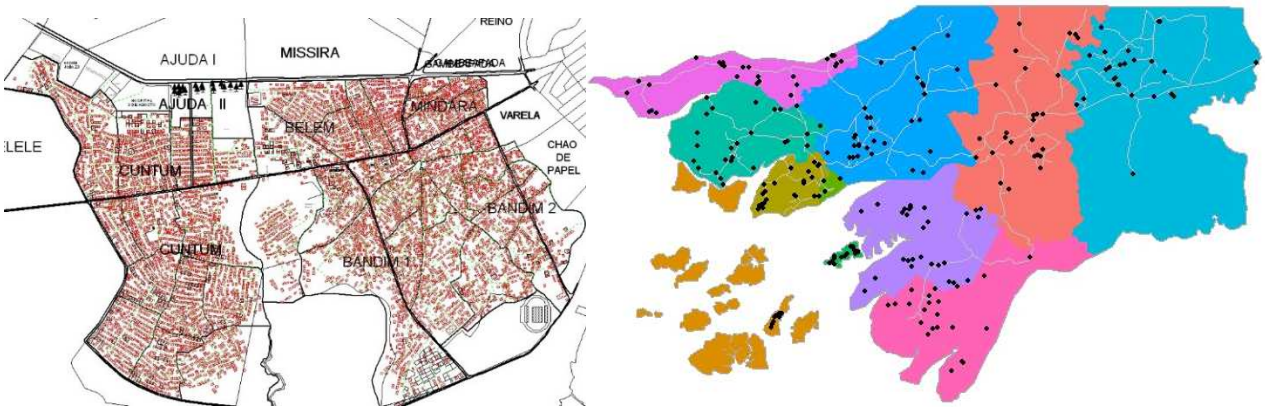

Figure 3. Map of the BHP urban HDSS with 6 suburbs and map of the rural HDSS with village clusters marked with dots ·

The rural HDSS covers all rural health regions in Guinea-Bissau, where women of fertile age and children aged 0-5 years are followed through biannual household visits. During some periods, visit frequency was higher in some regions due to randomised trials (2012-2016: monthly visits in Oio, Biombo, Cacheu and Sao Domingos due to a randomised trial of an additional early measles vaccination[36]; 2016-now: two-monthly visits in Oio, Biombo and Cacheu due to a cluster randomised trial of BCG vaccination provided at household visits within 72 hours after birth[85]). Data from the rural HDSS were used in paper I, paper II, paper III, and paper V. An overview of the data collected in the HDSS areas is displayed in figure 4 and 5, and details on the information collected are described in respective papers. Further details on the HDSS setup are provided elsewhere for both the urban HDSS[61] and the rural HDSS[99].

As a supplement to the routine BHP HDSS data, several studies and trials collect additional data, and there are even cohorts nested within the urban HDSS. An example of this is the TB register, where all diagnosed TB patients above 15 years of age from the urban study area are registered. The register was established in 2003, and contains among other information of place of residence for TB cases. This allowed us to identify houses with residents exposed to TB. The official infrastructure in Guinea-Bissau is scarce, and without the urban HDSS setup with an address system and all houses in the study area mapped and identified, we would not be able to conduct the study for paper IV.

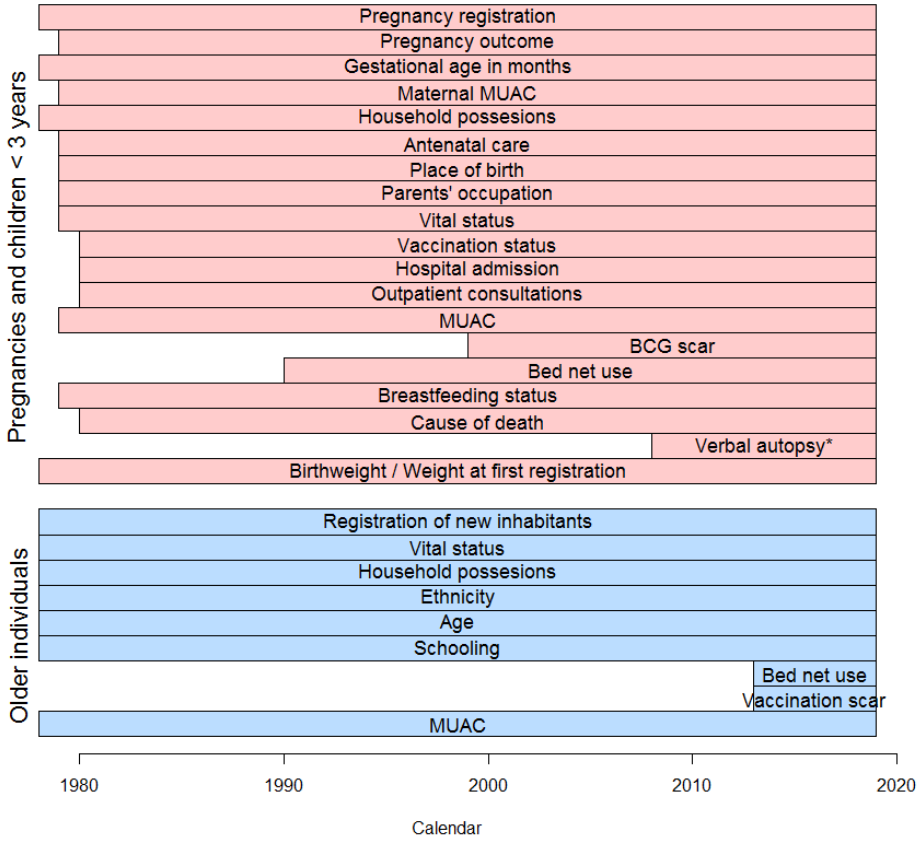

Figure 4: Gantt diagram of data collected through the BHP routine HDSS data collection in urban Guinea-Bissau between 1978 and 2019.

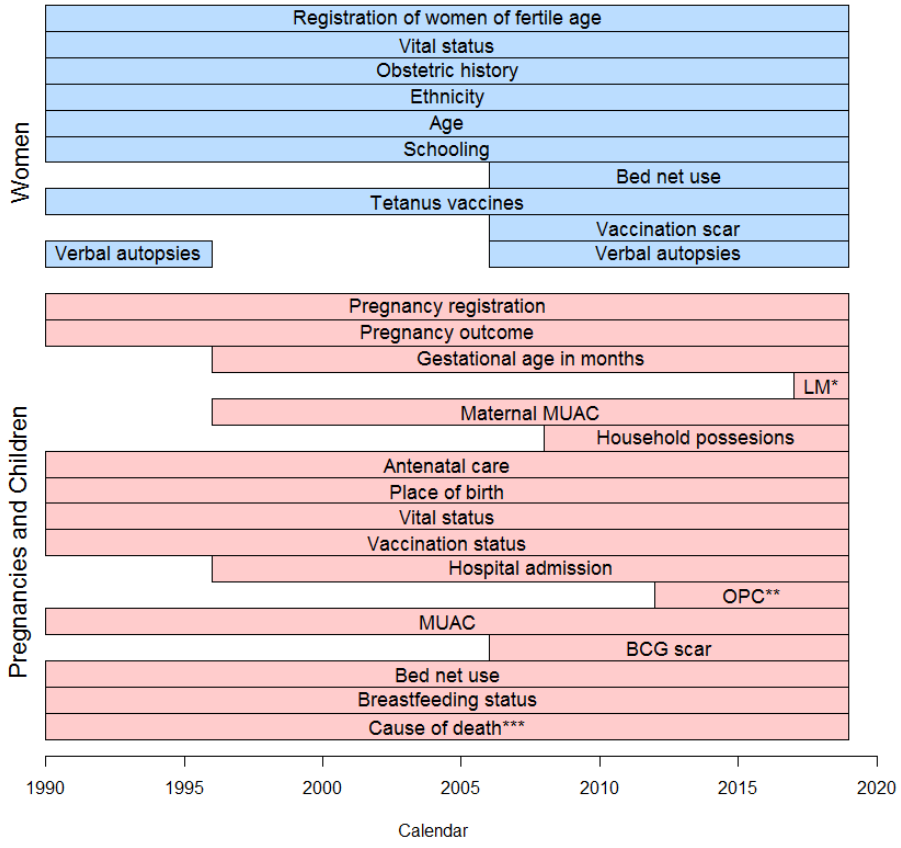

Figure 5: Gantt diagram of data collected through the BHP routine HDSS data collection in rural Guinea-Bissau between 1990 and 2019.

Observational studies

BCG coverage (Paper I and V)

The HDSS setups with routine data collection allow the realisation of observational studies. In paper I, we exploited the routine data collection of vaccination status, registration of vaccination card inspection and place of birth to estimate the current and potential BCG coverage at different ages in all rural health regions to assess the timeliness of vaccination. Vaccination coverage by 12 months of age is commonly assessed by using survey data from children between 12 and 23 months of age. To obtain comparable estimates for younger children, we assessed vaccination coverage at a certain "coverage age" among children with inspected vaccination card on a visit within the year following the coverage age. This approach was used to assess the BCG coverage used in paper I and in the baseline scenario in paper V. In both paper I and V, we calculated the potential BCG coverage in the non-restrictive scenario (disregarding the restrictive vial-opening policy) as the hypothetical BCG coverage if the child had been vaccinated at the first health facility contact. As we used registered health facility contacts to calculate the potential BCG coverage, the coverage estimates in the non-restrictive scenario in paper I and paper V are conservative estimates. We do not account for other health facility contacts that might lead to earlier vaccination such as outpatient consultations or child controls.

Barriers to timely BCG (Paper I and II)

In paper I, we assessed background factors associated with delayed BCG vaccination, and assessed whether background factors associated with delayed BCG changed when BCG was available at monthly village visits. In paper I and paper II, we complemented the risk factor information with interviews of mothers of BCG-unvaccinated children about barriers for timely BCG vaccination.

As BCG vaccination was provided at monthly visits in the four regions closest to Bissau (data used in paper III), we only collected information for paper II in the remaining regions (plus three clusters in Oio, where monthly visits were not conducted and BCG vaccination not provided). In the period between May 2014 and December 2016, we added a questionnaire for mothers of children registered prior to birth. At the first visit after birth, mothers were interviewed about how many times they had sought BCG vaccination for their child, how much money they had spent on transport and how many hours used away from home. Mothers of children not BCG-vaccinated or where the mother was not present at the first interview after birth were (re)interviewed at the next village visit. We calculated how many times mothers on average sought BCG vaccination and the household costs related with seeking BCG vaccination (time and money for transport).

A natural experiment (Paper III)

While the age of BCG vaccination is dependent on availability of BCG and that the mother brought the child for BCG vaccination on a day where BCG is available, we exploited the natural experiment created with the implementation of monthly visits in four rural regions (Oio, Biombo, Cacheu and Sao Domingos). Within this setup, we assessed the effect of BCG on neonatal mortality and morbidity as reported in paper III. To assess the effect on morbidity for this study we added questions on outpatient consultations to the routine data collection in 2012 (Figure 5). Furthermore, we trained a fieldworker to collect consultation information from the health centres. The fieldworker had a list of reported consultations at the health centre (and other health centres in villages with more than one health centre), and another list with children with no information on outpatient consultations. The fieldworker searched

for the outpatient consultations in intervals from one month prior to a consultation to one month after the reported date of consultation. Furthermore, we introduced silver stickers with ID information on all vaccination cards, and the health centre staff were asked to register the ID number in the consultation registers. We did not pay the health centre staff for this favour, and at some health centres children were easier to identify than at other health centres[100].

Picture of silver sticker with ID number on a child's vaccination card.

The natural experiment allowed us to mimic the random allocation of BCG vaccination as seen in a randomised trial design: Children received BCG at a random age within the first month of life dependent on when the child was born in relation to the village visit. As few children entering the analyses were BCG-vaccinated prior to the village visits, self-selection to vaccination was reduced. To avoid survival bias, all children were classified as BCG-unvaccinated until the village visit, which was the first possible registration of a BCG vaccine. Children, who had not been vaccinated prior to the BHP visit, were BCG-vaccinated by the BHP nurse at the visit. The restrictive vial-opening policy was disregarded, and a vial was opened even if only one child was present. Children not present entered the analysis in an intention to treat manner, thus, all children were considered BCG-vaccinated in the period between the first village visit after they were born and 28 days of life. Children obtaining BCG-vaccination elsewhere prior to the village visit were censored at the village visit (Figure 6).

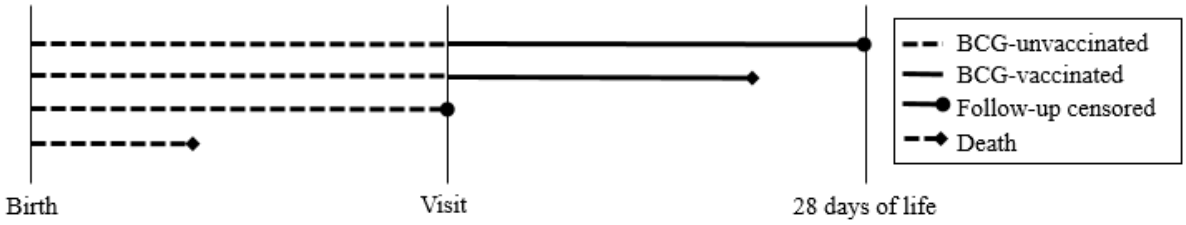

Figure 6. Observation time of 4 children from Paper III who happen to receive a village visit 14 days after birth. Child 1 is followed as unvaccinated until date of visit, hereafter as BCG-vaccinated. Follow-up is censored at day 28. Follow-up for child 2 is terminated at day 25 due to a death. Child 3 is followed as unvaccinated until time of visit, where a BCG vaccine obtained elsewhere is registered and follow-up therefore censored. Child 4 dies before visit and is followed only as BCG-unvaccinated.

TB exposure (Paper IV)

The address system allowed us to link the address information of all children alive in the study period with information on which houses had TB cases as residents. We used routine vaccination data and vital registration to determine whether each child had received neonatal BCG or not, and assessed the effect of neonatal BCG on mortality by TB-exposure status. We used the date of TB diagnosis for each TB patient, and classified the house as TB exposed from 3 months prior to TB diagnosis until 2 weeks after TB diagnosis. A child entered the analysis the first time a vaccination card was inspected after 28 days of age. Observation time for all children was split at the first exposure to TB, and children contributed time as TB-unexposed until first registered TB exposure. Once TB-exposed, the child remained classified as TB-exposed throughout follow-up.

Additional cost estimates (Paper V)

In paper V, we wanted to estimate the cost-effectiveness of disregarding the restrictive vial-opening policy from a societal perspective. Using a societal perspective, we do not only account for costs incurred by the health system as in traditional cost-effectiveness analyses, but also include costs incurred outside the health system, for instance costs of time and transport. As in a previous cost-effectiveness study from Guinea-Bissau[101], we decided to estimate the value of the mothers' time based on the 2011 average monthly earning for Guinea-Bissau estimated in a regression model by Knight and colleagues[102]. Assuming 22 work days per month, the 2017-value of the mother's time per day amounted to USD 2.98.

To calculate the cost of a mother/caregivers time of accompanying the child to the hospital during a hospital admission, we estimated median length of hospital admissions. As we did not have information about the length of TB hospital admissions in children in Guinea-Bissau, we obtained information from the national TB programme that almost all identified TB cases are hospitalised for 60 days [personal communication: Victor Gomes, Programmatic Manager of MDR-TB, National TB Programme]. Thus, the value of the mother's time per TB hospital admission amounted to USD 178.8.

For all-cause hospital admissions, we were able to calculate the median bed days per hospital admission using BHP routine data: At the national hospital Simão Mendes in Bissau, we register all children admitted to the hospital and follow the children daily while admitted. Based on this data, we calculated the median number of bed days per hospital admission for urban hospital admissions. In the rural area, we do not register hospital admissions at the health facilities. However, as part of the routine data collection, when a hospital admission is registered at the village visits, an extra questionnaire with information about the hospital admission is filled in. We calculated the median number of bed days per hospital admission separately for urban and rural Guinea-Bissau. In both urban and rural Guinea-Bissau, the median number of bed days per all-cause hospital admission was 5 days. The value of the mother's time per all-cause hospital admission amounted to USD 14.9.

To obtain the cost of a hospital bed day incurred by the health system, we conducted a micro-costing study in 2014, which included 4 hospitals and 12 randomly selected rural health centres. At the hospitals, accounts and activity reports were obtained. The accounts data included detailed information on recurrent costs including personnel costs, medicine and medical supplies, supplies for diagnostic services, and administration. To be able to calculate the average annual value of infrastructure, we measured the area of the hospital buildings, including the proportion of space used for child health services, and registered

major equipment and vehicles. For health centres, we obtained information on number and type of personnel, salaries and incentives, medicines and other supplies, as well as size of building structure, medical and office equipment and transport means. We also collected information on activities as well as distribution of staff time and building space for child health care and other activities. Annual recurrent costs were extracted from accounts data (hospitals) or calculated from reported data (health centers). All assets were assigned a replacement value using official standard square meter prices by type of health facility and equipment price lists[103,104], and the average annual capital cost calculated. A step-down allocation method was applied to distribute costs and used to estimate the costs incurred by the health system per child hospital bed day. The average cost per hospital bed day was estimated to USD 14.92, corresponding to a 2017-value of USD 15.58.

As cost estimates are estimated in different years, we used the World Bank Consumer Price Index[105] to adjust all costs to 2017-values. The Consumer Price Index describes information on the development of prices, and is thus a measure of inflation.

Modelling the impact and cost-effectiveness of BCG vaccination policy change

To estimate the impact and cost-effectiveness of disregarding the restrictive vial-opening policy, we developed a static mathematical model. We based the model on the static mathematical model developed by Roy and colleagues[106]. In the original model, Roy and colleagues had assumed all-or-nothing BCG vaccine efficacy, which means that a proportion of vaccinated children (corresponding to the vaccine efficacy) are assumed to be fully protected against the disease, and the remainder of vaccinated children and unvaccinated children are assumed to be unprotected. As we wanted to estimate the effect of changing BCG policy on both TB-specific mortality and all-cause mortality, we instead adapted the model to an assumption of leaky BCG vaccine efficacy, which means that all BCG-vaccinated children are assumed to be partly protected (corresponding to the vaccine efficacy). For our research question, it would not have been plausible to assume that some vaccinated children would be completely protected from dying due to any cause.

The model consisted of two parts, one assessing the impact on TB-specific mortality and one assessing the impact on all-cause mortality. The model parts were not completely identical as the HDSS data allowed for greater details in estimates of all-cause mortality and all-cause hospital admissions. We calculated the risk of all-cause mortality as the risk per person per day in age-intervals during the first 5 years of life (more detail is provided in Paper V, appendix A). Similarly, we estimated the risk of all-cause hospital admission. For the TB-specific mortality, we assumed that the risk of dying from TB was constant between 0 and 5 years of age, which is most likely not true, but an assumption we had to make, as data did not allow for more details. We therefore calculated the daily risk of dying from TB, as the risk per person per day based on the GBD estimates[107] of TB deaths in children aged 0-4 years in Guinea-Bissau. Based on BCG coverage estimates described previously, we defined a baseline scenario with the current BCG coverage and a non-restrictive scenario, where children were assumed to be BCG vaccinated at the first registered health facility contact. In figure 7, please see a model overview. As all HDSS data was collected per region, and we also had regional estimates of the 2017-birth cohort in Guinea-Bissau, we also estimated the impact of the policy change separately for urban and rural Guinea-Bissau. The existing evidence on the NSEs of BCG do not allow for firm evidence-based assumptions of the duration of NSEs of BCG vaccine. In the base case analyses, we assumed no waning of protection, and duration of

protection of more than 5 years. However, as mentioned previously, evidence suggest that vaccines interact, and that the NSEs of vaccines may depend on the most recent vaccine received[51,54,108]. We therefore conducted a sensitivity analysis, estimating the effect between age 0 and age 42, as this is the period where BCG and oral polio vaccines are the only recommended vaccines, and several other vaccines are scheduled at 6 weeks of age.

Further details are provided in paper V, where model equations are described step-by-step in Appendix B.

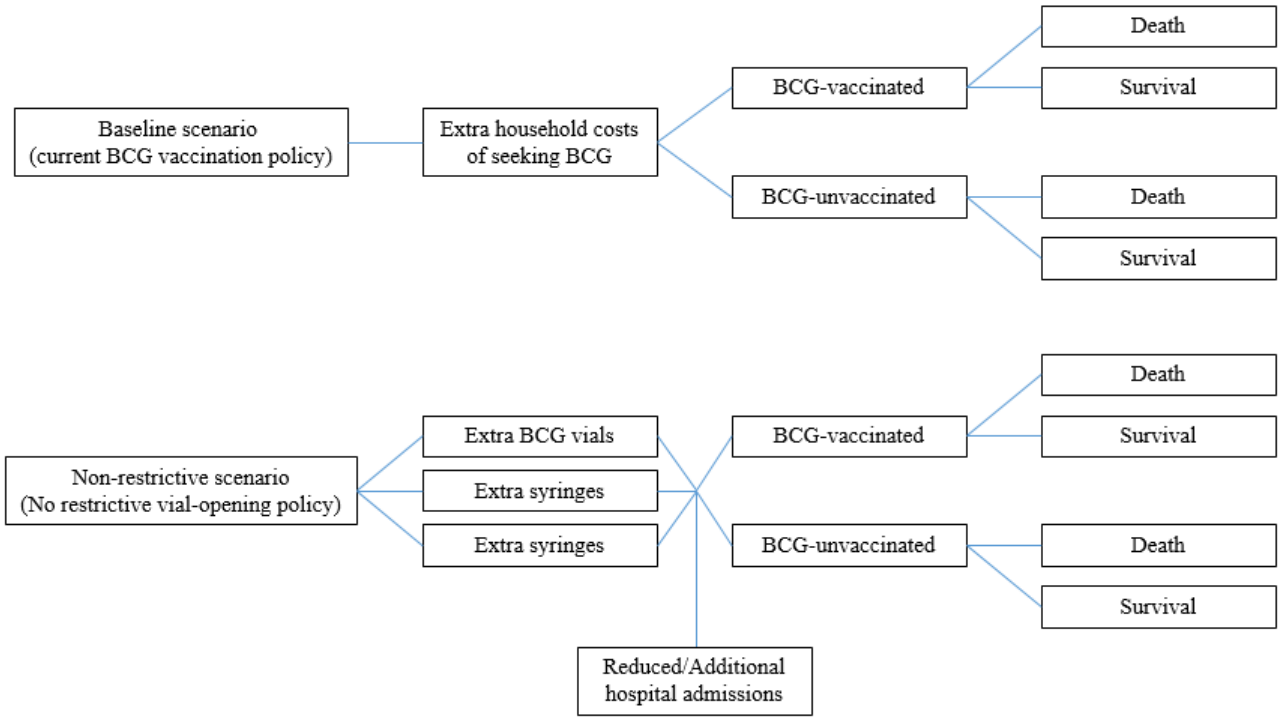

Figure 7. Overview of model structure. The model was conducted both for TB-specific mortality and all-cause mortality.

We assessed methodological and parameter uncertainties as recommended by Bilcke and colleagues[109]. We assessed the choice of BCG vaccine efficacy assumption, by adapting the model to an all-or-nothing BCG vaccine efficacy assumption. Furthermore, we assessed the choice of TB data source by conducting sensitivity analyses using TB data from WHO[110] and Dodd and colleagues[13].

Parameter uncertainty was assessed by running 100,000 Monte-Carlo simulations using Oracle® Crystal Ball. Further details are described in Paper V.

Statistical and methodological assessments

For papers I and II, we primarily used simple descriptive statistics. In Paper I, we compared determinants for BCG vaccination at different ages using logistic regression analyses with standard errors allowing for intragroup correlation within village clusters.

Survival analyses

In paper III, we calculated crude mortality rates, and compared mortality in Cox-proportional hazards models with age as underlying time scale and standard errors allowing for intragroup correlation within village clusters and allowing different baseline hazards by sex. In paper IV, we also calculated crude mortality rates and hazard rations in Cox-proportional hazards models with age as underlying time scale and allowing different baseline hazards by sex and place of birth. By using age as underlying time scale, we obtained age adjusted estimates and ensured that no child was compared to itself even if a child

contributed with more than one observation. The assumption of proportional hazards was tested using Schoenfeld residuals and displaying log-log plots.

For paper V, we estimated the daily risk of all-cause mortality and the daily risk of all-cause hospital admission in survival models with age as underlying time scale using the Kaplan-Meier estimates. We then modelled the effect of disregarding the restrictive vial-opening policy on TB mortality and all-cause mortality in a static mathematical model (Paper V, Appendix B for details on model equations).

Summary of results

Paper I: BCG coverage and barriers to BCG vaccination in Guinea-Bissau: an observational study

In the 2010-birth cohort, we estimated the actual BCG coverage and the potential BCG coverage, and found large differences between actual and potential coverage in early life: At 1 week of age the BCG coverage was 11% in rural Guinea-Bissau, whereas the potential BCG coverage was 54%. At 12 months of age, the differences had waned and the actual BCG coverage was 92%, whereas the potential BCG coverage was 99% had all children been vaccinated at their first health facility contact (Figure 8a).

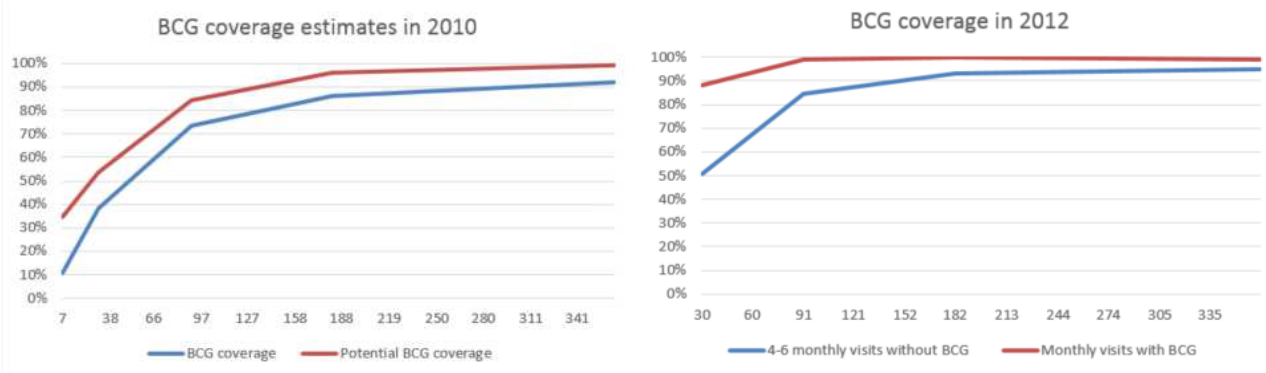

Figure 8a display BCG coverage in 2010 (blue), and the potential BCG coverage had BCG been given at the first contact with a health facility (red). Figure 8b displays the BCG coverage in 2012 in regions where BCG is provided at monthly village visits (red), and in villages where BHP do not provide BCG (blue).

As monthly visits were implemented in some regions in 2012, we assessed the impact on BCG coverage of providing BCG at monthly village visits. In regions with monthly village visits with BCG vaccination, we found a 1-month coverage of 88%, whereas the coverage was 51% in regions with 4-6 monthly visits where BCG was not provided by BHP. The potential BCG coverage was 93% in regions with monthly visits, and 65% in regions without monthly visits (Figure 8b).

We evaluated background factors associated with delayed BCG vaccination and found several factors associated with delayed BCG in the 2010-birth cohort: region of residence, education of caretaker, birth place, prenatal consultations, and socioeconomic status. Importantly, when BCG was provided at monthly visits, we found no association between neonatal BCG and socioeconomic status or indicators of contact with the healthcare system.

Paper II: Household costs of seeking BCG vaccination in rural Guinea-Bissau

Among the 2271 children for whom we obtained information on number of times they had been brought for BCG vaccination, mothers had on average sought BCG vaccination 1.17 times. However, for the 81% of mothers who had succeeded in getting their child BCG-vaccinated prior to the interview, mothers had on average sought BCG vaccination 1.26 times.

Figure 9 shows the distribution of how many times mothers had sought BCG vaccination for their child according to the age of the child at the time of interview.

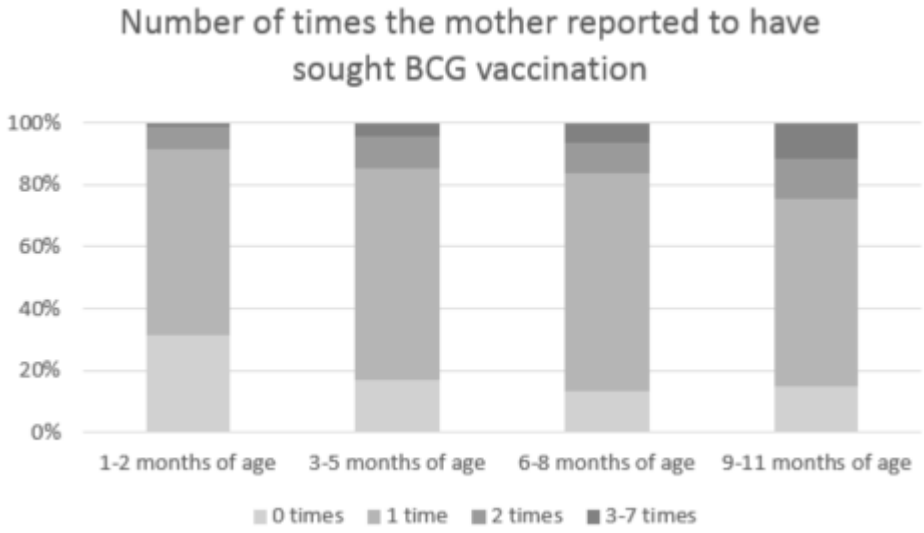

Figure 9: The number of times mothers reported to have sought vaccination by the age of the child at the time of interview.

The median time mothers reported to have spent on seeking BCG vaccination per time was 2 hours (Range: 0-14 hours). However, 11 mothers (0.1%) reported to have spent more than 24 hours seeking vaccination. To provide a realistic picture of the time most mothers spent on seeking vaccination, we excluded these children from the range, but they contributed to the calculation of the median value. Among the mothers who reported to have spent more than 24 hours, some reported that they would go to the village of the health centre the day before seeking vaccination to be able to be at the health centre early in the morning.

Mothers were asked how much money they had used on transportation, adding this to the value of the mother's time spent on seeking vaccination, we estimated an average cost of USD 1.89 per child, who had been brought for BCG-vaccination. The average cost was USD 1.93 for children delivered at home and USD 1.71 for children delivered in a health facility. Among the BCG-unvaccinated children, 42% had been brought for vaccination at an average cost of USD 2.83.

Paper III: The effect of early BCG vaccination on neonatal mortality and morbidity – a natural experiment

In the natural experiment, a total of 3622 children registered before birth entered the analysis at day 1 after birth. Most children were not BCG vaccinated prior to the BHP visit (at the visit, 9% of children had a BCG vaccination obtained elsewhere registered, and were therefore censored at the date of visit). Among BCG-unvaccinated children the mortality rate (MR) was 293.1 per 1000 PYRS (45 deaths during 154 PYRS), and in BCG-vaccinated children the MR was 99.1 per 1000 PYRS (10 deaths during 101 PYRS). The age-adjusted hazard ratio (HR) of BCG-vaccinated compared with BCG-unvaccinated children was 1.26 (0.60-2.64), and thus, we were not able to confirm our hypothesis that BCG reduced neonatal mortality.

We also assessed the effect of BCG on morbidity: The crude incidence rates of hospital admission were 85.2 per 1000 PYRS among BCG-unvaccinated and 69.8 per 1000 PYRS. In the model check of the Cox-proportional hazards assumption, we found that this assumption was violated, from a log-log plot we sought to identify where to split to obtain piecewise proportionality. However, as events were very few, we were not able to compare the hazard ratios of hospital admission. The crude rates of out-patient consultations among BCG-unvaccinated children were 494.0 per 1000 PYRS and 377.6 per 1000 PYRS. We also found no effect of BCG outpatient consultations (HR: 0.90 (0.56-1.45)). The results were robust to planned sensitivity analyses.

In explorative analyses, we assessed whether the effect of BCG differed by maternal BCG-scar status. We did not find evidence that the effect of BCG differed by maternal BCG-scar status, although mortality rates were lower in children of mothers with a BCG scar than in children of mothers without a BCG scar: The hazard ratio comparing mortality of children of mothers with a BCG scar with children of mothers without a BCG scar stratified by child BCG-vaccination status was 0.68 (95% CI: 0.40-1.16).

As strain of BCG has received increasing attention lately and as the majority of the children in the study had received BCG-Denmark, we explored whether censoring children who did not receive BCG Denmark at the date of visit changed the results, and found a HR of 1.38 (95% CI: 0.65-2.92). And there were no deaths reported among those who received other strains than BCG-Denmark.

Paper IV: Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children

We compared mortality of children who had received neonatal BCG with mortality of children who had not received neonatal BCG among 39,105 children in urban Guinea-Bissau between 2003 and 2017. Of these 84% had received neonatal BCG. Neonatal BCG was associated with 47% lower mortality between 28 days and 3 years of life (HR: 0.53 (95% CI: 0.44-0.64)) in a combined analysis of TB-exposed and TB-unexposed children.

Among TB-exposed children, neonatal BCG was associated with 65% lower mortality (aHR: 0.35 (0.17-0.71)) and among TB-unexposed children, neonatal BCG was associated with 45% lower mortality (HR: 0.55 (0.46-0.67)). The results were robust to the sensitivity analyses conducted, and the effect did not differ by sex (Figure 10).

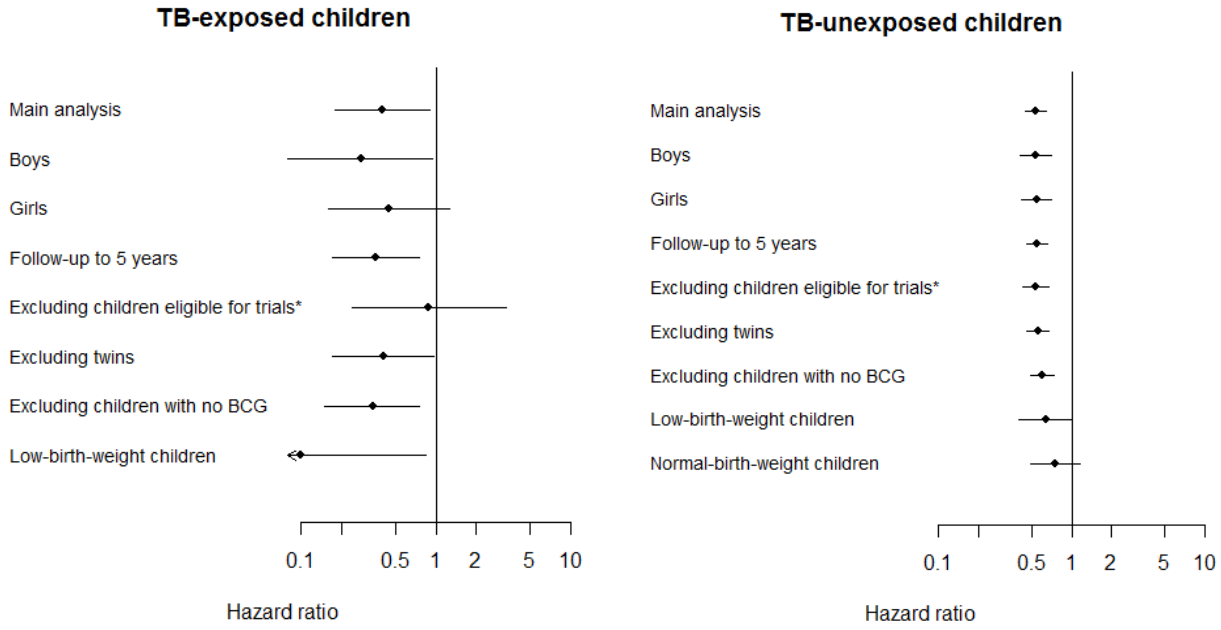

Figure 10a displays the effect of neonatal BCG among TB-exposed children with the main analysis at top and subgroups and sensitivity analyses below. There is no estimate for normal-birth weight children, as there was no deaths registered among the 101 children with no neonatal BCG. Likewise, figure 10b displays the effect-estimates obtained in TB-unexposed children with the main analysis on top and subgroup and sensitivity analyses below. *Excluding children eligible for trials of preventive TB treatment in children.

We also compared mortality of TB-exposed children with mortality of TB-unexposed children by BCG-vaccination status and found that among children with neonatal BCG, mortality of TB-exposed and TB-unexposed children were similar (HR: 1.09 (0.74-1.60)). However, among children with no neonatal BCG, mortality of TB-exposed children were 92% higher than TB-unexposed children (HR: 1.92 (95% CI: 1.06-3.46)). Stratifying the results by sex, we found that TB-exposure was associated with increased mortality among both girls with and without neonatal BCG, whereas TB-exposure was only associated with increased mortality among boys without neonatal BCG, and not for boys with neonatal BCG.

Paper V: Disregarding the restrictive vial-opening policy for BCG vaccine in Guinea-Bissau: impact and cost-effectiveness for tuberculosis mortality and all-cause mortality in children aged 0-4 years

Disregarding the restrictive vial-opening policy was estimated to reduce TB mortality by 11.0% (UR: 0.5-28.8%), corresponding to 4 (UR: 0-15) averted TB deaths per birth cohort in Guinea-Bissau during the first 5 years of life. Accounting for BCG's effects on all-cause mortality, the policy change was estimated to reduce all-cause mortality by 8.1% (UR: 3.3-12.7%), corresponding to 392 (UR: 158-624) fewer all-cause deaths per birth cohort.

As health seeking behaviour and vaccination opportunities are different in urban and rural Guinea-Bissau, we also estimated the impact of disregarding the restrictive vial-opening policy for the urban population and the rural population. In urban Guinea-Bissau, the current delay in BCG vaccination is not as large as in rural Guinea-Bissau (Figure 11).

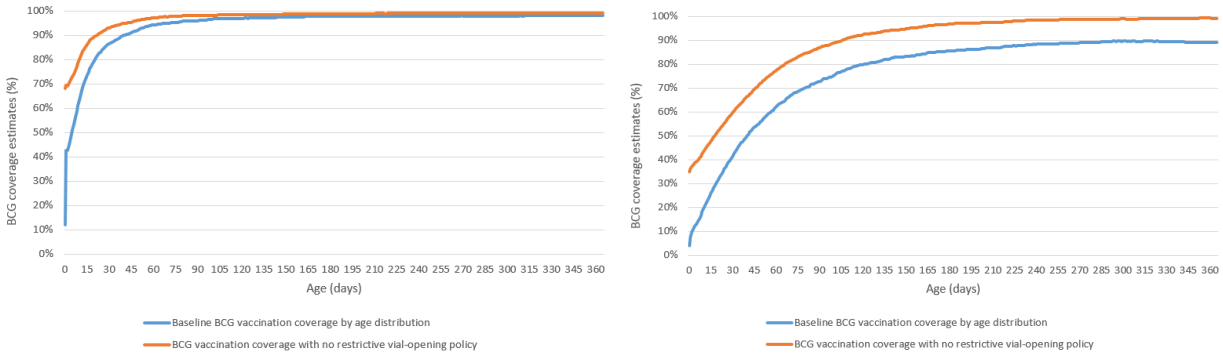

Figure 11a: BCG coverage in the baseline scenario (blue) and in the non-restrictive scenario (orange) in urban Guinea-Bissau.
Figure 11b: BCG coverage in the baseline scenario (blue) and in the non-restrictive scenario (orange) in rural Guinea-Bissau.

Disregarding the restrictive vial-opening policy in the urban area was estimated to reduce TB mortality by 2.6% (0.1-8.3%), corresponding to less than 1 TB death averted per birth cohort, and to reduce all-cause mortality by 10.4% (UR: 4.5-15.3%), corresponding to 111 (UR: 47-172) fewer all-cause deaths per birth cohort. In rural Guinea-Bissau, the policy change was estimated to reduce TB mortality by 16.4% (0.8-38.6%), corresponding to 4 (UR: 0-17) averted TB deaths, and reduce all-cause mortality by 8.4% (3.3-13.5%), corresponding to 319 (UR: 124-527) averted all-cause deaths. As delays in BCG-vaccination in rural Guinea-Bissau are larger than in urban Guinea-Bissau the relative and absolute differences in TB mortality was expected, but the that relative change in all-cause mortality in urban Guinea-Bissau was larger than in rural Guinea-Bissau was surprising. However, as most children are born in health facilities in urban Guinea-Bissau (and sometimes BCG-vaccinated the following day before discharge from the hospital), there was a large gap between the baseline scenario and the non-restrictive scenario at age 0, where the daily risk of dying was also high. Excluding age 0 and estimating the impact of policy change between 1 day and 5 years of age, the estimated reduction in all-cause mortality was 3.4% (24 averted all-cause deaths) in urban Guinea-Bissau, and 7.5% (240 averted all-cause deaths) in rural Guinea-Bissau.

We calculated the incremental cost-effectiveness of disregarding the restrictive vial-opening policy, as the cost-effectiveness per LYG, per discounted LYG (as recommended by WHO), and per death averted according to TB-specific effects and all-cause effects of BCG, respectively. The incremental cost-

effectiveness ratio (ICER) was estimated to USD 900 (UR: 142-9,085) per discounted LYG by averted TB deaths, and USD 24,269 (UR: 3827-244,869) per TB death averted, and USD 9 (UR: 5-23) per discounted LYG by averted all-cause deaths, and USD 249 (UR: 144-615) per all-cause death averted. Figure 12 displays ICERs for the total population and stratified by urban and rural population. There are large differences between ICERs per LYG and death averted, and also large differences between ICERs on TB-specific effects and all-cause effects. Thus, please notice the y-axis displaying USD in all four parts of the figure.

Figure 12a-d. Figure 12a displays the ICER of disregarding the restrictive vial-opening policy calculated based on TB-specific effects. Figure 12b displays the ICER based on all-cause effects. Figure 12c displays the ICER per TB death averted. Figure 12d displays the ICER per all-cause death averted. All ICERs are calculated in USD. Please notice of the different scales on the y-axes (USD).

Discussion

The work presented in the thesis demonstrates some of the important consequences of the current BCG vaccination practice in Guinea-Bissau. First, we identified that the current BCG vaccination policy was associated with delays in BCG vaccination, in particular for the children who were worse off. Monthly village visits with BCG vaccination reduced the inequity, and resulted in increased vaccination coverage, with a 1-month BCG coverage of 88% (equal to the global BCG coverage estimate at 12 months of age[111]) (Paper I). When we asked mothers about their experience in seeking and not obtaining BCG vaccination, many told us they had been told to return to the health facility another day. Data to support this observation were obtained and presented in paper II, where mothers reported to have sought BCG vaccination 1.26 times before getting their child vaccinated. Thus, by not opening a 20-dose vial of BCG vaccine unless more than 10 children were present for vaccination, savings by the programme transferred cost to the mothers that was equivalent to the UNICEF price range of a BCG vial. On average, the household cost of seeking BCG vaccination was USD 1.89, corresponding to a 2017-value of USD 1.92.

In paper III, utilising a natural experiment to assess the effect of BCG on neonatal mortality, we found no beneficial effect of BCG on neonatal mortality. Contrary to our hypothesis, we found that BCG vaccination was associated with higher neonatal mortality. The finding was robust to sensitivity analyses conducted. While we found no effect on neonatal mortality, paper IV indicated that early BCG is important: neonatal BCG was associated with lower all-cause mortality among both TB-exposed and TB-unexposed children, supporting that timely BCG vaccination is important and that BCG has beneficial NSEs.

In the light of the identified barriers, costs and the beneficial effects on mortality demonstrated in other studies from Guinea-Bissau[34,75-77] and including our finding of no benefit from paper III, we then estimated the impact of disregarding the restrictive vial-opening policy. BCG vaccination was estimated to reduce both TB mortality and all-cause mortality. The policy change was estimated to cause larger relative reductions in TB mortality compared with all-cause mortality, but while the policy change was estimated to avert 4 TB deaths per birth cohort, the corresponding number was 392 averted all-cause deaths per birth cohort. The resulting cost-effectiveness analyses showed that including the all-cause effects of BCG in the evaluation resulted in higher cost-effectiveness than merely including TB-specific effects.

Estimating the effects of disregarding the restrictive vial-opening policy separately for urban and rural Guinea-Bissau, we found larger absolute and relative changes in TB mortality in rural Guinea-Bissau than in urban Guinea-Bissau. However, the policy change was estimated to reduce mortality in urban Guinea-Bissau by a larger percentage than in rural Guinea-Bissau. This was surprising, as the delay in BCG vaccination in rural Guinea-Bissau was larger, and likewise, the gap between the baseline coverage and the coverage in the non-restrictive scenario was larger. Exploring the coverage in the baseline scenario and the non-restrictive scenario separately for the urban and the rural area, revealed that much of the difference in coverage in the urban area was at age 0. Many children in the capital are born at the national hospital, where BCG is provided before discharge, thus, while some children will receive BCG at the day of birth, many will be vaccinated the following day. Beneficial effects of BCG-at-birth have been demonstrated already 3 days after vaccination[34], but the existing evidence do not allow for conclusions as

to whether BCG will affect mortality on the same day that it has been given. We therefore conducted a sensitivity analysis of the effect of BCG on all-cause mortality between 1 day of age and 5 years. Disregarding the restrictive vial-opening policy resulted in 3.4% reduction in urban all-cause mortality and 7.5% reduction in rural all-cause mortality, corresponding with the difference in coverage between the two scenarios. Moreover, as it may be unlikely that BCG affects mortality immediately at the day of administration, the estimate excluding age 0 may provide a more realistic estimate of the effect of disregarding the restrictive vial-opening policy.

Strengths and limitations

All studies used data from the BHP HDSS sites in Guinea-Bissau. The continuous data collection platform allows for nested study designs, which are only possible in the timeframe of a PhD since they are conducted within an already existing HDSS. The data collection at the BHP has been ongoing for many years, and the fieldworkers are experienced. On top of that, fieldworkers are frequently supervised to ensure high and consistent data quality. Mothers in both the urban and the rural areas know the fieldworkers and BHP has a good reputation among mothers. All of these factors are very important in ensuring correct and timely information. The continuous pregnancy registration allows for precise estimates of neonatal mortality, as women registered during pregnancy are interviewed on pregnancy outcome, rather than assuming full information when interviewed months later. Thus, despite not capturing complete information, the HDSS setup allows for valid estimates of neonatal mortality, which is important for evaluating effects of interventions in early life.

Official coverage estimates are based on the number of doses provided and estimated population below 1 year of age. Imprecisions in the assessed target population have led to local coverage estimates of more than 100%. By estimating the coverage only among children with assessed vaccination status, we were able to obtain accurate estimates of BCG coverage and evaluate BCG coverage at different ages during the first year of life. The routine data collection of vaccination status, vital status and hospital admissions allowed for more precise estimates of vaccine coverage, mortality and morbidity outcomes than usually available in low-income settings. This was a major strength in paper V, as we were able to use real data rather than modelled estimates as data inputs in the model.

It can be difficult to obtain information about barriers for timely BCG as mothers are often met with reprimands and even penalties at some health centres if they have not brought their child for vaccination on time, have not given birth at the health facility, or in other ways have not followed the instructions of the health-facility staff. Therefore, some mothers are likely to try to provide the correct answer when being interviewed. The fieldworkers are instructed in asking the questions neutrally, but as the interviews are conducted on verandas with other women present, some answers may be influenced by social desirability bias. While we cannot infer that the entire delay in coverage is due to the restrictive vial-opening policy, the fact that more than half of the mothers of unvaccinated children recalled being told to return another day to obtain BCG vaccination, supports that the restrictive vial-opening policy is important for coverage of BCG.

In paper III, we tried to imitate the random allocation from a randomised trial. The ideal setup for studying the effect of BCG on mortality would have been a randomised trial with random allocation to BCG or no BCG immediately after birth. However, as BCG is recommended at birth none of the existing

vaccination contacts can be easily utilised to provide BCG at the recommended age. Creating vaccination opportunities immediately after birth in a rural setup, with many women giving birth at home, is a challenging and expensive setup. We therefore decided on the natural experiment design limiting the risk of healthy vaccinee bias. With this design, most children enter the analysis as BCG-unvaccinated, and gradually become vaccinated as village visits are conducted. Thus, the unvaccinated group decreases in size and the vaccinated group increases in size resulting in skewed distributions. Using age as underlying time scale in the Cox proportional hazards model ensures that the estimates are adjusted for age, and the skewed distribution should not be problematic.

Unlike in paper III, the observational study with self-selection to BCG vaccination in paper IV did not limit the healthy vaccine bias, and thus, there is a considerable risk that the estimates could be influenced by healthy vaccinee bias. BCG vaccination may be delayed for frail children. During the study period, the policy was to delay BCG vaccination for low-birth-weight neonates until they had gained weight to 2500g. In most of the study period, randomised trials of BCG-at-birth among low-weight neonates were conducted at the national hospital[34], and some low-weight children will therefore have received neonatal BCG. We conducted several sensitivity analyses to explore the robustness of our results. To assess whether confounding could explain our findings, we included available background factors in the analysis one by one, and adjusted for factors changing the estimate by more than 5%. However, with this method, we will not identify confounding due to interaction of two or more factors, and residual confounding due to not identified confounding or unmeasured confounding can affect the results. Since the results were robust to sensitivity analyses, we find it unlikely that healthy vaccine bias would explain all of the effect of BCG.

In Paper V, we estimated the impact of disregarding the restrictive vial-opening policy. To our knowledge, this was the first model to estimate the impact of a BCG policy change including both TB-specific and all-cause effects of BCG. The fact that we used a country-level model was a big strength as we were able to use more detailed and more accurate data in the analyses. Data input to global models are often based on output estimates from other mathematical models, increasing the uncertainty of the estimates. Also, using data originating specifically from Guinea-Bissau makes it relevant to the setting. However, it makes the generalisability of our estimates less apparent. As delays in BCG vaccination and the use of the restrictive vial-opening policy have been observed in other countries[112,113], our study suggests that it is likely to reduce both TB-specific and all-cause mortality elsewhere than Guinea-Bissau, however the magnitude and values of cost-effectiveness estimates are not directly transferable. It is however likely that it will also be cost-effective in other settings, but as always with research results, the context of implementation should be taken into consideration.

For the estimates of the all-cause effects of BCG, most data inputs were obtained from the HDSS and applied to estimates of the regional number of livebirths in 2017. However, as we did not have data on TB mortality in children in the HDSS, we used country-level estimates obtained from mathematical modelling. We used estimates from the Global Burden of Disease study[107], as they provided age-stratified data on both TB incidence and TB mortality, allowing to directly use estimates for children aged 0-4. We accounted for the differences between the TB mortality estimates from different TB sources by conducting sensitivity analyses using country-specific estimates for Guinea-Bissau in children aged 0-4

WHO[110] and from Dodd and colleagues[13]. It is not clear how the WHO estimates of TB incidence and TB mortality are linked. Since we for children aged 0-4 in Guinea-Bissau only had access to TB incidence data from WHO[110], we used the case fatality rate from a systematic review and meta-analysis conducted by Jenkins and colleagues[114] as previously used in a global model[106] to calculate the number of TB deaths.

We chose a static mathematical model instead of a transmission model as TB in children is less infectious than in adults[115], and we therefore assumed the effect of BCG on transmission of disease to be minimal. The increased complexity of a transmission model would therefore not necessarily result in more precise estimates. For the all-cause effects of BCG, it may have been bold to assume that the all-cause effects of BCG would not affect the risk of transmission of all-cause diseases. However, as evidence was insufficient to allow for evidence-based assumptions, we chose a static mathematical model, well aware that the resulting estimates were likely to be conservative.

Comparison with other studies:

Delays in BCG

We found that the current BCG vaccination policy was associated with delays in BCG vaccination. As mentioned previously, such delays are not measured in official coverage estimates, as only coverage at 12 months of age is reported. However, delayed BCG vaccination is not a problem isolated to Guinea-Bissau and it has been observed in several other countries[21,112,116]. In an ecological study from Ghana, the decline in median age at BCG from 46 days in 1996 to 4 days in 2012 coincided with a decline in neonatal mortality from 46 per 1000 livebirths in 1996 to 12 per 1000 livebirths in 2012, suggesting that removing the delay in BCG vaccination can reduce neonatal mortality[117].

BCG vaccination and mortality

Randomised trials of BCG-at-birth in low-weight children in Guinea-Bissau have found that BCG was associated with reduced neonatal mortality[34,71,72]. We did not find that BCG was associated with reduced neonatal mortality (Paper III). In two recent trials among low-birth-weight infants in India, they found no effect of BCG on neonatal mortality[73]. The discrepancy between previous findings and the trials in India and our results rise many questions. It has previously been suggested that strain of BCG is important for both TB-specific effects and non-specific effects[27]. The authors of the trials from India suggest that their use of BCG-Russia may explain why they do not find effects similar of the effects in the trials in Guinea-Bissau, where BCG-Denmark was used[73]. The current evidence do however not allow for conclusions of whether difference in strain is the reason for these different results. Nonetheless, in our study, we used mainly BCG-Denmark, and censoring children who had received other strains at the day of visits did not alter the conclusion, thus use of strain is unlikely to explain why our results are not comparable to the previous trials from Guinea-Bissau, although there may be batch-to-batch variations[31]. A recent trial from Denmark also found no effect of BCG on hospital admissions[97], but among children of BCG-vaccinated mothers, BCG was associated with 35% (6-55%) lower mortality[98]. In Guinea-Bissau, we did not have vaccination information on mothers, and we would not be able to collect correct information about whether or not they had received BCG vaccination. As a successful BCG vaccination usually causes a small scar, we used the BCG-scar status of women to evaluate whether effect of BCG differed by maternal BCG-scar status. However, with limited power, we were as expected not able to make any

conclusions. Another study from Guinea-Bissau also used BCG scar as a proxy for maternal BCG vaccination, and found that children with a BCG scar had 66% (33-83%) lower mortality than children without a BCG scar if the mother also had a BCG scar, whereas the reduction in mortality was only 8% (-83-53%) if the mother had no BCG scar[93]. As we only assessed neonatal mortality, we were not able to account for whether or not the child developed a BCG scar.

In paper IV, we found that neonatal BCG was associated with reduced mortality in both TB-exposed and TB-unexposed children. Our results support that BCG has beneficial non-specific effects as found in other studies[34,77]. Most studies have compared BCG-vaccinated children with unvaccinated children, and have not evaluated the timing of BCG. However, an observational study among low-weight children in Guinea-Bissau found a stronger effect of BCG for children vaccinated within the first week of life[76]. Our results support that timing of BCG is important, and while we do not find a beneficial effect of BCG on neonatal mortality, our results from paper IV suggest that BCG reduces later child mortality.

Impact of a BCG policy change

Disregarding the restrictive vial-opening policy was estimated to reduce TB mortality in Guinea-Bissau by 11% (0.5-28.8%). Roy and colleagues estimated that increasing global BCG coverage to 92% at birth would reduce global TB mortality by 2.8% (UR: 0.1-0.7%), corresponding to 5,449 (UR: 218-15,071) TB deaths per year[106]. The larger relative change in TB mortality could be due to delays of BCG in Guinea-Bissau being larger than average, but could also be greatly influenced by the more precise estimates of current BCG coverage. Roy and colleagues assess several potential BCG coverage scenarios, and also evaluate the consequences of providing BCG together with DTP at 6 weeks of age. All of the scenarios evaluated are hypothetical scenarios, however, some of these are not feasible to implement in a country like Guinea-Bissau without straining health system, where resources are already too few. In our study, we estimate the impact of an easy implementable policy change.

There is however, a challenge in suggesting all countries to disregard the restrictive vial-opening policy, as sufficient BCG vials should be available. In 2015, there was a global shortage of BCG vaccines, which led some countries to change their BCG policy[118]. Harris and colleagues estimated that the global BCG shortage in 2015 was associated with 7,433 (UR: 320-19,477) excess TB deaths in children less than 15 years of age[119]. The effect of BCG on all-cause mortality was not included in the model, and based on our results, it is likely that the BCG shortage caused many all-cause deaths. Recently, a study from South Africa reported an increase in TB meningitis following the BCG shortage[120]. Their study design does not allow for conclusions on causality, but the coincidence of the shortfall of BCG and the increase in TB meningitis also suggests that timely BCG is important. Thus, it will be important to ensure that sufficient BCG vials are available so that all countries can disregard the restrictive vial-opening policy, and no mother should seek BCG in vain.

Interpretation

We demonstrated that the current BCG vaccination policy is associated with large delays in BCG vaccination. Importantly, when BCG vaccination was provided at monthly village visits, a 1-month coverage of BCG corresponding to the global 12-month BCG coverage (88%) was obtained, and furthermore, inequity in delays waned as socio-economic factors and health seeking behaviour were no longer associated with delayed BCG. This suggests that vaccination outreach can improve BCG coverage

significantly, and could be a way of ensuring BCG coverage. However, vaccination outreach is a strain on the health system, and in paper II, we were able to show that on average mothers sought BCG-vaccination 1.26 times before getting their child vaccinated. Thus, utilising the already existing contacts with the health facility could be a way of increasing the BCG coverage without adding additional strain on the health system. Furthermore, as the average cost spent on seeking BCG vaccination corresponded to the UNICEF prices of a BCG vial, not opening a vial for every child will transfer costs rather than save costs. As we estimated in paper V, it is highly cost-effective to open a vial of BCG for every child.

We were not able to confirm our hypothesis that BCG would be associated with reduced neonatal mortality. We did not find any factors explaining this finding. The crude mortality rates were almost three-fold higher in BCG-unvaccinated children compared with BCG-vaccinated children. With only 10 deaths among BCG-vaccinated children, the results are not sufficiently strong to refute that BCG has beneficial effects on mortality. However, in the light of BCG having no effect on neonatal mortality in two randomised trials in India[73], and the finding of no overall effect on hospital admissions in the trial from Denmark[98], it is important to identify what causes fluctuations in the effects of BCG on overall health outcomes, and which children benefit from early vaccination. As strain variations have been suggested to have different effects[27], the effects of different strains should be evaluated further. As batch-to-batch variations have been demonstrated[31], it is important to consider how this affects mortality. Immunological research on the effects of BCG and immune training has progressed rapidly during the past years, and it would be interesting to pursue whether laboratory results and epidemiological beneficial effects could be linked, as this might help identify under which conditions and which strains or types of BCG vaccines have beneficial NSEs. As BCG vaccination is a cheap intervention it is worthwhile to identify how best to exploit the full potential.

Timing of BCG has not been studied much, and the results from paper IV suggest that neonatal BCG is associated with beneficial NSEs of BCG, also beyond the initial early effects. Studies assessing the effect of timing of BCG are warranted. Currently, randomised trials assessing the effect of BCG on all-cause mortality are being conducted in Uganda in HIV-1-exposed children, and a similar trial in all children is expected. However, in the trials the main outcome is mortality between birth and 14 weeks of age[84]. As BCG may interact with other vaccines, it is important to clarify what is actually assessed, and we have therefore suggested additional analyses: 1) assessing the effect of BCG between birth and 6 weeks of age (the effect of BCG on all-cause mortality), and 2) assessing the effect between 6 weeks and 14 weeks of age (the effect of BCG combined with subsequent vaccines)[121], as this will help to shed light on the interaction between vaccines. In our modelling study, we were not able to account for interaction with other vaccines. More data studying the interaction between vaccines would be useful to make sure that the vaccination programme is designed in the best possible way, and when such data exist, it will be important to incorporate them when conducting modelling studies.

The results from this thesis support that BCG should be provided timely, despite the finding of no effect of BCG on neonatal mortality. Practices of the restrictive vial-opening policy should be removed. It would be highly cost-effective to do so and would reduce both TB-mortality and all-cause mortality.

Future perspectives

At the beginning of this PhD, WHO had not yet acknowledged the existence of NSEs, all trials of BCG on all-cause mortality suggested beneficial NSEs, and several observational studies supported these findings. Much has happened within the field of NSEs and BCG during the period of this PhD: 1) The WHO-commissioned review concluded that BCG may have beneficial NSEs[2], 2) Several immunological studies suggest that BCG can induce immune training of the innate immune system[122,123], which could possibly be linked to the beneficial effects on all-cause mortality, 3) Trials from India found no effect of BCG on neonatal mortality[73], and 4) A trial from Denmark found no overall effect of BCG on hospital admissions in children aged 0-15 months[97], but found reduced hospital admissions due to infectious disease among BCG-vaccinated children of BCG-vaccinated mothers[98]. Thus, there is still much to be explored concerning the effects of BCG.

We need further trials assessing the effect of BCG on mortality, and it will be important to identify what causes the different effects. As most trials on BCG have been conducted in low-birth-weight children in hospitals, we are now conducting a cluster-randomised trial in rural Guinea-Bissau of providing BCG and oral polio vaccine within 72 hours after birth. The trial enrols both low-birth-weight children and normal-birth weight children. In the trial, we use BCG-Japan, and thus not BCG-Denmark or BCG-Russia as used in the previous trials from Guinea-Bissau and India. However, two other trials are comparing different strains of BCG at the national hospital in Guinea-Bissau. Also another trial is currently being conducted in India, which will help to identify whether strain of BCG is important or other explanations should be investigated.

In the thesis, I have not included immunological effect of BCG. This field has made rapid and interesting progress within the last decade, and for the coming years it will be important to try to link immunological and epidemiological findings, maybe by nesting immunological studies within randomised trials. Identifying immunological links could be an important tool in the development of new vaccines. As mentioned in the beginning of the thesis, there are several new TB vaccines in the pipeline. Trials assessing the effects of these vaccines should include overall health outcomes, and not focus merely on the TB-specific effects. Replacing BCG by another TB vaccine based on the TB-specific protection if the NSEs are not taking into account. As we demonstrated in paper V, large relative reductions in TB mortality corresponded to small absolute changes compared with the effect on all-cause mortality. Thus, it is important to assess the effect on all-cause mortality also for new vaccines before replacing the BCG vaccine.

To exploit the full potential of BCG and other vaccines, further studies on the interaction between vaccines should be conducted, as this information is important to create the most optimal vaccination programme. Furthermore, the effects of vaccines should be assessed by sex, as boys and girls may benefit differently from different vaccines, and maybe it would be better with different recommendations for boys and girls, which could be implemented by vaccination cards of different color, which has already been implemented in Guinea-Bissau.

Furthermore, the unexploited potential of BCG should be further explored. BCG has for many years been used in bladder cancer therapy, but recent studies have also suggested that BCG might affect non-infectious diseases like diabetes[124].

Lastly, what we already know about the effects of BCG should be used to optimise the current BCG vaccination policies. As stated throughout this thesis, the restrictive vial-opening policy should be disregarded, and all children should be BCG vaccinated at their first contact with a health facility. To create incentives to ensure timely BCG vaccination. Proportion of children with neonatal BCG could be reported in addition to the already existing BCG coverage at 12 months of age.

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Paper I

BCG coverage and barriers to BCG vaccination in Guinea-Bissau: an observational study

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BCG coverage and barriers to BCG vaccination in Guinea-Bissau: an observational study

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Abstract

Background: BCG vaccination is recommended at birth in low-income countries, but vaccination is often delayed. Often 20-dose vials of BCG are not opened unless at least ten children are present for vaccination ("restricted vial-opening policy"). BCG coverage is usually reported as 12-month coverage, not disclosing the delay in vaccination. Several studies show that BCG at birth lowers neonatal mortality. We assessed BCG coverage at different ages and explored reasons for delay in BCG vaccination in rural Guinea-Bissau.

Methods: Bandim Health Project (BHP) runs a health and demographic surveillance system covering women and their children in 182 randomly selected village clusters in rural Guinea-Bissau. BCG coverage was assessed for children born in 2010, when the restricted vial-opening policy was universally implemented, and in 2012–2013, where BHP provided BCG to all children at monthly visits in selected intervention regions. Factors associated with delayed BCG vaccination were evaluated using logistic regression models. Coverage between intervention and control regions were evaluated in log-binomial regression models providing prevalence ratios.

Results: Among 3951 children born in 2010, vaccination status was assessed for 84%. BCG coverage by 1 week of age was 11%, 38% by 1 month, and 92% by 12 months. If BCG had been given at first contact with the health system, 1-week coverage would have been 35% and 1-month coverage 54%. When monthly visits were introduced in intervention regions, 1-month coverage was higher in intervention regions (88%) than in control regions (51%), the prevalence ratio being 1.74 (1.53-2.00). Several factors, including socioeconomic factors, were associated with delayed BCG vaccination in the 2010-birth cohort. When BCG was available at monthly visits these factors were no longer associated with delayed BCG vaccination, only region of residence was associated with delayed BCG vaccination.

Conclusion: BCG coverage during the first months of life is low in Guinea-Bissau. Providing BCG at monthly vaccination visits removes the risk factors associated with delayed BCG vaccination.

Keywords: BCG, Coverage, Timeliness of vaccines, Implementation of the vaccination programme

Background

Bacillus Calmette Guérin (BCG) vaccine is recommended at birth to normal-birth-weight children in Guinea-Bissau. However, BCG vaccination is often delayed for several reasons, one of them being the "restrictive vial-opening policy": BCG is a freeze-dried vaccine supplied in vials with 20 infant doses [1] and once reconstituted, the vaccine should only be used for a maximum of six hours. In Guinea-Bissau and other low-income countries [2,3], the focus on not wasting vaccines [4] has led to a policy of not opening a BCG vial unless 10 children are present to be vaccinated [5]. Previous studies have shown that BCG has beneficial non-specific or heterologous effects, providing protection also against many non-tuberculosis causes of death [6-15]. In two randomised trials among low-birth-weight (<2500 g, LBW) neonates, BCG at birth compared with the usual delayed BCG, lowered neonatal mortality by 48% (95% CI: 18%-67%) [6,7], the reduction being 58% (8%-81%) the first three days after vaccination [6,7]. The rapidly occurring effect suggests that BCG stimulates the innate immune system. This is supported by recent immunological studies

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showing that BCG induces epigenetic changes which reprogram monocytes to increased pro-inflammatory responses against unrelated pathogens [16,17].

Most infant deaths occur during the neonatal period, particularly in the first week of life [18] and thus any delays in BCG vaccination may have major consequences because children do not benefit from BCG when their mortality risk is highest. Hence, it is important to identify obstacles to early BCG vaccination, as this will help target interventions to lower the age at vaccination.

We assessed BCG coverage at different ages among children born in 2010 in rural Guinea-Bissau to identify factors associated with delayed BCG vaccination in a context with a restrictive vial-opening policy. In 2012, we implemented monthly visits and provided BCG vaccination to newborns in three intervention regions (Oio, Biombo and Cacheu) but not in six control regions. We evaluated how not adhering to the restrictive vial-opening policy affected BCG coverage and affected factors associated with delayed BCG vaccination.

Methods

Setting and study population

The study was conducted in the rural study area of the Bandim Health Project (BHP) in Guinea-Bissau. The BHP maintains a health and demographic surveillance system following 182 randomly selected clusters of 100 women and their children in rural Guinea-Bissau. The clusters were initially selected using the Expanded Programme on Immunizations (EPI) methodology for immunisation surveys sampling 20 clusters of 100 women in each of the eight larger health regions, and 10 and 12 clusters in the two smallest regions. Later two regions have been joined and rural Guinea-Bissau now has nine health regions; Oio, Biombo, Gabu, Cacheu, Bafata, Quinara, Tombali, Bubaque, and Bolama. All women of fertile age and children below the age of 5 years are followed through home visits every four to six months.

At the home visits women of fertile age are registered and information on ethnicity and schooling is collected. If a pregnancy is registered, a special form is completed collecting information on prenatal consultations and socioeconomic factors. For all newborn children information on date of birth, place of birth, and prenatal consultations is collected. At all visits the child's vaccination card is inspected and vaccination dates are recorded. If the child has no vaccination card a vaccination card is provided from the BHP team.

BCG vaccination possibilities

Children in Guinea-Bissau should receive BCG and oral polio vaccine (OPV) at birth, pentavalent vaccine (diphtheria-tetanus-pertussis-H. influenza type B-Hepatitis B vaccine) and OPV at 6, 10, and 14 weeks, and measles and yellow fever vaccines at 9 months. These vaccines are provided free of charge at the health centres as part of the national programme and during outreach to villages when additional funding is available. Due to the restricted vial-opening policy some vaccines are only provided once a week at health centres and only if there is a sufficient number of eligible children present.

Since 2007 the BHP teams visiting the surveyed villages in all regions have been accompanied by a nurse, who offered OPV, pentavalent vaccine, measles vaccine, and yellow fever vaccine to all children below the age of 1 year. The vaccines were supplied through the national programme and the BHP nurse had to follow the national policy. Hence she did not bring BCG as she would very rarely encounter sufficient eligible children in a village.

In 2012 the BHP increased the frequency of visits from four-six-monthly visits to monthly visits in three regions (Oio, Biombo and Cacheu). In these intervention regions BCG vaccination was offered to all children below the age of 1 year regardless of the number of children present, thus a BCG vial was opened for one child. In the remaining control regions national policy was followed and BCG was not offered during village visits.

Information about reasons for delay of BCG vaccination

During the year from 1st July 2012 to 30th June 2013 mothers of BCG unvaccinated infants in all regions were interviewed on their BCG vaccination attempts/experiences during home visits. They were asked if they knew that their child was due to receive BCG and whether they had taken the child for vaccination. Mothers who reported to have sought vaccination were asked why the child had not been vaccinated. Mothers who had not sought BCG vaccination were interviewed about the reasons.

Ethical approval

BHP's HDSS which has been in place in Guinea-Bissau since 1978 and is conducted by request from the Guinean Ministry of Health. The current surveillance system in the rural areas has been approved by the National Ethics Committee in Guinea-Bissau and the Central Ethics Committee in Denmark. No separate ethical and consent approval was sought.

Statistical analyses

Assessing BCG coverage in the 2010-birth cohort

Standard estimates for vaccination coverage are usually based on vaccinations obtained by 12 months of age, assessed among children aged 12 to 23 months [19,20]. In the 2010-birth cohort we assessed BCG coverage by 1 month of age in children aged 1–12 months at the time of the home visit, the coverage by 3 months of age in children aged 3–14 months, the coverage by 6 months

in children aged 6–17 months, and the coverage by 12 months in children aged 12–23 months (Table 1). Since most neonatal deaths occur within the first week of life, we also assessed the 1-week coverage using vaccination status assessed within the 12 months after day 7. Vaccination status was determined at the first visit in the relevant time period at which the vaccination card was seen. Children who had lost their vaccination card or for whom the vaccination card was not seen were excluded from the analysis.

Missed opportunities among BCG unvaccinated children were defined as contact with the health system, either being born at a health facility or having received other vaccines (based on the registered date of another vaccination on the vaccination card). We calculated the potential coverage if BCG had been given at the first contact with the health system.

Assessing BCG coverage after implementation of monthly visits, 2012 cohort

In 2012 monthly visits were introduced in the intervention regions, whereas four-six-monthly visits continued in control regions. We considered a village as belonging to the intervention regions when there was less than 6 weeks between two subsequent visits. BCG coverage was assessed among all children visited from 1st July 2012 to 30th June 2013. Like in the 2010-birth cohort, we assessed BCG coverage at 1, 3, 6, and 12 months of age. To take into account that children in intervention regions with monthly visits had a larger possibility of having their card seen within a 12 months period compared with children in control regions with four-six-monthly visits, we considered only data collected in the month after 1, 3, 6, and 12 months, respectively (Table 1).

Comparisons of coverage between regions with different BCG provision strategies were evaluated in log-binomial regression models providing prevalence ratios (PR).

Factors associated with delayed BCG vaccination

Factors associated with delayed BCG vaccination (vaccination after 1 month of age) were assessed in the 2010-birth cohort and after implementation of monthly visits separately for intervention and control regions in 2012/2013. This study focused on background factors assessed among the users of the vaccination services. The factors evaluated were: sex, birth place, antenatal care, region, type of roof, toilet, household possessions (radio, cell phone, and generator), ethnic group (Fula, Pepel, Balanta, Manjaco, and other), age of mother, and education of caretaker. All continuous variables were tested for linear relationship with BCG coverage by inspecting the BCG coverage in quintiles of the variable. Where inspection suggested a linear relationship, the quadratic value of the continuous variable was included in the model to assess departure from linearity. We tested all variables one by one in a simple model using logistic regression to calculate the odds ratio (OR) of being unvaccinated. As children were not individually sampled but selected for the study based on residence within a geographical cluster, we adjusted the standard error for cluster. In a larger multivariable model we included all factors associated with delayed BCG. In this large model we excluded ethnic group since ethnic group and region were highly correlated with more than 90% of the Pepels living in Biombo, and more than 75% of the Manjacos living in Cacheu. We chose to include region rather than ethnicity since region would be feasible to target through interventions.

Results

BCG coverage in the 2010-birth cohort

We assessed vaccination status at a visit within the first 2 years of life for 84% (3318/3951) of all children (Additional file 1). In the 2010-birth cohort, the BCG coverage by 1 week of age was 11% (327/3020) and 38% (1140/2984) by 1 month of age but increased to 92% (2385/2594) coverage by 12 months of age (Figure 1). If all children had received BCG vaccine at their first contact with the healthcare system, coverage would have been at least 35% by 1 week of age, 54% by 1 month of age and 99% by 12 months of age (Figure 1). The median age of BCG vaccination among children vaccinated within the first 12 months of life was 39 days. This could be reduced to 27 days if BCG vaccine had been given at first contact with the healthcare system.

BCG coverage after implementation of monthly visits, 2012 cohort

In the 2010-birth cohort, BCG coverage did not differ significantly between the regions which subsequently became intervention regions compared with the control

Table 1 Coverage assessment methods for standard coverage estimates and coverage estimates in the 2010-birth cohort and the 2012 cohort

	Standard coverage estimates	Coverage in 2010-birth cohort	Coverage in 2012 cohort
Children		Children born in 2010	Children aged 1, 3, 6, or 12 months of age, when visited from 1st July 2012 to 30th June 2013
Assessment ages	12 months	1 week, 1, 3, 6 and 12 months	1, 3, 6, and 12 months
Vaccination status	12-23 months	First visit with seen vaccination card within 12 months after assessment age	Visit with seen vaccination card in the month after the assessment age

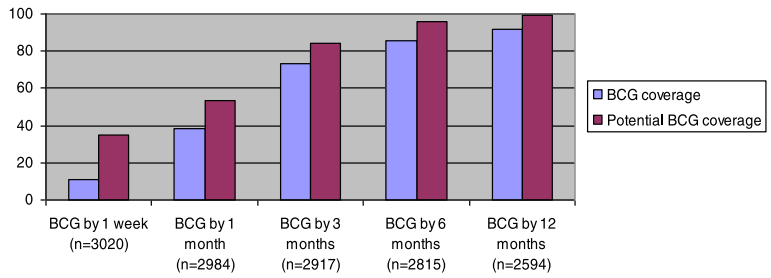

Figure 1 Observed BCG coverage and potential BCG coverage by different ages. Observed BCG coverage (blue) and potential BCG coverage if all vaccination opportunities had been used (purple). Bandim Health Project, Guinea-Bissau, 2010-birth cohort.

regions (PR = 1.03 (0.85-1.24)) (data not shown). After implementation of monthly visits, we assessed BCG coverage among a total of 2812 children (Additional file 2). Coverage by 1 month of age was 88% (769/872) in intervention regions and 51% (141/279) in control regions (Figure 2), the PR being 1.74 (1.53-2.00). The 3-months coverage was 99% (769/776) in intervention regions and 85% (304/359) in control regions. By 12 months of age, it had increased to 99% (257/259) in intervention regions and 95% (284/299) in control regions. The potential 1-month coverage if BCG vaccine had been given at first contact with the healthcare system was 93% in intervention regions and 65% in control regions.

Factors associated with delayed BCG vaccination in the 2010-birth cohort

A number of factors were associated with being BCG vaccinated by 1 month of age (Table 2). Region of residence was strongly associated; only 25% of children in Oio had received BCG compared with 60% on Bolama. Caretaker's education was significantly associated with delayed BCG vaccination in both the univariate analysis (OR = 1.15 (1.10-1.20) per year of schooling), and the multivariable analysis (OR = 1.07 (1.02-1.12)). Previous contact with the health system was associated with higher BCG coverage in both the univariate and the multivariable analysis: Children born at health centres or hospitals were more likely to be BCG vaccinated (OR = 1.70 (1.26-2.30) and OR = 2.88 (2.06-4.01), respectively) than children born at home. Also, children of mothers who attended prenatal consultation were more likely to be BCG vaccinated (OR = 1.78 (1.23-2.57). The children born to mothers with better economic status reflected in possession of a latrine and possession of a cell phone had higher coverage; however, other socioeconomic factors were not significantly associated with coverage in the multivariable analysis. Maternal age was not associated with BCG coverage, and finally BCG coverage did not differ significantly for girls compared with boys (OR = 1.19 (0.99-1.43)) (Table 2).

The risk factor analysis for BCG coverage by 3, 6, and 12 months identified the same factors but most associations were weaker. Prenatal consultation was significantly associated with BCG coverage at all ages (data not shown).

Factors associated with delayed BCG vaccination after implementation of monthly visits, 2012 cohort

After implementation of monthly visits, factors associated with delayed BCG vaccination were studied among the 2812 children who had a vaccination status assessed by 1, 3, 6, or 12 months of age. In the control regions (n = 1147) the factors strongest associated with being BCG vaccinated by 1 month of age in the 2012 cohort were region, contact with the healthcare system (being born at a hospital; OR = 1.81 (1.20-2.73)), and living in a house with hard roof (OR = 1.74 (1.30-2.32)). Other factors were significantly associated with delayed BCG in the

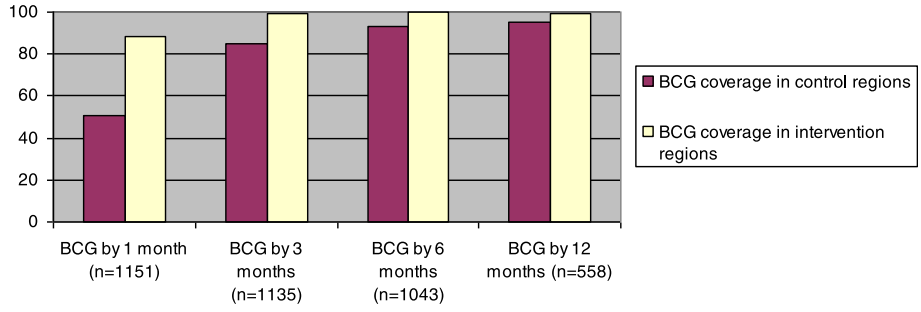

Figure 2 Observed BCG coverage by different ages in intervention and control regions. Bandim Health Project, Guinea-Bissau, 2012 cohort.

Table 2 Factors associated with BCG vaccination by 1 month of age

	Total number of children	BCG by 1 month n (%)	OR of BCG vaccination[1]	Multivariable analysis OR (95%CI)[1]	P-value for the univariate/multivariable analyses
Gender					0.202/0.063
Male	1493	553 (37)	Ref	Ref	
Female	1491	587 (39)	1.10 (0.95-1.28)	1.19 (0.99-1.43)	
Age of mother[2]					0.616/0.662
1.quartile (<21)	763	304 (40)	Ref	Ref	
2. quartile (21–24)	614	239 (39)	0.96 (0.77-1.21)	1.06 (0.80-1.40)	
3. quartile (25–30)	825	306 (37)	0.89 (0.71-1.11)	1.08 (0.82-1.43)	
4. quartile (>30)	745	275 (37)	0.88 (0.71-1.10)	1.18 (0.90-1.55)	
Education of caretaker[3,4]					**<0.001/0.006**
None	1889	627 (33)	**1.15 (1.10-1.20)**	**1.07 (1.02-1.12)**	
1-4 years	648	286 (44)			
5+ years	347	185 (53)			
Region[5]					**<0.001/0.007**
Oio	416	102 (25)	**0.37 (0.21-0.64)**	**0.39 (0.21-0.73)**	
Biombo	445	183 (41)	0.79 (0.50-1.24)	0.90 (0.60-1.35)	
Gabu	459	132 (29)	**0.45 (0.28-0.75)**	**0.50 (0.30-0.82)**	
Cacheu	582	274 (47)	Ref	Ref	
Bafata	370	122 (33)	**0.55 (0.31-0.98)**	**0.49 (0.27-0.89)**	
Quinara	277	143 (52)	1.20 (0.69-2.09)	1.07 (0.61-1.87)	
Tombali	277	105 (38)	0.69 (0.46-1.03)	0.84 (0.55-1.29)	
Bubaque	86	36 (42)	0.81 (0.46-1.42)	0.50 (0.24-1.01)	
Bolama	72	43 (60)	1.67 (0.98-2.84)	0.59 (0.33-1.04)	
Ethnic group[6]					**<0.001**/NA
Balanta	739	265 (36)	1.27 (0.88-1.82)		
Mandinga/Fula	1116	342 (31)	Ref		
Manjaco	254	149 (59)	**3.21 (1.89-5.44)**		
Pepel	391	151 (39)	1.42 (0.92-2.20)		
Other	481	231 (48)	**2.09 (1.46-2.99)**		
Contact with the health system					
Birth place[7]					**<0.001/<0.001**
At home	1888	576 (31)	Ref	Ref	

Table 2 Factors associated with BCG vaccination by 1 month of age *(Continued)*

Healthcare centre	527	263 (50)	**2.27 (1.68-3.07)**	**1.70 (1.26-2.30)**	
Hospital	380	228 (60)	**3.42 (2.61-4.48)**	**2.88 (2.06-4.01)**	
Other	29	4 (14)	0.36 (0.13-1.02)	0.43 (0.16-1.18)	
Prenatal consultations[8]					**<0.001/0.002**
Yes	2313	942 (41)	**2.88 (2.06-4.02)**	**1.78 (1.23-2.57)**	
No	301	58 (19)	Ref	Ref	
Socioeconomics					
Type of roof[9]					**<0.001**/0.324
Straw	1427	482 (34)	Ref	Ref	
Hard	1522	649 (43)	**1.46 (1.17-1.81)**	1.13 (0.89-1.43)	
Toilet[10]					**0.001/0.005**
None	903	286 (32)	Ref	Ref	
Latrine/ toilet in house	2037	839 (41)	**1.51 (1.18-1.94)**	**1.54 (1.14-2.08)**	
Household possessions					
Cell phone[11]					**<0.001/0.025**
Yes	1339	571 (43)	**1.41 (1.20-1.64)**	**1.24 (1.03-1.49)**	
No	1575	545 (35)	Ref	Ref	
Radio[12]					0.211/0.192
Yes	2104	825 (39)	1.13 (0.94-1.36)	0.87 (0.71-1.07)	
No	810	295 (36)	Ref	Ref	
Generator[13]					**0.025**/0.563
Yes	187	88 (47)	**1.47 (1.05-2.07)**	1.14 (0.73-1.80)	
No	2753	1036 (38)	Ref	Ref	

Bandim Health Project, Guinea-Bissau, 2010-birth cohort.
[1]Standard error adjusted for clustering by robust variance estimates.
[2]Numbers do not add up due to some not living with their mother.
[3]Education of caretaker per year's schooling; linear.
[4]100 had missing information on years of schooling.
[5]When including ethnic group rather than region in the final model the estimates changed less than 10% for all parameters assessed.
[6]3 had missing information on ethnic group.
[7]160 had missing information on place of birth.
[8]370 had missing information on prenatal consultations.
[9]35 had missing information on type of roof.
[10]44 had missing information on possession of a latrine.
[11]70 had missing information on possession of a cell phone.
[12]70 had missing information on possession of a radio.
[13]44 had missing information on possession of a generator.
Note: significant ($p < 0.05$) findings in bold.

univariate analysis, but not when adjusted for the other factors (Table 3). When the visit frequency was increased to monthly visits (n = 1665) socioeconomic factors and contact with the healthcare system were no longer significantly associated with BCG coverage, only region was significantly associated with BCG coverage in the multivariable analysis (Table 3).

Information about reasons for not being BCG vaccinated

The year following implementation of monthly visits in intervention regions, 1470 interviews were conducted with mothers of BCG unvaccinated children from all regions. Among the mothers 229 (16%) reported to have sought vaccination for their child, 135 (59%) recalled to be told to return another day to get the vaccine, and 76 (33%) had received other vaccines (Additional file 3). Among the 1239 mothers, who reported not to have sought vaccination, 760 (61%) reported that their main reason was lack of money whereas 481 (39%) said that the distance to the vaccination post kept them from seeking vaccination (Additional file 3).

Discussion

Main findings

In 2010, BCG coverage by 1 week of age was only 11% in rural Guinea-Bissau. By 1 month of age the coverage was 38%, increasing to 73% by 3 months of age and 92% by 12 months of age. Contact with the health system was one of the main factors associated with BCG vaccination, but socioeconomic factors also played a role. When monthly visits were introduced in intervention regions and BCG was available for all children the inequity was reduced and the 1-month BCG coverage was 88% compared with 51% in control regions.

Strengths and weaknesses

A major strength of this study is the set-up in the form of the health and demographic surveillance system covering a representative part of the population in rural Guinea-Bissau. Data was collected through frequent home visits by experienced field workers. Weaknesses include that children who died before the assessment age did not enter the coverage analysis; however, children dying before 12 months of age usually do not enter the standard coverage estimation either. Also it should be noted that the vaccination coverage was estimated using slightly different approaches in the 2010-birth cohort and the 2012 cohort. However, we do not directly compare coverage between the cohorts, but only compare coverage between intervention and control regions within the 2012 cohort. Information on reasons for not being vaccinated was collected based on the mothers' recall.

Consistency with other studies

Vaccination coverage is usually reported by 12 months of age. We found 92% BCG coverage by 12 months in 2010, which corroborates the 94% coverage from 2009 reported by WHO [21]. Others have found that the median vaccination coverage across 31 low- and middle-income countries was 98% and ranged from 56% to 100% [19].

We found a much lower coverage by 1 and 3 months, which concurs with reports of a median coverage across the 31 countries of 65% by age of 4.3 weeks, ranging from 15% to 97% [19]. This supports the need for assessing BCG coverage at earlier ages to disclose the delay in BCG vaccination.

Other studies [22-24] defined timely BCG vaccination as vaccinated before 8 weeks of age. The percentage of timely vaccinated children ranged from 69% vaccinated [22] in a large survey in 45 low-income and middle-income countries, to 99% [24] in a study from three areas in South Africa. This still does not fully disclose the poor coverage in the neonatal period, with only 49% coverage by 4 weeks [22], quite similar to the 1-month coverage of 38% in the present study.

We found that giving birth at a hospital or health centre increased the likelihood of being BCG vaccinated. Similarly, a study from South Africa found that birth at a health facility reduced the risk of being unvaccinated by 47% (26%-42%) [24]. In Ethiopia there was also higher BCG coverage for those born at a health facility [25]. In Guinea-Bissau it is not general practice that children born at a hospital or health centre are vaccinated before they leave the health facility, however, they are often told to return to the health centre for vaccination.

It has previously been reported from South Africa [24] and 31 low- and middle-income countries [19] that low socioeconomic status was related to delay in BCG vaccination. We found a similar tendency, especially during the first month of life. Importantly, this inequity in getting BCG vaccinated was no longer apparent when we provided BCG at monthly village visits.

Interpretation and implications

WHO vaccination coverage estimates are reported as the coverage by 12 months of age not taking into account the timeliness of vaccines received. This does not disclose delays in administration of the BCG vaccine. It has been shown that BCG vaccination can reduce neonatal mortality by 48% in LBW children when administered at birth [6]. When donors only ask for BCG coverage by 12 months of age, there is no incentive to provide BCG in the neonatal period. Therefore the 1-month coverage or the median age at vaccination would be better indicators of BCG coverage and its likely effect on child survival.

Obstacles to timely BCG were identified through the interview with mothers of unvaccinated children. Among

Table 3 Factors associated with BCG vaccination by 1 month of age after implementation of monthly visits in intervention regions

	Intervention regions				Control regions			
	BCG by 1 month n (%)	OR of early BCG vaccination[1]	Multivariable analysis OR (95% CI)[1]	P-value for the univariate/ multivariable analyses	BCG by 1 month n (%)	OR of early BCG vaccination[1]	Multivariable analysis OR (95% CI)[1]	P-value for the univariate/multivariable analyses
Gender[2]				0.492 / 0.662				0.099/0.070
Male	658 (77)	Ref	Ref		284 (50)	Ref	Ref	
Female	620 (76)	0.93 (0.75-1.15)	1.05 (0.84-1.32)		260 (45)	0.82 (0.65-1.04)	0.78 (0.59-1.02)	
Age of mother[3]				0.582 / 0.615				0.998/0.979
1.quartile (<21)	339 (78)	Ref	Ref		125 (47)	Ref	Ref	
2. quartile (21–26)	244 (78)	1.01 (0.68-1.49)	0.95 (0.59-1.53)		113 (47)	1.00 (0.69-1.47)	1.09 (0.69-1.71)	
3. quartile (27–31)	357 (76)	0.90 (0.64-1.27)	0.86 (0.60-1.25)		153 (48)	1.01 (0.71-1.45)	1.03 (0.65-1.63)	
4. quartile (>31)	335 (75)	0.83 (0.59-1.17)	0.80 (0.53-1.22)		153 (48)	1.03 (0.75-1.40)	1.07 (0.72-1.59)	
Education of caretaker[4, 5]				**0.003** / 0.377				**0.018**/0.771
None	607 (73)	**1.08 (1.03-1.14)**	1.03 (0.96-1.10)		317 (46)	**1.07 (1.01-1.13)**	0.99 (0.92-1.07)	
1-4 years	323 (79)				118 (47)			
5+ years	270 (83)				84 (59)			
Region[6]				**<0.001 / 0.002**				**0.003**/0.161
Oio	271 (70)	**0.67 (0.46-0.95)**	0.70 (0.49-1.02)		81 (40)	**0.49 (0.31-0.77)**	0.67 (0.40-1.09)	
Biombo	528 (81)	1.21 (0.87-1.68)	1.34 (0.91-1.98)		42 (47)	0.67 (0.41-1.11)	0.92 (0.56-1.51)	
Gabu		NA	NA		83 (40)	**0.50 (0.31-0.82)**	0.63 (0.36-1.10)	
Cacheu	479 (77)	Ref	Ref		148 (57)	Ref	Ref	
Bafata		NA	NA		42 (39)	**0.48 (0.27-0.88)**	**0.54 (0.30-0.98)**	
Quinara		NA	NA		112 (57)	1.01 (0.59-1.73)	1.18 (0.68-2.04)	
Tombali		NA	NA		24 (37)	0.44 (0.17-1.15)	0.69 (0.26-1.89)	
Bubaque		NA	NA		6 (60)	1.13 (0.43-2.95)	1.61 (0.43-6.07)	
Bolama		NA	NA		7 (70)	1.75 (0.52-5.94)	1.82 (0.30-10.99)	
Ethnic group[7]				0.109 / NA				**<0.001**/NA
Balanta	401 (73)	Ref			124 (41)	Ref		
Mandinga/Fula	194 (76)	1.18 (0.81-1.73)			175 (39)	0.92 (0.66-1.28)		
Manjaco	140 (79)	1.37 (0.79-2.38)			64 (67)	**2.84 (1.49-5.40)**		
Pepel	426 (81)	**1.60 (1.12-2.27)**			35 (48)	1.31 (0.80-2.13)		
Other	115 (74)	1.07 (0.66-1.73)			147 (63)	**2.45 (1.65-3.65)**		

Table 3 Factors associated with BCG vaccination by 1 month of age after implementation of monthly visits in intervention regions *(Continued)*

Contact with the health system								
Birth place[8]				0.369 / 0.515				**<0.001/<0.001**
At home	793 (76)	Ref	Ref		337 (43)	Ref	Ref	
Healthcare centre	255 (81)	1.38 (0.95-1.99)	1.30 (0.83-2.03)		82 (53)	**1.48 (1.01-2.16)**	1.15 (0.76-1.74)	
Hospital	192 (78)	1.17 (0.83-1.66)	0.96 (0.64-1.44)		106 (62)	**2.18 (1.54-3.08)**	**1.81 (1.20-2.73)**	
Prenatal consultations[9]				0.410 / 0.504				0.057/0.558
Yes	1093 (79)	1.16 (0.81-1.66)	0.86 (0.55-1.34)		460 (49)	1.55 (0.99-2.44)	1.20 (0.71-2.05)	
No	124 (76)	Ref	Ref		42 (38)	Ref	Ref	
Socioeconomics								
Type of roof[10]				**0.048** / 0.128				**<0.001/<0.001**
Straw	493 (74)	Ref	Ref		151 (37)	Ref	Ref	
Hard	777 (79)	**1.35 (1.00-1.83)**	1.28 (0.93-1.77)		386 (54)	**1.97 (1.50-2.58)**	**1.74 (1.30-2.32)**	
Toilet[11]				**0.039** / 0.188				0.136/0.706
None	445 (74)	Ref	Ref		100 (44)	Ref	Ref	
Latrine/ toilet in house	819 (79)	**1.32 (1.01-1.73)**	1.24 (0.90-1.71)		439 (49)	1.23 (0.94-1.62)	0.93 (0.67-1.30)	
Household possessions								
Cell phone[12]				**0.011** / 0.136				**0.009**/0.347
Yes	747 (80)	**1.38 (1.08-1.78)**	1.25 (0.93-1.66)		311 (51)	**1.39 (1.08-1.79)**	1.15 (0.84-1.55)	
No	501 (74)	Ref	Ref		221 (43)	Ref	Ref	
Radio[13]				0.183 / 0.303				0.159/0.841
Yes	958 (78)	1.21 (0.91-1.59)	1.17 (0.87-1.56)		422 (49)	1.24 (0.92-1.67)	1.05 (0.75-1.48)	
No	295 (74)	Ref	Ref		109 (43)	Ref	Ref	

Table 3 Factors associated with BCG vaccination by 1 month of age after implementation of monthly visits in intervention regions *(Continued)*

Generator[14]			**0.020** / 0.083				0.234/0.636
Yes	149 (83)	**1.51 (1.07-2.13)**	1.46 (0.95-2.24)	53 (53)	1.27 (0.86-1.87)	0.89 (0.54-1.47)	
No	1115 (76)	Ref	Ref	485 (47)	Ref	Ref	

Bandim Health Project, Guinea-Bissau, 2012 cohort.
[1]Standard error adjusted for clustering by robust variance estimates.
[2]1 had missing information on gender.
[3]Numbers do not add up due to some not living with their mother.
[4]Education of caretaker per year's schooling; linear.
[5]157 had missing information on years of schooling.
[6]When including ethnic group rather than region in the final model the estimates changed less than 10% for all parameters assessed.
[7]3 had missing information on ethnic group.
[8]56 had missing information on place of birth, 26 were born elsewhere and have been excluded due to small numbers.
[9]202 had missing information on prenatal consultations.
[10]33 had missing information on type of roof.
[11]36 had missing information on possession of a latrine.
[12]71 had missing information on possession of a cell phone.
[13]58 had missing information on possession of a radio.
[14]37 had missing information on possession of a generator.
Note: significant ($p < 0.05$) findings in bold.

the mothers having sought vaccination but had not obtained BCG more than half the mothers recalled being told to return another day. We speculate that the restricted vial-opening policy is one of the main obstacles to early BCG vaccination but the information disclosed to the mothers does not allow any final conclusion. Among the mothers who had not sought vaccination not having money and distance to health facility were the main obstacles to taking their child for vaccination. Routine childhood vaccinations are provided free of charge in Guinea-Bissau, but health workers charge fees (~1$) for vaccination cards.

Twenty-five percent of BCG unvaccinated children had been in contact with a health facility by 1 month of age. The potential coverage by 1 month of age was 54% in 2010, if all children had been BCG vaccinated at first contact with the health system. Monthly village visits with BCG vaccination for all children significantly increased BCG vaccination coverage, especially at early ages, and would provide a very efficient tool for increasing especially coverage among the youngest children.

Conclusions

Our study showed a large delay in BCG vaccination in Guinea-Bissau with less than half of the children being BCG vaccinated by 1 month and only 11% being BCG vaccinated by 1 week of age. Our risk factor analysis identified many factors associated with delay of BCG vaccination, including a number of socioeconomic factors, but these factors were no longer associated with delayed BCG vaccination when BCG-vaccination became available to all children at monthly visits.

Additional files

Additional file 1: Flowchart. Bandim Health Project, Guinea-Bissau, 2010 rural birth cohort.

Additional file 2: Flowchart. Bandim Health Project, Guinea-Bissau, 2012 rural cohort.

Additional file 3: Reasons for not being BCG vaccinated. Bandim Health project, Guinea-Bissau, BCG unvaccinated children when met by the BHP team in 2012.

Abbreviations

BCG: Bacillus Calmette-Guérin vaccine; BHP: Bandim Health Project; CI: Confidence interval; LBW: Low-birth-weight; OPV: Oral polio vaccine; OR: Odds ratio; PR: Prevalence ratio; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ST contributed to coordination of the study and interpretation of the data, conducted the data analysis and drafted the manuscript. MP and SB participated in coordination of the study. HR helped with statistical analysis. AF designed the study, helped with data analysis and contributed to the draft and write up of the manuscript. All authors contributed to the design, the interpretation of data, and read and approved the final manuscript.

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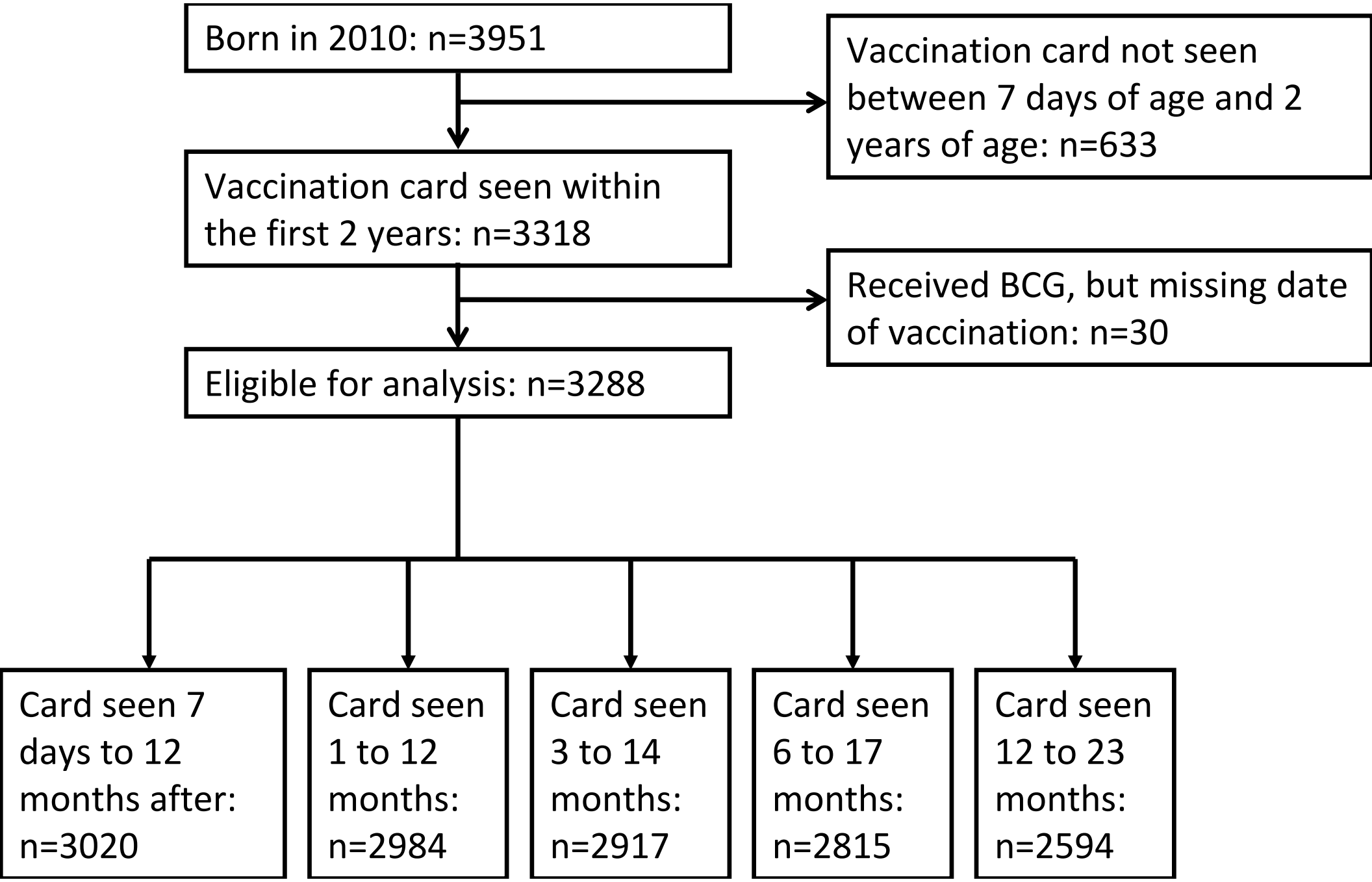
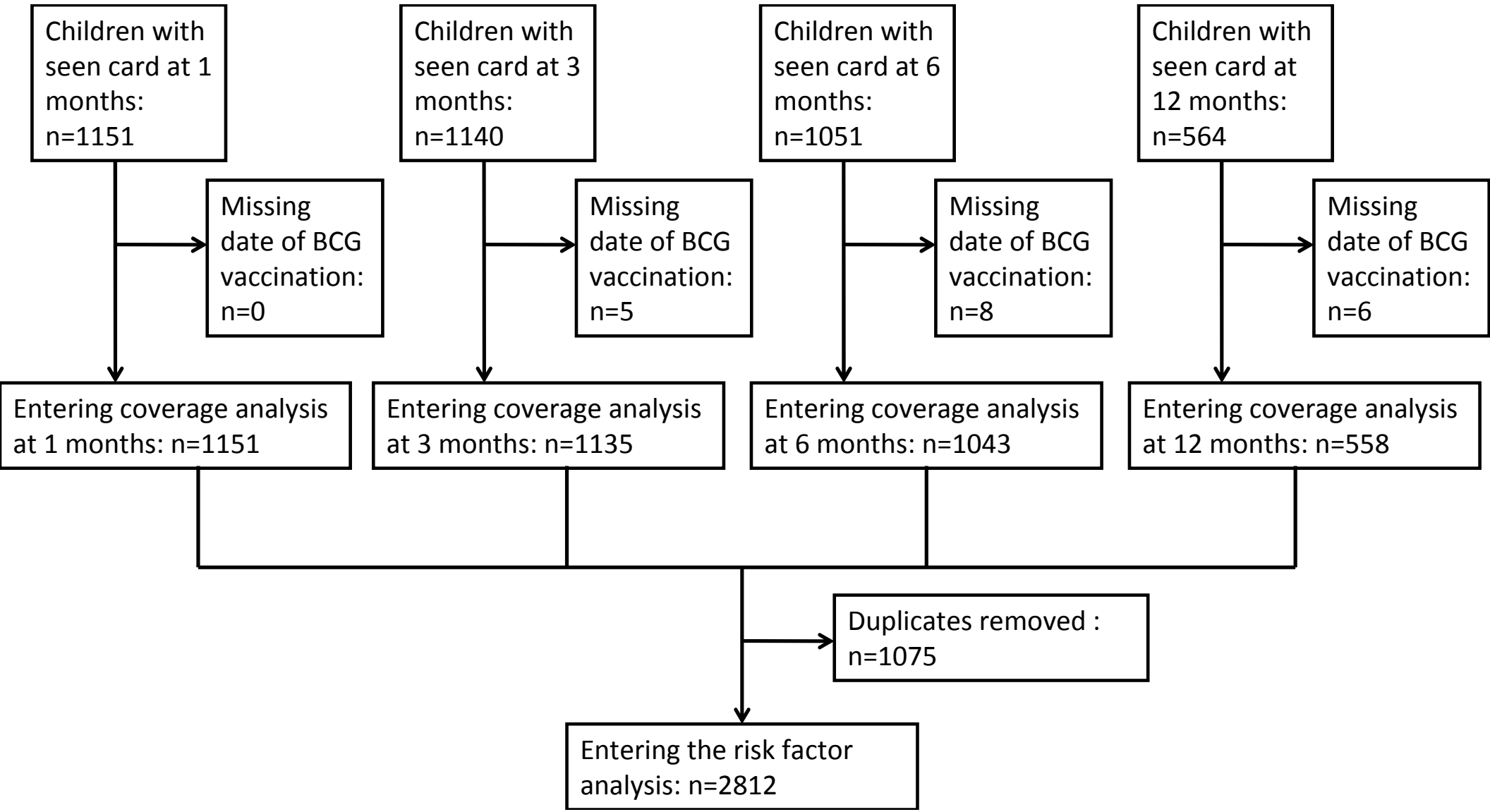

Born in 2010: n=3951
Vaccination card not seen between 7 days of age and 2 years of age: n=633
Vaccination card seen within the first 2 years: n=3318
Received BCG, but missing date of vaccination: n=30
Eligible for analysis: n=3288
Card seen 7 days to 12 months after: n=3020
Card seen 1 to 12 months: n=2984
Card seen 3 to 14 months: n=2917
Card seen 6 to 17 months: n=2815
Card seen 12 to 23 months: n=2594


Children with seen card at 1 months: n=1151
Children with seen card at 3 months: n=1140
Children with seen card at 6 months: n=1051
Children with seen card at 12 months: n=564
Missing date of BCG vaccination: n=0
Missing date of BCG vaccination: n=5
Missing date of BCG vaccination: n=8
Missing date of BCG vaccination: n=6
Entering coverage analysis at 1 months: n=1151
Entering coverage analysis at 3 months: n=1135
Entering coverage analysis at 6 months: n=1043
Entering coverage analysis at 12 months: n=558
Duplicates removed : n=1075
Entering the risk factor analysis: n=2812

		Number[1]
Number		1470
Median age (days) at the time of the interview (Inter quartile range)		56 (24-111)
Reported to have sought vaccination[2]		229 (16%)
	Recalled being told to return another day	135 (59%)
	Received other vaccinations	76 (33%)
Reported not to have sought vaccination[3]		1239 (84%)
	Received other vaccinations	39 (3%)
	Knew the child was due to be vaccinated	1123 (91%)
	Lack of money	760 (61%)
	Distance to vaccination place too long	481 (39%)
	Waiting for outreach services in the village.	396 (32%)

[1] Number (%) unless reported otherwise
[2] Children can be included in more than one category
[3] Mothers can report several reasons not having sought vaccination

Paper II

Household costs of seeking BCG vaccination in rural Guinea-Bissau

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(submitted)

Household costs of seeking BCG vaccination in rural Guinea-Bissau

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Abstract

In Guinea-Bissau, a vial of BCG vaccine is often not opened unless 10 infants are present for vaccination, with the aim of reducing vaccine wastage. This causes delays in vaccination, as previously demonstrated in Guinea-Bissau and other low-income countries. Reducing wastage of BCG vaccine to save money may deprive infants of important health benefits and transfer costs from the vaccination programme to mothers.

Using the Bandim Health Project's rural Health and Demographic Surveillance System, we interviewed mothers of infants aged 1-11 months about household costs of seeking BCG vaccination.

On average mothers took their infant for BCG vaccination 1.26 times before obtaining the vaccine. For mothers who had sought BCG vaccine for their infants the average cost was 1.89 USD for each BCG-vaccinated infant. Among BCG-unvaccinated infants at the time of interview, 42% had brought their infant for BCG vaccination in vain at an average cost of 2.83 USD.

Highlights

- BCG vaccine vials contain 20 doses and must be used within 6 hours of reconstitution
- BCG is recommended at birth but to reduce wastage, a vial of BCG is not opened unless sufficient children are present for vaccination
- We assessed missed opportunities of BCG vaccination and household cost of seeking BCG in Guinea-Bissau
- Infants were on average brought to health centers 1.26 times before obtaining BCG
- The household cost was 1.89 USD per vaccinated child - similar to the price of a BCG vial

Introduction

Investments in vaccination programme strengthening are justified by their impact on target infections, commonly measured by vaccination coverage as proxy, and decisions are supported by cost-effectiveness assessments. A way to increase the efficiency of vaccination programmes is by reducing costs, for example by reducing vaccine wastage.

Bacillus Calmette-Guérin (BCG) vaccine is recommended to be given at birth in countries with high tuberculosis burden[1]. The vaccine is supplied in 20-dose vials, and like other live vaccines, once reconstituted the vaccine must be used within 6 hours. The official wastage target for BCG was 25% in Guinea-Bissau in 2013-2017[2]. In an attempt to reduce vaccine wastage, most health centres only provide live vaccines on a specific week day. However, even on these days a vial is not opened unless a sufficient number of infants (10 in Guinea-Bissau) are present for vaccination, causing mothers to seek vaccination in vain[3-5]. Thus, BCG is often delayed in Guinea-Bissau[4], and in other low-income countries[6,7].

Delays in BCG vaccination are not monitored through administrative data, and the high BCG coverage (88% globally in 2017[8]) reported at 12 months of age does not call for action. Despite BCG being developed to protect against tuberculosis, increasing evidence supports that the vaccine has beneficial non-specific effects (NSE)[9], reducing mortality by more than can be explained by the prevention of tuberculosis. Even small delays in BCG vaccination may be important for the benefits of NSEs: In a meta-analysis of three randomised trials among low-weight infants in Guinea-Bissau for whom vaccination is normally delayed, BCG-at-birth was associated with 38% (17-54%) lower neonatal mortality compared with infants with delayed vaccination, with an effect of 45% (7-68%) within three days after vaccination[10].

Thus, reducing wastage of BCG vaccine to save costs may deprive infants of important health benefits and also transfer costs from the vaccination programme to mothers. The objectives of this study were to determine the average number of times a mother sought BCG vaccination for her infant and to estimate the household costs of seeking BCG vaccination in rural Guinea-Bissau.

Methods

The Bandim Health Project runs a Health and Demographic Surveillance System (HDSS) in rural Guinea-Bissau where women and children are followed through biannual household visits. Between May 24, 2014 and December 29, 2016, we interviewed mothers of infants living in seven regions (Oio, Gabu, Bafata, Quinara, Tombali, Bolama and Bijagos). Mothers of infants registered prior to birth were interviewed at the first visit after the neonatal period. If the mother and infant were not present or the infant had not yet been BCG vaccinated, the mother was (re-)interviewed at the next visit if the infant was still below one year of age.

All mothers were asked whether their infant had received the BCG vaccine against tuberculosis (explained as “the vaccine given in the arm that often makes a small scar”), and date of BCG was obtained from the infant’s vaccination card. Mothers of infants born in a health facility were asked whether their infant had received BCG at birth. The interview was terminated for infants vaccinated at birth at a health facility. However, some mothers stated that their infant received BCG vaccine at birth, although the date of BCG vaccination was registered to be later. Since very few infants are hospitalised longer than 7 days after birth, infants stated to be BCG-vaccinated at birth in a health

facility and with a registered date of BCG vaccination within 7 days after birth were classified as brought for vaccination 0 times. Infants stated to be BCG-vaccinated at birth in a health facility and with a date of BCG vaccination after 7 days after birth were assumed to have been brought for BCG vaccination once.

All mothers were asked if and how many times they had sought BCG vaccination. We asked for details on time spent seeking BCG vaccination (from leaving the house to returning home). We asked for number hours up to 24 hours, and categorised mothers who had used more than 24 hours in one group (>24 hours). To provide a realistic picture of the time most mothers use to seek BCG vaccination, these mothers were excluded in the reported range of transportation time, but contributed to the median estimate and were classified as having spent 24 hours. We furthermore asked for money spent on transportation. To evaluate if there were other missed opportunities of BCG vaccination, we asked whether the mother had brought her infant for other vaccinations or consultations prior to obtaining BCG.

All costs were collected in West-African Francs (CFA), and converted into US dollars (USD) using the 2016 average exchange rate of 594 CFA to 1 USD. The value of time spent seeking BCG vaccination was calculated based on an estimated average monthly earning of 61 USD (2011, Guinea-Bissau) by Knight et al[11]. Using World Bank Consumer Price Index[12] this corresponded to an average monthly earning of 69.94 USD in 2016. We assumed 176 working hours per month as in a previous study[3], resulting in a value of 0.36 USD per hour of a mother's time. We calculated the costs of seeking BCG vaccine per infant among those who were stated not to be BCG vaccinated at birth, by multiplying the value of the mother's time by time spent seeking vaccination and adding transportation costs. If the mother had sought BCG vaccination for her infant more than once, the cost of seeking BCG was multiplied by the number of times. We calculated an average cost of seeking BCG vaccination per infant among children who had been brought for vaccination.

Results

We interviewed 2203 mothers of 2271 infants aged 1 to 11 months. Among these 1480 (65%) were born at home, 780 (34%) were born in health facilities, and 11 infants had missing information on place of birth. For infants born in health facilities, mothers stated that 287 (37%) were BCG vaccinated at birth with 96 (12%) having received BCG vaccine at birth, and 175 (22%) before 7 days of age. These infants were counted as BCG vaccinated with 0 times seeking the vaccine. Among infants stated to be BCG-vaccinated at birth, the date of BCG according to the vaccination card was more than 7 days after birth for 112 infants (39%), and these infants were recoded to have been brought for BCG vaccination once.

Among the 2271 infants where information was obtained, 1850 (81%) were BCG vaccinated at time of interview. On average mothers had sought BCG vaccination 1.17 times; 1.26 times among BCG-vaccinated infants and 0.82 times among infants who had not yet been vaccinated. Among the 1753 infants for whom BCG vaccination had been sought, a median of two hours was spent away from home (Range: 0-14 hours) (Table 1), but 11 (0.1%) mothers had spent more than 24 hours away from home. 315 (16%) of the infants' mother paid for transport to the health facility. Among these, the median transport cost was 0.84 USD (Range: 0.17-11.78) (Table 1). The average total cost of seeking BCG vaccination was 1.89 USD per infant among those who had sought BCG vaccination. The total cost of seeking BCG vaccination differed according to birth location: The average cost of

seeking BCG for infants born at home was 1.93 USD. The average cost for infants born in health facilities was 1.71 USD (Table 1). When stratifying by BCG vaccination status at time of interview the average cost of seeking BCG vaccination among infants BCG-vaccinated was 1.89 USD. Among the BCG-unvaccinated infants, 169 (42%) had been brought for BCG vaccination at an average cost of 2.83 USD (Table 2). The older the infant, the more likely the mother was to have sought BCG vaccination several times (Figure 1).

Among BCG-unvaccinated infants at time of interview, mothers to a subset of infants (275) were asked for reasons for the infant not being vaccinated. The majority of these, 205 (75%) knew that vaccines were recommended at birth, and 53 (19%) had sought vaccination. Of these, 42 (79%) were told to return another day since no BCG vaccine vial was opened. Among the 222 infants who had not been brought for vaccination, mothers gave several reasons for not seeking BCG vaccination: distance (116 (52%)), lack of money (124 (56%)) and waiting for vaccination outreach (116 (52%)).

Discussion

Utilising the HDSS setup in rural Guinea-Bissau, we were able to assess household costs of seeking BCG vaccination. We found that mothers on average brought their infant for BCG vaccination 1.26 times before obtaining the vaccine and that average household cost of seeking BCG vaccination was 1.89 USD per BCG-vaccinated infant. At the time of interview, 42% of unvaccinated infants had been brought for BCG vaccination with an average household cost of 2.83 USD. This is equivalent to the UNICEF price ranges of 1.36-3.24 USD per vial of BCG in 2016[13]. Mothers seeking BCG vaccination, spent an average of 2 hours (Range: 0-14 hours) on obtaining the vaccine for their infant, with 0.1% of mothers spending more than 24 hours.

Most mothers in rural Guinea-Bissau are not aware of which vaccines are recommended at which age. BCG and oral polio vaccine are scheduled at birth and further vaccines are scheduled after 6 weeks of age, and only BCG and yellow fever vaccine are administered in the arm. Thus, mothers were told that we were asking about “the vaccine against tuberculosis given in the left arm and that often leaves a small scar”. Mothers do not take their infant for a specific vaccine and we were therefore not able to disentangle the costs of bringing the infant for BCG vaccination from the costs of bringing the infant for other vaccines. Aside from vaccination contacts, the mothers were asked if they had taken their infant for consultations. All reported health contacts (seeking vaccination or consultation) prior to date of BCG were counted as possible opportunities for BCG vaccination. Outreach vaccination is part of the national vaccination programme and was conducted in some villages during the study period. We were not able to account for outreach vaccination, although mothers of infants vaccinated during outreach vaccination most likely would report to have spent little time and no money on seeking vaccination.

In a country like Guinea-Bissau with few national registries and large informal sector, it is difficult to assign a value to mothers’ time. We used estimates of average monthly earning to calculate the value of an hour of a mother’s time. However, this estimate contains much uncertainty, and is likely to differ significantly between urban and rural women. In absence of better estimates, we assumed that the monthly earning on average was representable for the mothers in rural Guinea-Bissau.

To our knowledge, no other study has assessed the household costs of seeking BCG vaccination. We have previously assessed the household costs of seeking measles vaccination in Guinea-Bissau,

and found that mothers on average took their children for vaccination 1.4 times with an average cost of 2.04 USD[3]. We found that mothers of BCG-vaccinated children on average took their infants for vaccination 1.26 times with an average cost of 1.89 USD. We did not assign a cost to the vaccination opportunity at the time of birth for children born in health facilities. 79% of mothers of unvaccinated infants who had sought BCG vaccination were told to return another day. One could speculate that seeking BCG vaccination in vain may affect subsequent behaviour. Hence, the time and money spent may prevent the mother from seeking vaccination again, or may even prevent other mothers from seeking BCG vaccination for their infant, but these potential wider consequences were not assessed in our study.

Conclusion

Not opening a vial of BCG vaccine to save costs not only delays BCG vaccination, but also increases household costs and time spent on seeking BCG vaccination. A vial of BCG vaccine should be opened for every unvaccinated infant to ensure timely vaccination, and that no mother seeks BCG vaccination in vain.

Competing interests: None

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Figure 1. Number of times a mother brought her infant for BCG vaccination according to the infant's age at time of visit.

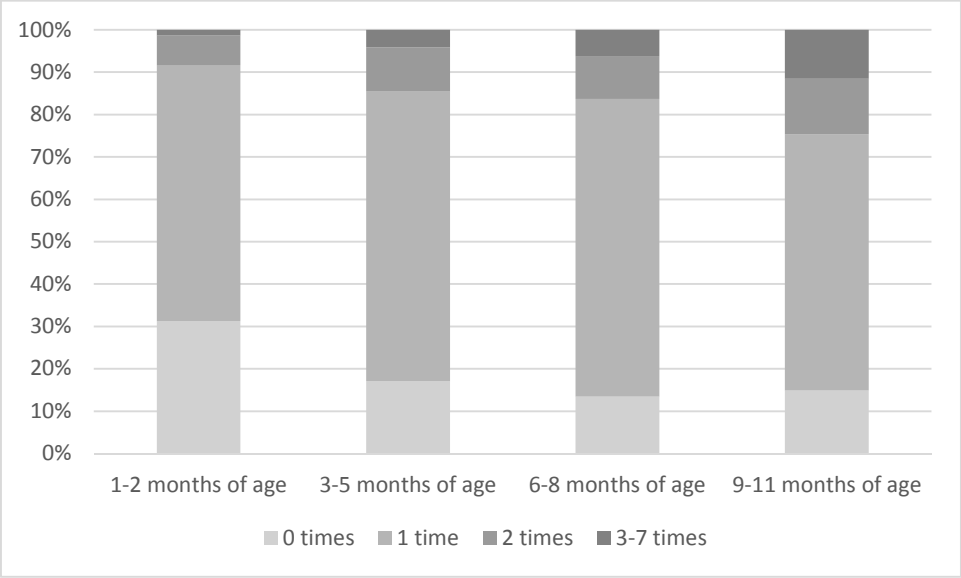

Table 1. BCG vaccination opportunities and costs of seeking BCG vaccination according to birth location

	Total number of mothers present for interview	Infants stated to be vaccinated at the health facility at birth	Infants with BCG at time of interview	Infants brought for BCG vaccination at least once	Number of possible BCG vaccination contacts	Time spent on seeking BCG vaccination (hours)	Number of mothers who paid for transport	Transport costs of seeking BCG vaccination (USD)	Average costs of seeking BCG vaccination (USD)[2]
Birth place	n	n (%)	n (%)	n (%)	Mean (sd)	Median (Range[1])	n (%)	Median (Range)	Mean (sd)
All	2271	286 (13%)	1850 (81%)	1753 (88%)	1.17 (0.82)	2 (0-14)	315 (16%)	0.84 (0.17-11.78)	1.89 (2.67)
Home	1481	0	1150 (78%)	1278 (86%)	1.13 (0.80)	2 (0-14)	234 (16%)	0.84 (0.17-11.78)	1.93 (2.76)
Health facility	779	286 (37%)	690 (89%)	464 (94%)	1.28 (0.86)	2 (0-12)	79 (16%)	0.84 (0.17-3.37)	1.71 (2.13)

[1] With exception of 11 mothers, who reported to have spent more than 24 hours seeking BCG vaccination per time

[2] Among children for whom BCG vaccination was sought (excluding infants who received BCG at birth in a health facility)

Table 2. BCG vaccination opportunities and costs of seeking BCG vaccination according to BCG vaccination status at time of interview

	Total number of mothers present for interview	Infants stated to be vaccinated at the health facility at birth	Infants brought for BCG vaccination at least once	Number of possible BCG vaccination contacts	Time spent on seeking BCG vaccination (hours)	Number of mothers who paid for transport	Transport costs of seeking BCG vaccination (USD)	Average costs of seeking BCG vaccination[2] (USD)
	n	n (%)	n (%)	Mean (sd)	Median (Range[1])	n (%)	Median (Range)	Mean (sd)
BCG vaccinated prior to interview	1850	266 (14%)	1584 (100%)	1.26 (0.67)	2 (0-14)	290 (18%)	0.84 (0.17-11.78)	1.89 (2.67)
Not BCG vaccinated prior to interview	421	20 (5%)	169 (42%)	0.82 (1.18)	1 (0-11)	25 (6%)	1.01 (0.34-3.37)	2.83 (4.51)

[1] With exception of 11 mothers, who reported to have spent more than 24 hours seeking BCG vaccination per time

[2] Among children for whom BCG vaccination was sought (excluding infants who received BCG at birth in a health facility)

Paper III

The effect of early BCG vaccination on neonatal mortality and morbidity: a natural experiment

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Effect of early BCG vaccination on neonatal mortality and morbidity: a natural experiment

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Abstract

Background

Bacillus Calmette-Guérin (BCG) vaccine is recommended at birth in countries with high TB burden. Increasing evidence supports that the BCG vaccine, in addition to the protection against TB, has beneficial non-specific effects reducing all-cause mortality.

Methods

Within Bandim Health Project's rural Health and Demographic Surveillance System, we visited villages and provided BCG vaccines at monthly visits. This created a natural experiment in which children were vaccinated at a random day during the first month of life, depending on when they were born in relation to the monthly visits. We assessed mortality, hospital admission, and outpatient consultation rates in the neonatal period by BCG vaccination status, and compared the rates between day 1 and 28 in Cox-proportional hazard models with age as underlying time scale and standard errors allowing for intragroup correlations within village cluster.

Results

Among registered pregnancies, the stillbirth proportion was 5.4% (309/5682). Between day 1 and 28 among BCG-unvaccinated children the mortality rate (MR) was 293.1 per 1000 person years (PYRS) (45 deaths during 154 PYRS) compared with 99.1 per 1000 PYRS (10 deaths during 101 PYRS) among BCG-vaccinated children. The Cox-analyses did not suggest that BCG lowered mortality (Hazard Ratio (HR): 1.26 (95% CI: 0.60-2.64)) compared with BCG-unvaccinated children. Similarly, we found no effect on morbidity.

Conclusion

In this natural experiment with limited healthy-vacinee bias, but also limited power, our analyses did not support beneficial non-specific effects of BCG. Based on this study, we are not able to make firm conclusions on the effect of BCG on neonatal outcomes. The effects of BCG should be evaluated in randomised trials assessing the effect on all-cause outcomes in newborns.

Introduction

Bacillus Calmette-Guérin (BCG) vaccine is recommended at birth to protect against tuberculosis[1]. The official BCG coverage estimates are high in most countries, and the global BCG coverage in 2017 was 88%[2]. Despite high coverage of BCG at 12 months of age, BCG is often delayed in low-resource settings[3-5]. In rural Guinea-Bissau only 38% of children were BCG vaccinated at 1 month of age, and there were many missed opportunities for BCG vaccination[6]. A major reason for delayed BCG vaccination is the local policy of not opening a vial of BCG unless 10 children are present for BCG vaccination[6 7]. On average, mothers in rural Guinea-Bissau bring their child for BCG vaccination 1.26 times to obtain BCG vaccination[7].

Aside from the protection against tuberculosis, BCG has non-specific effects (NSE)[8 9], reducing infant mortality by more than can be explained by the protection against tuberculosis. Delayed BCG has previously been recommended for low-birth-weight infants[10 11], which made it possible to conduct randomised trials of BCG-at birth compared with the usual delayed BCG vaccination among low-weight children in Guinea-Bissau: A meta-analysis of the three randomised trials assessing the effect of BCG-at-birth found that BCG vaccination was associated with 38% (17-54%) lower neonatal mortality[12]. Findings from an ecological study in Ghana also suggest that earlier BCG may be associated with lower neonatal mortality: In Ghana, the decline in median age of BCG vaccination from 46 days in 1996 to 4 days in 2012 coincided with a decline in neonatal mortality from 46 to 12 per 1000 live births[13].

Under-5 mortality has declined significantly during the last decades[14] and part of the success is generally ascribed to vaccines[15]. The mortality decline has been particularly marked for post-neonatal infants and older children, while the rate of decline in neonatal mortality has been slower[14]. If BCG has marked effects on neonatal mortality, removing the delay in BCG vaccination could make a substantial contribution to reducing neonatal mortality.

In 2014, a WHO commissioned working group reviewed the NSE of BCG, and concluded that evidence suggests that receipt of BCG reduces overall mortality by more than expected through the effect on tuberculosis[9]. No policy changes were recommended, but more randomised trials were called for[9]. Since BCG is recommended at birth, depriving children of BCG vaccination would be unethical. It is therefore difficult to conduct trials of the effect of BCG on neonatal mortality. In the present study, we exploited monthly village visits, which created a natural experiment in which we could assess the effect of BCG on neonatal mortality and morbidity. Due to monthly visits, children received BCG at a random age within the first month of life depending on when they were born relative to the village visit.

Methods

Setting and study population

The Bandim Health Project (BHP) runs a Health and Demographic Surveillance System (HDSS) in rural Guinea-Bissau[16], where women of fertile age and children below 5 years of age are followed through regular home visits. At the time of registering women of fertile age, we document age,

ethnicity, education, and register BCG scars. At all home visits, new pregnancies are registered, the outcome of registered pregnancies is ascertained, and mothers are interviewed about vital status and health status of their children (including hospital admissions and outpatient consultations). When a pregnancy is registered, we record the mother's arm circumference, household possessions, and collect information on antenatal care; at the first visit after the birth of a child, information on antenatal care is updated. For all children, the mid-upper-arm circumference is measured, the BCG-scar status is assessed and vaccination information is obtained by inspecting the child's vaccination card.

Between September 3, 2012 and July 27, 2016, monthly visits were conducted in the four health regions closest to the capital (Oio, Biombo, Cacheu and Sao Domingos) due to a randomised trial of an additional early dose of measles vaccine[17]. In January 2015, we ended trial enrolments in Sao Domingos and monthly visits were discontinued in this region. At the monthly visits, the BHP nurse administered routine vaccines according to national vaccination policy. For BCG, we did however not follow the national policy of vaccinating only if 10 children were present to be vaccinated[7]. Instead, we opened a vial of BCG at all visits, even if there was only one child present for vaccination. BCG vaccines were obtained from the national vaccination programme, but to account for the higher wastage than accepted by the programme, we supplemented the stock from the national programme with BCG vaccines (BCG-Denmark strain) purchased from the Serum Institute in Denmark[18]. BCG was administered as an intradermal injection of 0.05mL in the deltoid area on the left arm. We have previously used a subsample of the cohort to assess determinants for BCG scarification, and found that vaccination technique and vaccine strain were associated with development of a BCG scar, whereas nutritional and socioeconomic statuses were not[18].

At the registration of a death, a short interview was conducted to obtain information about cause of death; we had planned to compare the causes of death in BCG-vaccinated and BCG-unvaccinated children. In the study protocol, the expected neonatal mortality after day 1 was 2.9% and we had anticipated that 3,551 children would enter the analysis. However, we had lower mortality than expected and though 3,622 children entered the analysis, we observed only 55 neonatal deaths. Since we had fewer events than expected, we did not have sufficient power to compare causes of death.

Statistical analyses

We compared baseline characteristics of children who were BCG-vaccinated by BHP during the neonatal period with children for whom a BCG vaccine obtained elsewhere was registered within 28 days of life, since these children BCG-vaccinated elsewhere would be censored at the visits. To assess whether the timing of our visit resulted in balanced distribution of background factors, we also compared baseline characteristics of children who were BCG-vaccinated by BHP during the neonatal period with children who had no BCG vaccine registered before day 28.

In Cox-proportional hazards models with age as underlying time scale and standard errors allowing for intragroup correlations within village cluster, we aimed to assess the effect of BCG versus no BCG on all-cause mortality, all-cause self-reported hospital admissions and all-cause self-reported

outpatient consultations during the neonatal period. Only children registered during pregnancy were included in the analyses. Children entered the analyses on the day after birth, thereby excluding the period immediately after birth where mortality is particularly high, and where it is unlikely to be feasible to affect neonatal health by BCG vaccination in a rural setting. Follow-up time was split after the first village visit by the BHP surveillance team where a BCG vaccine was offered. Thus, children can contribute time as both BCG unvaccinated and BCG vaccinated, but with age as underlying time scale, no child can be compared to itself. Children who were absent at the first visit or whose mother declined BCG vaccination entered the analyses in an intention to treat manner, contributing time in the BCG-vaccinated group after the first village visit, where the child could have been vaccinated. Some children would already be BCG vaccinated elsewhere prior to the village visit, but most children are not BCG vaccinated within the first month of life in rural Guinea-Bissau[6]. Children, who had received BCG vaccination elsewhere were censored from the analyses at the date of obtaining information about BCG vaccination, thus avoiding survival bias[19].

As stated in the study protocol, we excluded a) Children born at health centres and hospitals outside the village, since these larger health facilities were more likely to provide BCG at birth. As children were only counted as BCG-vaccinated after information was obtained, including these children would have added misclassification of vaccination status. b) Children of mothers travelling at both visits during the first two months of life, since in addition to vaccination status, it would not have been possible to obtain accurate information on timing of birth and outpatient consultations.

Since previous studies have demonstrated sex-differential NSEs[9], all analyses were stratified by sex. We had furthermore planned to evaluate whether the effect of BCG differed by age of vaccination. In three different analyses, we assessed whether receiving BCG before day 3, between day 4 and day 7 or after day 8 was associated with different survival. In all three analyses, we used BCG-unvaccinated as the reference groups allowing all children to enter the analysis at day 1, day 4 and day 8 in the three analyses.

For all outcomes, we assessed whether baseline factors changed the estimate by more than 5% by adjusting for the baseline factors one by one in the analyses. Since no baseline factor changed any of the main outcome estimates by more than 5%, no adjusted estimates are presented.

Sensitivity analyses

Children not present at the time of the first visit after birth were included in the main analysis in an intention to treat manner, contributing time as BCG-unvaccinated until village visit and BCG-vaccinated after village visit. In a sensitivity analysis, we censored these children at the time of visit, thus including only children BCG-vaccinated at the day of visit in the BCG-vaccinated group.

In Guinea-Bissau, BCG and oral polio vaccine (OPV) are scheduled to be given at birth, and the next vaccines are scheduled at 42 days of life. In sensitivity analyses, we prolonged follow-up to 42 days of life.

Explorative analyses

After the planning of the present study, but before any analyses were done, we decided to perform secondary analyses of the effect of BCG by maternal BCG-scar status, since recent studies have found stronger beneficial NSEs among children, where the mother was BCG-vaccinated[20 21]. In rural Guinea-Bissau, we are unlikely to obtain accurate information about maternal BCG-vaccination status, but a correctly administered BCG vaccine usually leaves a small scar[22], which can be used as a lifelong marker of a successful BCG vaccination. We therefore used maternal BCG-scar status as a proxy for a successful BCG vaccination.

It has previously been suggested that the effect of BCG vaccines differ by strain[23]. The majority of children BCG vaccinated by BHP received BCG-Denmark. In a sensitivity analysis, children who did not receive BCG-Denmark were censored at the time of village visit.

Ethics

The BHP rural HDSS was established in 1990, and the data collection by the mobile teams has been conducted at the request of the Ministry of Health in Guinea-Bissau. In rural Guinea-Bissau, only 38% of children are BCG vaccinated within 1 month of life[6]. Through the present project, we ensured a more timely delivery of BCG vaccines.

The study was approved by the Guinean Ethical Committee (Ref. 044/CNES/INASA/2012).

Results

A total of 6,197 pregnancies were registered between September 3, 2012 and July 27, 2016. Among these, 515 were still pregnant at end of study, 81 pregnancies resulted in miscarriage, 309 pregnancies resulted in stillbirth, and 56 women moved out of the study area before delivery. Excluding women who were still pregnant by the end of study. The stillbirth proportion among registered pregnancies was 5.4% (309/5682).

We excluded 46 children who died within 1 day after birth and 1,270 children born in health facilities outside the village. As specified in the protocol, we excluded 295 children, where the mother was not present at the first two visits after birth. Three children did not enter the analysis, as they were censored at the date of entry, leaving 3,622 children in the main analysis (Supplementary Figure).

Baseline characteristics

Most children (2237, 62%) were BCG-vaccinated by BHP within 28 days and only 9% (328) had received BCG elsewhere before the BHP visit. Compared with children who had received BCG vaccine prior to the BHP visit, children vaccinated by BHP were more likely to be born in the rainy season, and more likely to be born at home, mothers had attended fewer antenatal care visits and had less education, and lower socioeconomic status. Furthermore, region, ethnicity, and number of pregnancies differed between the groups (Table 1).

Effect of BCG on neonatal mortality

Among the 3,622 children contributing with observation time in the main analysis, 3,470 children contributed with time as BCG-unvaccinated, 152 children were vaccinated early and only contributed with time as BCG-vaccinated. In total, 2,506 children contributed with analysis time in the BCG-vaccinated group. The crude mortality rate (MR) of BCG-unvaccinated children was almost threefold higher than the MR of BCG-vaccinated children (MR of BCG-unvaccinated children: 293.1 per 1000 person years (PYRS), 45 deaths during 154 PYRS, and MR of BCG-vaccinated children: 99.1 per 1000 PYRS, 10 deaths during 101 PYRS) (Table 2). In a Cox-proportional hazards model with age as underlying time scale, the BCG-vaccinated children had a hazard ratio (HR) of 1.26 (95% Confidence Interval: 0.60-2.64) compared with BCG-unvaccinated children. Stratifying the results by sex, we found no significant difference between boys and girls (p-value=0.41) (Table 2).

The MRs of BCG-vaccinated and BCG-unvaccinated children showed less difference when children BCG-vaccinated at different ages were compared with BCG-unvaccinated children under follow-up in the corresponding analysis time. In all time windows, the MRs of BCG-unvaccinated children were higher than the MRs of BCG-vaccinated children (Supplementary Table 1).

The information on cause of death was scarce, and for more than half of the deaths (29 deaths), the information was insufficient to classify the cause of death. Among those with information on cause of death, most deaths were classified as due to infection, the proportion being higher among the older BCG-vaccinated infants (Supplementary table 2). Due to limited power, we did not compare causes of death in BCG-vaccinated and BCG-unvaccinated children.

Effect of BCG on neonatal hospital admissions

There were only 20 hospital admissions during the neonatal period. In BCG-unvaccinated children, the crude incidence rate (IR) of hospital admissions was 85.2 per 1000 PYRS (13 hospital admissions during 153 PYRS), while BCG-vaccinated children had an IR of 69.8 per 1000 PYRS (7 hospital admissions during 100 PYRS) (Table 3). Model control of the Cox-proportional hazards assumptions indicated that hazards were not proportional, and the low number of event limited the possibility of assessing piecewise proportionality, thus we could not compare the rates in an age-adjusted model.

Effect of BCG on neonatal outpatient consultations

We registered a total of 115 outpatient contacts occurring during the neonatal period. The IR of outpatient consultations was 494.0 per 1000 PYRS (77 outpatient consultations during 156 PYRS) in BCG-unvaccinated children, and 377.6 per 1000 PYRS (38 outpatient consultations during 101 PYRS) in BCG-vaccinated children. In a Cox-proportional hazards model, we obtained a HR of 0.90 (0.56-1.45) comparing BCG-vaccinated with BCG-unvaccinated children (Table 4). The effect of BCG did not differ between boys and girls (p=0.92).

Sensitivity analyses

Censoring follow-up at the first visit for the 279 children, who were not present and therefore not BCG-vaccinated at the visit, resulted in a similar effect of BCG on neonatal mortality (Supplementary Table 3).

In sensitivity analyses of all outcomes, we prolonged follow-up to 42 days of life, which resulted in similar effects as in the main analyses (Supplementary Table 4).

Effect of BCG by maternal BCG-scar status

In explorative analyses, we assessed the effect of BCG by maternal BCG-scar status. The mortality rates tended to be lower in children of mothers with a BCG scar than in children of mothers without a BCG scar, the hazard ratio being 0.68 (0.40-1.16) stratified for child BCG-vaccination status. However, there was no clear indication that maternal scar affected the morbidity (Supplementary table 5). Given the small number of events it was not surprising that we found no strong evidence that the effect of BCG vaccination of the child differed by maternal scar status.

The majority of children received BCG Denmark. Censoring children who did not receive BCG Denmark at the time of visit removed two deaths among the children who were not vaccinated on the date of visit (entered the analysis in an intention to treat analysis). The resulting HR was 1.38 (0.65-2.92) (Supplementary Table 3). We had no deaths among the 294 children who received other strains than BCG-Denmark.

Discussion

Main findings

We could not confirm the hypothesis of BCG having beneficial effects on neonatal mortality and morbidity: BCG appeared to be associated with slightly higher mortality (HR: 1.26 (0.60-2.64)) compared with BCG-unvaccinated children, and we found no effect of BCG on morbidity outcomes.

Strengths and limitations

The natural experiment design of the study was a major strength, since there was limited risk of self-selection to vaccination, which is often a problem in observational studies of vaccines[9]. With this design, the situation resembles the random allocation to BCG vaccination found in a randomised trial; however, aside from some children obtaining BCG elsewhere there is an important difference that might affect the results: With the natural-experiment setup, very few children are vaccinated on day 1 (at observation start). The BCG-vaccinated group increases in size over time as children get vaccinated, whereas the BCG-unvaccinated group decreases in size over time, thus, the distribution between the groups is very skewed (Figure 1). Since the mortality is very high in the first week of life and decreases rapidly during the neonatal period, the skewness between the two groups may become a problem in our natural experiment. Due to fewer children under risk in the BCG-vaccinated group early in life (when mortality is highest), a death among BCG-vaccinated children will carry more weight than among the greater number of BCG-unvaccinated children, when performing statistical analyses such as a Cox proportional hazards model. In a

randomised trial, this would not occur, as the group sizes would be balanced also in the period where mortality is particularly high.

The study was conducted within the setup of BHP's rural HDSS with experienced field assistants collecting data through monthly household visits. Aside from enabling the study design, the frequent visits also ensured high quality of data with little risk of misclassification of main events (deaths and hospital admissions). However, there are also some limitations: while a hard endpoint like death is very unlikely to be misclassified, the precision of the date may decrease with time since the event. It is likely that we do not have complete information about hospital admissions and outpatient consultations as these were self-reported outcomes, and some mothers may report any contact with a health facility as an outpatient consultation.

Consistency with other studies

Trials of low-weight children from urban Guinea-Bissau have shown that BCG-at-birth lowers neonatal mortality[12]. However, two large randomised trials from India recently found no effect of BCG on neonatal mortality[24]. The Indian trials used BCG-Russia, and the authors suggest that the strain of BCG may be part of the explanation why their results differ from previous findings[24]. In our study, we used mainly BCG-Denmark, which has also been used in the previous trials of BCG on neonatal mortality in Guinea-Bissau[12]. Censoring children who did not receive BCG-Denmark did not alter our conclusions. This suggests that strain of BCG is not the reason why we do not find a beneficial effect of BCG. However, taking into account the strain may not be sufficient as batch-to-batch variation of the same strain may also contribute to explaining variability and BCG-Denmark clearly had variations before production was stopped[25]. Thus, the importance of strain of BCG should be explored further.

Our natural experiment had little power to assess effects on hospital admissions, an outcome which was rarer than death in the present setting. A combined analysis of trials from urban Guinea-Bissau, has recently found no effect of BCG on hospital admissions during the neonatal period[26].

Previous studies suggest that maternal BCG may prime the effect of BCG in children[20 21]. We found a tendency towards lower mortality among children of mothers with a BCG scar, but with the limited number of deaths among BCG-vaccinated children, we had no power to test whether maternal BCG primed the response in BCG-vaccinated children.

Interpretation

Our analyses indicated no beneficial effect of BCG on neonatal mortality and morbidity. However, power was low, and despite the strength of limiting the risk of self-selection to vaccination and healthy vaccine bias in the study, we are not able to make firm conclusions of the effect of BCG on the neonatal outcomes assessed. The effect of BCG on all-cause mortality in early life should be further assessed in randomised trials.

Conclusion

BCG-vaccinated children had lower crude mortality rates, hospital admission rates and outpatient-consultation rates in the neonatal period compared with BCG-unvaccinated children. This pattern was not reflected in the Cox analyses, where we found that BCG was associated with slightly higher mortality, and no effect on morbidity.

Authors' contributions

ABF designed the study with inputs from PA. SMT, SB, KMF, CC, IDSB, and ABF supervised the data collection and data entry. SMT, KMF and CC cleaned the data. SMT analysed the data, and wrote the first manuscript with inputs from CCJ, PA, and ABF. All authors received and approved the final manuscript.

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Conflicts of interest

All authors have no interests to declare.

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Figure 1. Observation time under study as BCG-unvaccinated and BCG-vaccinated

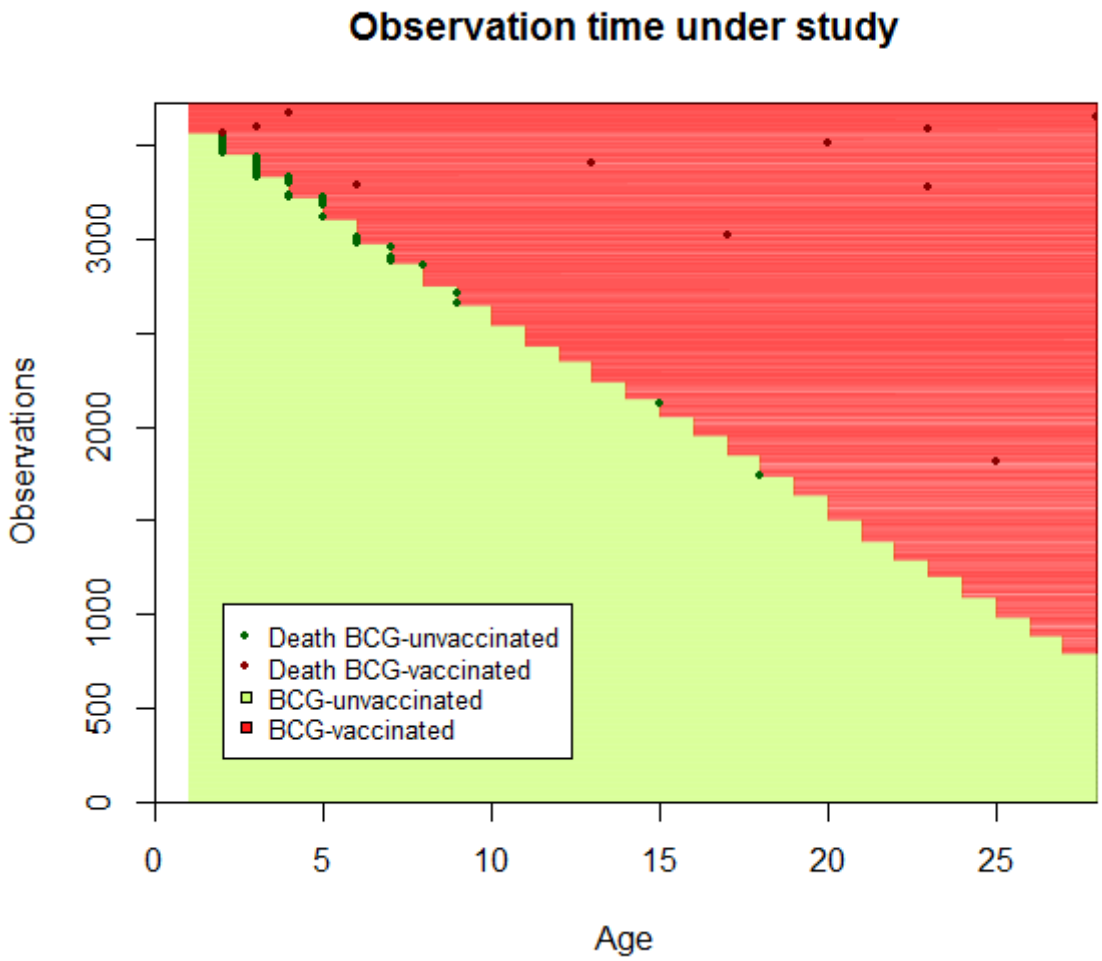


Table 1. Baseline characteristics according to BCG-vaccination status in neonatal period.

	BCG-vaccinated by BHP before 28 days of life	BCG vaccine registered before 28 days of life	No BCG vaccine register by day 28 of life	
	n (%)	n (%)	n (%)	p-value[1]
Number	2237 (61.7%)	328 (9.1%)	1058 (29.2%)	
Sex[2]				0.94
Male	1125 (50.3%)	162 (49.4%)	526 (49.9%)	
Female	1112 (49.7%)	166 (50.6%)	528 (50.1%)	
Twin				0.36
Yes	63 (2.8%)	14 (4.3%)	32 (3.0%)	
No	2174 (97.2%)	314 (95.7%)	1026 (97.0%)	
Season of birth				0.01
Dry season	979 (43.8%)	173 (52.7%)	493 (46.6%)	
Rainy season	1258 (56.2%)	155 (47.3%)	565 (53.4%)	
Birth location[3]				<0.0001
Home	1909 (86.3%)	202 (62.7%)	804 (78.5%)	
Health centre	248 (11.2%)	93 (28.9%)	160 (15.6%)	
Hospital	33 (1.5%)	19 (5.9%)	29 (2.8%)	
Other	22 (1.0%)	8 (2.5%)	31 (3.0%)	
Antenatal consultations[4]				<0.0001
0	358 (16.9%)	23 (7.2%)	148 (21.4%)	
1 - 2	896 (42.3%)	94 (29.5%)	239 (34.6%)	
3 +	862 (40.7%)	202 (63.3%)	303 (43.9%)	
Number of pregnancies[5]				.01
1	99 (5.2%)	16 (5.6%)	63 (7.1%)	
2 - 3	492 (25.8%)	99 (34.7%)	235 (26.5%)	
4 or more	1314 (69.0%)	170 (59.6%)	588 (66.4%)	
Maternal MUAC[6]				0.76
1st quartile (<244mm)	281 (18.1%)	34 (15.7%)	121 (17.5%)	
2nd quartile (244-257 mm)	337 (21.7%)	48 (22.2%)	159 (23.0%)	
3rd quartile (258-275 mm)	432 (27.9%)	66 (30.6%)	176 (25.5%)	
4th quartile (>275 mm)	500 (32.3%)	68 (31.5%)	235 (34.0%)	
Maternal BCG scar[7]				0.44
Yes	1070 (53.6%)	166 (57.2%)	529 (55.0%)	
No	928 (46.4%)	124 (42.8%)	433 (45.0%)	

Maternal age[8]				0.50
1st quartile (<22 years)	494 (22.3%)	73 (22.8%)	246 (23.5%)	
2nd quartile (22-25 years)	609 (27.4%)	99 (30.9%)	289 (27.6%)	
3rd quartile (26-30 years)	523 (23.6%)	77 (24.1%)	259 (24.7%)	
4th quartile (>30 years)	593 (26.7%)	71 (22.2%)	255 (24.3%)	
Education of mother[9]				<0.0001
0 year	1195 (57.1%)	119 (38.9%)	527 (53.4%)	
1-4 years	523 (25.0%)	97 (31.7%)	269 (27.3%)	
+4 years	373 (17.8%)	90 (29.4%)	191 (19.4%)	
Region				<0.0001
Oio	760 (34.0%)	62 (18.9%)	365 (34.5%)	
Biombo	639 (28.6%)	128 (39.0%)	236 (22.3%)	
Cacheu	420 (18.8%)	90 (27.4%)	235 (22.2%)	
Sao Domingos	418 (18.7%)	48 (14.6%)	222 (21.0%)	
Ethnicity[10]				<0.0001
Pepel	523 (23.4%)	118 (36.0%)	212 (20.0%)	
Balanta	885 (39.6%)	99 (30.2%)	386 (36.5%)	
Mandinga / Fula	443 (19.8%)	36 (11.0%)	205 (19.4%)	
Manjaco / Mancanha	181 (8.1%)	53 (16.2%)	143 (13.5%)	
Socioeconomic factors				
Type of roof[11]				0.16
Hard	830 (37.3%)	106 (32.6%)	367 (34.9%)	
Straw	1398 (62.7%)	219 (67.4%)	685 (65.1%)	
Toilet[12]				<0.0001
Yes	1327 (59.7%)	230 (70.6%)	654 (62.3%)	
No	896 (40.3%)	96 (29.4%)	396 (37.7%)	
Telephone[13]				<0.0001
Yes	1054 (48.5%)	189 (59.1%)	528 (52.0%)	
No	1120 (51.5%)	131 (40.9%)	488 (48.0%)	
Radio[14]				0.03
Yes	1720 (78.2%)	272 (84.7%)	812 (78.5%)	
No	480 (21.8%)	49 (15.3%)	223 (21.5%)	
Generator				0.63
Yes	371 (16.7%)	49 (15.0%)	181 (17.3%)	
No	1848 (83.3%)	277 (85.0%)	865 (82.7%)	

[1] P-value calculated by chi square test of the distribution of baseline characteristics in the three vaccination groups

[2] Missing information for 4 children with no BCG registered at day 28

[3] Missing information for 25 children BCG vaccinated by BHP, 6 children BCG vaccinated elsewhere, and 34 children with no BCG registered at day 28
[4] Missing information for 121 children BCG vaccinated by BHP, 9 children BCG vaccinated elsewhere, and 368 children with no BCG registered at day 28
[5] Missing information for 332 children BCG vaccinated by BHP, 43 children BCG vaccinated elsewhere, and 172 children with no BCG registered at day 28
[6] Missing information for 687 children BCG vaccinated by BHP, 112 children BCG vaccinated elsewhere, and 367 children with no BCG registered at day 28
[7] Missing information for 239 children BCG vaccinated by BHP, 38 children BCG vaccinated elsewhere, and 96 children with no BCG registered at day 28
[8] Missing information for 18 children BCG vaccinated by BHP, 8 children BCG vaccinated elsewhere, and 9 children with no BCG registered at day 28
[9] Missing information for 146 children BCG vaccinated by BHP, 22 children BCG vaccinated elsewhere, and 71 children with no BCG registered at day 28
[10] Missing information for 205 children BCG vaccinated by BHP, 22 children BCG vaccinated elsewhere, and 112 children with no BCG registered at day 28
[11] Missing information for 9 children BCG vaccinated by BHP, 3 children BCG vaccinated elsewhere, and 6 children with no BCG registered at day 28
[12] Missing information for 14 children BCG vaccinated by BHP, 2 children BCG vaccinated elsewhere, and 8 children with no BCG registered at day 28
[13] Missing information for 63 children BCG vaccinated by BHP, 8 children BCG vaccinated elsewhere, and 42 children with no BCG registered at day 28
[14] Missing information for 37 children BCG vaccinated by BHP, 7 children BCG vaccinated elsewhere, and 23 children with no BCG registered at day 28
[15] Missing information for 18 children BCG vaccinated by BHP, 2 children BCG vaccinated elsewhere, and 12 children with no BCG registered at day 28

Table 2. Neonatal mortality according to BCG-vaccination status for all children, and by sex.

	Number of observations n	MR per 1000 PYRS[1] (deaths / PYRS)	Hazard ratio[2] (95% CI)
All children			
BCG-unvaccinated children	3470	293.1 (45/154)	Ref
BCG-vaccinated children	2506	99.1 (10/101)	1.26 (0.60-2.64)
Boys[3]			
BCG-unvaccinated children	1734	351.4 (27/77)	Ref
BCG-vaccinated children	1253	99.7 (5/50)	1.03 (0.42-2.53)
Girls[3]			
BCG-unvaccinated children	1732	222.0 (17/77)	Ref
BCG-vaccinated children	1252	98.5 (5/51)	1.64 (0.63-4.27)

[1] MR: Mortality rate, PYRS: Person years

[2] Hazard ratio estimated in a Cox-proportional hazards model with age as underlying time scale, allowing different baseline hazards by sex, and with confidence intervals adjusted for village cluster using robust standard errors.

[3] 4 children had missing information on sex

Table 3. Neonatal hospital admissions according to BCG-vaccination status for all children, and by sex.

	Number of observations n	IR per 1000 PYRS[1] hospital admissions / PYRS)
All children		
BCG-unvaccinated children	3457	85.2 (13/153)
BCG-vaccinated children	347	69.8 (7/100)
Boys[2]		
BCG-unvaccinated children	1730	130.8 (10/76)
BCG-vaccinated children	1248	60.1 (3/50)
Girls[2]		
BCG-unvaccinated children	1723	39.4 (3/76)
BCG-vaccinated children	1240	79.6 (4/50)

[1] IR: Incidence rate, PYRS: Person years

[2] 4 children had missing information on sex

Table 4. Neonatal outpatient consultations according to BCG-vaccination status for all children, and by sex.

	Number of observations n	IR per 1000 PYRS[1] (consultations / PYRS)	Hazard ratio[2] (95% CI)
All children			
BCG-unvaccinated children	3553	494.0 (77/156)	Ref
BCG-vaccinated children	2498	377.6 (38/101)	0.90 (0.56-1.45)
Boys[3]			
BCG-unvaccinated children	1775	589.8 (46/78)	Ref
BCG-vaccinated children	1243	444.1 (22/50)	0.89 (0.48-1.63)
Girls[3]			
BCG-unvaccinated children	1774	398.7 (31/78)	Ref
BCG-vaccinated children	1254	313.3 (16/51)	0.92 (0.52-1.63)

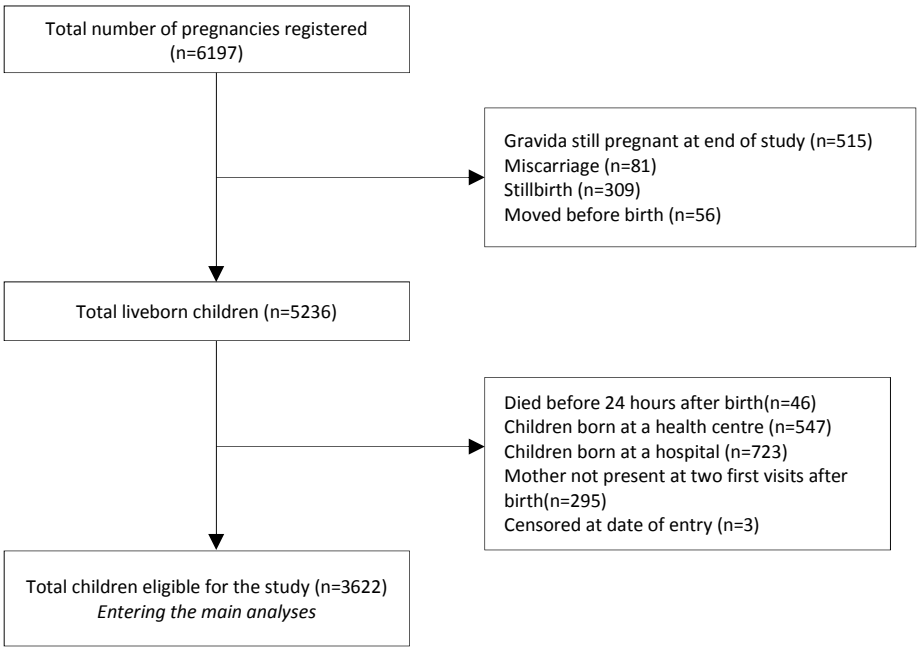
[1] IR: Incidence rate, PYRS: Person years

[2] Hazard ratio estimated in a Cox-proportional hazards model with age as underlying time scale, allowing different baseline hazards by sex, and with confidence intervals adjusted for village cluster using robust standard errors.

[3] 4 children had missing information on sex

Supplementary material for "Effect of early BCG vaccination on neonatal mortality and morbidity: a natural experiment"

Supplementary Figure. Flowchart of study participants.



Supplementary table 1. Mortality of BCG-vaccinated and BCG-unvaccinated children by age of BCG administration.

	Number of observations n	MR per 1000 PYRS[1] (deaths / PYRS)	Hazard ratio[2] (95% CI)
BCG-unvaccinated day 1-	3470	293.1 (45/154)	Ref
BCG-vaccinated day 1-3[3]	349	245.0 (6/24)	1.45 (0.59-3.57)
BCG-unvaccinated day 4-	3133	127.0 (16/126)	Ref
BCG-vaccinated day 4-7	410	120.3 (3/25)	1.54 (0.43-5.49)
BCG-unvaccinated day 8-	2668	42.7 (4/94)	Ref
BCG-vaccinated day 8-28	1747	19.4 (1/52)	0.52 (0.07-3.85)

[1] MR: Mortality rate, PYRS: Person years

[2] Hazard ratio estimated in a Cox-proportional hazards model with age as underlying time scale, allowing different baseline hazards by sex, and with confidence intervals adjusted for village cluster using robust standard errors.

[3] A child vaccinated on day 0 enters the analysis on day 1 in the BCG group

Supplementary table 2. Classified causes of deaths[1] according to BCG-vaccination status.

	BCG-unvaccinated children	BCG-vaccinated children
Infectious diseases	13 (29%)	5 (50%)
Preterm birth / low-birth weight	5 (11%)	1 (10%)
Congenital malformations	1 (2%)	0 (0%)
Other[2]	1 (2%)	0 (0%)
Missing/insuffient information	25 (56%)	4 (40%)

[1] Cause of death classified using information collected during an interview conducted when registering the death

[2] 1 child died due to respiratory failure

Supplementary table 3. Neonatal mortality in BCG-vaccinated and BCG-unvaccinated children in sensitivity analyses a) censoring children not present at first visit, b) including children born in health facilities, and c) censoring children who did not receive BCG Denmark at the BHP visit

	Number of observations	MR per 1000 PYRS[1] (deaths / PYRS)	Hazard ratio[2] (95% CI)
Censoring children not present at first visit			
BCG-unvaccinated children	3470	293.1 (45/154)	Ref
BCG-vaccinated children	2233	99.5 (9/90)	1.24 (0.58-2.64)
Including children born in health facilities			
BCG-unvaccinated children	4785	346.5 (74/214)	Ref
BCG-vaccinated children	3254	113.4 (15/132)	1.38 (0.75-2.56)
Censoring children not receiving BCG Denmark at date of visit			
BCG-unvaccinated children	3470	293.1 (45/154)	Ref
BCG-vaccinated children	1949	114.0 (9/79)	1.38 (0.65-2.92)

[1] MR: Mortality rate, PYRS: Person years

[2] Hazard ratio estimated in a Cox-proportional hazards model with age as underlying time scale, allowing different baseline hazards by sex, and with confidence intervals adjusted for village cluster using robust standard errors.

[3] Among the 2233 children BCG vaccinated by BHP at the first visit after birth, 1949 received BCG Denmark. All other children were censored on the date of first visit after birth.

Supplementary table 4. The effect of BCG on mortality, hospital admissions and outpatient contacts in children between day 1 and day 42.

	Number of observations	MR/IR per 1000 PYRS[1] (event / PYRS)	Hazard ratio[2] (95% CI)
Mortality for children day 1 - day 42			
BCG-unvaccinated children	3470	278.9 (46/165)	Ref
BCG-vaccinated children	2955	91.6 (19/207)	1.20 (0.56-2.57)
Hospital admissions for children day 1 - day 42			
BCG-unvaccinated children	3457	79.3 (13/164)	NA
BCG-vaccinated children	2933	38.9 (8/206)	NA
Outpatient consultations for children day 1 - day 42			
BCG-unvaccinated children	3553	487.9 (82/168)	Ref
BCG-vaccinated children	2945	547.1 (112/205)	1.04 (0.70-1.56)

[1] MR: Mortality rate, IR: Incidence rate, PYRS: Person years

[2] Hazard ratio estimated in a Cox-proportional hazards model with age as underlying time scale, allowing different baseline hazards by sex, and with confidence intervals adjusted for village cluster using robust standard errors.

Supplementary table 5. Mortality, hospital admission, and outpatient consultations in the neonatal period by maternal BCG-scar status and BCG-vaccination status.

	Number of observations	MR per 1000 PYRS[1]	Hazard ratio[2]
		(deaths / PYRS)	(95% CI)
Maternal BCG scar			
BCG-unvaccinated children	1698	268.4 (20/75)	Ref
BCG-vaccinated children	1216	60.6 (3/50)	0.95 (0.27-3.42)
No maternal BCG scar			
BCG-unvaccinated children	1416	347.2 (22/63)	Ref
BCG-vaccinated children	1026	122.5 (5/41)	1.38 (0.51-3.68)
Hospital admission for children by maternal scar status			
	Number of observations	IR per 1000 PYRS	Hazard ratio
		(hospital admissions / PYRS)	(95% CI)
Maternal BCG scar			
BCG-unvaccinated children	1689	108.2 (8/74)	Ref
BCG-vaccinated children	1204	81.6 (4/49)	NA
No maternal BCG scar			
BCG-unvaccinated children	1414	79.2 (5/63)	Ref
BCG-vaccinated children	1021	73.9 (3/41)	NA
Consultations for children by maternal scar status			
	Number of observations	IR per 1000 PYRS	Hazard ratio
		(consultations / PYRS)	(95% CI)
Maternal BCG scar			
BCG-unvaccinated children	1744	487.3 (37/76)	Ref
BCG-vaccinated children	1216	403.2 (20/50)	0.96 (0.50-1.84)
No maternal BCG scar			
BCG-unvaccinated children	1447	576.0 (37/64)	Ref
BCG-vaccinated children	1018	396.0 (16/40)	0.79 (0.42-1.48)

[1] MR: Mortality rate, IR: Incidence rate, PYRS: Person years

[2] Hazard ratio estimated in a Cox-proportional hazards model with age as underlying time scale, allowing different baseline hazards by sex, and with confidence intervals adjusted for village cluster using robust standard errors.

Paper IV

Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children

Sanne M. Thysen, Christine S. Benn, Victor F. Gomes, Frauke Rudolf, Christian Wejse, Adam Roth, Per Kallestrup, Peter Aaby, Ane B. Fisker (manuscript)

Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children: an observational study

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Word count: Abstract: 240, Manuscript: 3444

Abstract

Background

WHO recommends BCG vaccination at birth in countries with high tuberculosis (TB) burden. Unfortunately, BCG vaccination is often delayed. Since BCG, aside from the TB protection, has beneficial non-specific effects, delays in BCG vaccination may be important even among TB-unexposed children.

Methods

Bandim Health Project runs an urban Health and Demographic Surveillance site in Guinea-Bissau with registration of mortality, vaccination status and TB cases. We followed children between 28 days and 3 years of life. Children residing in the same house as a TB case were classified as TB-exposed from 3 months prior to case registration to the end of follow-up.

Using Cox-proportional hazards models with age as underlying time scale, we compared mortality of children with and without neonatal BCG between October 2003 and September 2017.

Findings

Among the 39,105 children who entered the analyses, 2,964 (7.6%) had observation time as TB-exposed. In total, 84% of children received neonatal BCG. Children with neonatal BCG had lower mortality both in TB-exposed (HR: 0·35 (95%CI: 0·17-0·71)) and in TB-unexposed children (HR: 0·55 (95% CI: 0·46-0·67)) than children without neonatal BCG. Adjusting for potential confounders did not alter the conclusions.

Interpretation

Neonatal BCG vaccination was associated with lower mortality among both TB-exposed and TB-unexposed children, consistent with neonatal BCG vaccination having beneficial non-specific effects. Interventions to increase timely BCG vaccination are urgently warranted.

Funding

Karen Elise Jensen Foundation, Augustinus Foundation, Novo Nordisk Foundation, DANIDA, and the Danish National Research Foundation (DNRF108).

Research in context

Evidence before this study

We searched PubMed for literature about the effects of BCG on all-cause mortality up to December 2018 using the search terms “BCG vaccine”, “child mortality”, “mortality” and “children”. The current literature suggest that BCG, in addition to the protection against tuberculosis (TB), protects against all-cause mortality. This has been coined the beneficial non-specific effects of BCG. Only few studies have assessed the effect of age of BCG vaccination on all-cause mortality, as most studies compare BCG versus no BCG. BCG is recommended at birth in TB endemic areas, but it has been demonstrated in several countries, that BCG is often given with delay. This delay is currently not captured by administrative coverage estimates. To our knowledge, no study has assessed the effect of BCG age on all-cause mortality in TB-exposed and TB-unexposed children. If age of BCG vaccination is important, identifying and removing barriers to timely BCG is essential.

Added value of this study

We compared all-cause mortality of children who had received neonatal BCG with mortality of children who had not received neonatal BCG among TB-exposed and TB-unexposed children, respectively. Neonatal BCG was associated with lower mortality in TB-exposed and TB-unexposed children. The results were robust to sensitivity analyses. Our results support that timing of BCG is important, both in children exposed to TB, but also in children not exposed to TB. This stresses the importance of timely BCG for all children, and supports that BCG has non-specific effects.

Implications of all the available evidence

Our results emphasize the importance of timely BCG vaccination. Neonatal BCG vaccination should be reported in addition to the currently reported vaccination coverage estimates to provide incentive for timely BCG vaccination. Delays in BCG vaccination should be avoided to ensure that no child is deprived of the early TB-specific and non-specific effects of timely BCG vaccination.

Background

Bacillus Calmette-Guérin (BCG) vaccine was developed to protect against tuberculosis (TB), and remains the only approved TB vaccine[1]. The efficacy of the BCG vaccine to protect against TB has varied between different trials[2]. However, trials of neonatal BCG vaccination consistently find that BCG is associated with reduced TB incidence[2].

BCG is recommended at birth in countries with high burden of TB[1]. According to WHO/UNICEF estimates, 88 percent of children are BCG vaccinated in countries where BCG is part of the routine vaccination programme[3]. However, WHO/UNICEF coverage estimates are based on coverage at 12 months of age, and BCG is often delayed in low-income countries[4,5]. If delayed BCG vaccination is associated with lower vaccine efficacy, delays may be critical.

Additionally, there is increasing evidence that BCG has non-specific effects (NSEs) on mortality. That is, BCG may affect mortality by more than can be explained by the protection against TB[6,7]. Three randomised trials in low-weight children in Guinea-Bissau found that BCG-Denmark vaccination at birth was associated with 38% (17-54%) lower neonatal mortality compared with children not BCG vaccinated at birth[8]. Already 3 days after enrolment, BCG was associated with 45% (7-68%) lower mortality compared with unvaccinated children[8], suggesting that even small delays in BCG vaccination may be important for survival.

A potential effect of early BCG on childhood mortality could be due to prevention of TB, NSEs of BCG or a combination. If the effect of BCG was merely specific, we should expect strong effects among children exposed to TB[9] and no effect in children not exposed to TB.

TB is difficult to diagnose, especially in children, and many TB cases are not diagnosed and treated[10,11]. Tuberculin skin test (TST) with purified protein derivatives (PPD) is commonly used to test for latent infection with *M. Tuberculosis*[12]. A response to PPD can be due to either *M. Tuberculosis* infection or BCG vaccination; however, BCG vaccination have been shown to result in lower PPD response compared with latent TB infection[12].

The main objective of this study was to assess the association between neonatal BCG versus later BCG vaccination and mortality by registered exposure to TB. We furthermore assessed the association between neonatal BCG vaccination and positive TST reactions by registered exposure to TB.

Methods

Setting and study population

Bandim Health Project (BHP) runs a Health and Demographic Surveillance System (HDSS) in six suburban districts in Bissau, the capital of Guinea-Bissau. Children are followed through tri-monthly home visits until 3 years of age. At the home visits, a field assistant collects information on vital status, measures mid-upper-arm circumference (MUAC) and registers vaccination status by transcribing information from the child's vaccination card. Each month, fieldwork assistants conduct home visits to follow-up on pregnant women and register new births. Children above 3 years of age and adults are followed through censuses conducted every 2-5 years.

The BCG vaccines used in Guinea-Bissau have been provided by UNICEF and mainly the Russian strain has been used. During large periods within the study period, however, the BHP provided vaccines for the study area; these were BCG-Denmark strain purchased at the Statens Serum Institut, Denmark.

Since 1996, all diagnosed TB cases living in the study area have been registered and followed[13]. In 2003, a register of information on all TB patients from the study area was established, making it possible to identify houses exposed to TB. We defined a house as exposed to TB from 3 months prior to diagnosis of a TB case in the house until 2 weeks after diagnosis. In Guinea-Bissau, more than one household usually shares a house, and the houses are constructed with a common roof, no ceiling and the walls separating the rooms do not reach the roof. Much of the everyday life takes place at the veranda of the house. Thus, children living in a house with a TB case were classified as TB-exposed, also if the TB case was from a different household within the same house. Children were classified as TB-exposed from the first registered TB exposure and remained classified as TB-exposed throughout the follow-up period. We expect some TB cases to be undiagnosed. Thus, some children classified as TB-unexposed may have been exposed.

Between September 1, 2005 and October 31, 2007, and between January 18, 2011 and August 20, 2013, studies of the effect of preventive treatment to TB-exposed children were conducted in the BHP study area[14,15]. We excluded these periods for all children in the present study (Figure 1). Thus, we included children who had their vaccination status assessed after 28 days of age between October 28, 2003 (Start of the TB registry) and September 15, 2017 excluding periods with studies of preventive TB treatment. From July 2002 to May 2006, a randomised trial of BCG-revaccination at 19 months of age was conducted in the study area, we therefore censored follow-up at 19 months of age for children eligible for the BCG-revaccination trial[16].

Between start of study and July 1, 2008, a sample of children living in the study area were TST tested using the Mantoux method with an intradermal application of 0·1 ml of PPD (2 tuberculin units RT23, Statens Serum Institut, Denmark) in the forearm at ages 6 and 12 months. The PPD reactions were measured after 48-72 hours using a ruler and ballpoint technique to measure two diameters[17]. Among children with measured PPD reactions, we assessed the effect of neonatal BCG vaccination versus later BCG vaccination on PPD reactions using cut-offs of 10mm and 15mm.

Statistical analyses

We compared baseline characteristics of children with and without neonatal BCG in TB-exposed and TB-unexposed children using chi^2 and paired t-tests. We also compared baseline characteristics of children registered and not registered as TB-exposed.

In Cox-proportional hazards models with age as underlying time scale, we compared mortality rates of children with neonatal BCG with mortality rates of children without neonatal BCG (delayed BCG or no BCG) separately for TB-exposed and TB-unexposed children, allowing for different baseline hazards according to sex and place of birth (maternity ward, health centre or home). Children entered the analysis the first time their vaccination status was assessed at a home visit after the neonatal period. Observation time was split at first registered TB exposure and thus, TB-exposed children could contribute with observation time in the TB-unexposed group until TB exposure. To control for potential confounding, we assessed whether baseline characteristics

changed the estimate by including the factors in the analysis one by one. We adjusted for baseline characteristics that changed the estimate by more than 5%.

In a secondary analysis, we explored whether the effect of neonatal BCG differed by timing of BCG within the neonatal period, thus we divided children with neonatal BCG in two groups, children with early neonatal BCG (vaccinated within 7 days after birth), and children with late neonatal BCG (vaccinated between day 8 and day 28).

In sensitivity analyses, we: A) Excluded all children, who potentially had been exposed to preventive TB treatment as part of the aforementioned studies (Supplementary Figure 1). B) Restricted the analysis to children with a registered BCG vaccine (i.e. allowing a child only to contribute time at risk from the first visit at which a BCG vaccine was registered, therefore, children with no registered BCG vaccine would not enter the analysis). C) Extended follow-up to 5 years of age. D) Excluded twins, as these are more likely to be low-birth-weight (LBW) children and thus receive delayed BCG. E) Stratified the analysis by LBW status for the subset of children for whom we had information on birthweight.

BCG vaccination may cause a transient weak response to PPD[18], but reactions above 15mm would be less likely to be caused by BCG[19]. Using log-binomial regression, we compared the prevalence of a positive TST in children with neonatal BCG with children without neonatal BCG among TB-exposed and TB-unexposed children, respectively. We limited comparison to children BCG vaccinated prior to TST assessment and evaluated PPD reaction at age 6 and 12 months using cut-offs of 10mm and 15mm.

Ethical considerations

Most data was obtained from the routine data collection in the urban HDSS of the BHP. The BHP data collection was initiated at the request of the Ministry of Health in Guinea-Bissau. Oral consent was obtained from mother/guardian of the children prior to TST.

Results

A total of 39,105 children contributed time at risk, and among these 2,964 had observation time while living in houses of registered TB cases. Among children with a vaccination card seen after 28 days, 32,938 (84%) had received neonatal BCG. The median age of vaccination in the neonatal BCG group was 2 days (interquartile range (IQR): 1-10). Among the 6,167 children not BCG vaccinated in the neonatal period, 5,498 (89%) had a BCG vaccine registered at some point of time during the follow up period (median age of vaccination: 48 days (IQR: 36-68)).

Baseline characteristics

We compared baseline characteristics of children with and without neonatal BCG according to TB-exposure status. In this large dataset, most statistically significant differences were small absolute differences. In both groups, mothers of children with neonatal BCG were better educated, older, and had better socioeconomic status (toilet, electricity, among not TB-exposed children also type of roof). Children with neonatal BCG were more likely to be born at a health facility and less likely to be twins. Ethnic groups also varied between children with and without neonatal BCG. Among TB-unexposed children, the distribution of season of birth, year of birth, number of pregnancies and

suburb differed between neonatal BCG vaccinated and not neonatal BCG vaccinated children (Table 1).

Several baseline characteristics were statistically significant different between TB-exposed and TB-unexposed children (birth location, number of pregnancies, maternal age, suburb, ethnicity, and socioeconomic factors) (Table 2).

Effect of neonatal BCG on mortality among children registered as TB-exposed

We included 2,964 TB-exposed children in the analyses, among these 2,566 (87%) were neonatally BCG vaccinated, whereas 398 (13%) were not. TB-exposed children with neonatal BCG had a mortality rate (MR) of 11·4 per 1000 person years (PYRS, 27 deaths during 2,377 PYRS), and children without neonatal BCG (delayed or no BCG) had a MR of 33·9 per 1000 PYRS (12 deaths during 354 PYRS). The hazard ratio (HR) comparing TB-exposed children with and without neonatal BCG was 0·28 (95% CI: 0·13-0·57). Adjusted for the baseline characteristics that changed the estimate by more than 5% resulted in an adjusted hazard ratio (aHR) of 0·35 (0·17-0·71) (Table 3). The effect of neonatal BCG among TB-exposed children did not differ according to sex (p-value=0·44) (Table 4).

The mortality was lowest among children with early neonatal BCG (aHR compared with children with no neonatal BCG: 0·24 (0·10-0·57)), but a lower mortality was also observed among children with late neonatal BCG (aHR of 0·53 (0·22-1·26)) (Supplementary table 1).

Extending follow-up to 5 years of age or excluding children with no registered BCG vaccination, or excluding twins from the analysis did not alter the conclusions (Supplementary table 2). Excluding children who had potentially been eligible for studies of preventive TB treatment (Supplementary figure 1) resulted in an aHR of 0·60 (95% CI: 0·21-1·72) (Supplementary table 2). Stratifying the analysis by LBW status (<2500g), had limited power (Supplementary table 3).

Effect of neonatal BCG on mortality among children registered as TB-unexposed

We included 37,824 children classified as TB-unexposed in this analysis, 31,836 (84%) were neonatal BCG vaccinated, and 5,988 (16%) were not. Children with neonatal BCG vaccination had a MR of 13·1 per 1000 PYRS (498 deaths during 37,943 PYRS) and not neonatal BCG vaccinated children had a MR of 22·7 per 1000 PYRS (148 deaths during 6,530 PYRS). Children with neonatal BCG had 45% lower mortality (HR: 0·55 (95% CI: 0·46-0·67) compared with not neonatal BCG vaccinated children (Table 3). No background factor changed the estimate by more than 5%, and thus adjusted estimates are not presented. The effect of neonatal BCG did not differ by sex (p=0·93) (Table 4).

The effect of BCG age was similar within the neonatal period: HR: 0·58 (0·47-0·73) for children with early neonatal BCG compared with no neonatal BCG and HR: 0·53 (0·42-0·66) for children with late neonatal BCG (Supplementary table 1).

Extending follow-up to 5 years of age, excluding children with no registered BCG vaccine, excluding twins from the analysis or excluding children potentially eligible for studies of preventive TB treatment (Supplementary figure 1) did not alter the conclusions (Supplementary table 2). Stratifying the analysis by LBW status, indicated that neonatal BCG was associated with an HR of

0.66 (0.41-1.05) in LBW children, and an HR of 0.75 (0.49-1.15) in NBW children (Supplementary table 3).

Mortality in TB-exposed and TB-unexposed children according to vaccination status

We also examined the effect of being TB-exposed on mortality among children with neonatal BCG and children without neonatal BCG, respectively. Among children with neonatal BCG, TB-exposed children had similar mortality as TB-unexposed children (HR: 1·09 (0·74-1·60)). Among not neonatally BCG-vaccinated children, TB-exposed children had higher mortality (HR: 1·92 (1·06-3·46)) (p=0·12 for interaction between neonatal BCG and TB-exposure, Table 5).

We explored whether the effect differed for boys and girls. TB-exposure was associated with higher mortality for girls both with and without neonatal BCG (HR: 1·53 (0·95-2·47) and 2·13 (0·92-4·89), respectively). In boys, TB-exposure tended to be associated with higher mortality for children without neonatal BCG (HR: 1·75 (0·76-4·01)), whereas this was not the case for children with neonatal BCG (HR: 0·69 (0·36-1·35)) (Table 5).

Tuberculin skin test reactions

Among 57 TB-exposed children with a TST assessed at 6 months, a total of 4 (7·5%) children (all with neonatal BCG) had a PPD reaction above 10mm. Only seven TB-exposed children had a TST assessed at 12 months and no child had a PPD reaction above 15mm (Supplementary Table 4).

Among 1379 TB-unexposed children with assessed TST at 6 months, neonatal BCG did not affect PPD reactions; among the 335 children with TST assessed at 12 months fewer children with neonatal BCG had large TSTs (Supplementary Table 4).

Discussion

Main findings

Neonatal BCG was associated with lower mortality among both TB-exposed (aHR: 0·35 (95%CI: 0·17-0·71)) and TB-unexposed children (HR: 0·55 (95%CI: 0·46-0·67)), resulting in a combined HR of 0.53 (0.44-0.64), not adjusted as no factor changed the estimate by more than 5%. The results were robust to sensitivity analyses of longer follow-up, exclusion of children with no registered BCG vaccine, exclusion of children who were twins and exclusion of children potentially eligible for studies of preventive TB treatment. Children exposed to TB had higher mortality than TB-unexposed children if they had not received neonatal BCG.

Strengths and weaknesses

The study was conducted within the setup of Bandim Health Project's urban HDSS that since 1978 has followed the population in the study area with regular home visits to collect information on health status of children. Information on vaccination status and vital status is collected by experienced field assistants at home visits every third month, and hard endpoints like death are therefore unlikely to be misclassified. Only children with assessed vaccination status after 28 days of life were included in the analysis, and children only entered the analyses when the vaccination status was assessed at a home visit, thus avoiding differential misclassification of vaccination status and survival bias.

To remove potential confounding, we controlled for baseline characteristics that changed the estimate by more than 5% when included in the analyses one by one. However, there may be residual confounding not accounted for, and the results should be interpreted with caution.

The registration of diagnosed TB cases from the study area allows for classifying children as TB-exposed. However, some TB cases will be undiagnosed, and some TB-exposed children may therefore have been misclassified as TB-unexposed. We expect any misclassification to be independent of timing of BCG vaccination. Unfortunately, we did not have HIV status for mothers or children, and we were therefore not able to assess whether HIV status influenced our results.

TST was only assessed for a subsample of our population, and we thus had scarce data in some groups. Our data did not allow for conclusions as to whether timing of BCG may have an impact on latent TB infection assessed by PPD reactions above 10mm or 15mm.

Comparison with other studies

The vaccine efficacy of BCG on preventing TB has been widely debated as different studies have yielded very different effects[20]. However, a meta-analysis of BCG's effect on TB found higher vaccine efficacy when BCG was given early in life[2]. Our results support that BCG in early life has beneficial effects, and furthermore suggest that timing of BCG is important. We did not study the vaccine efficacy on TB, and the results are therefore not directly comparable, but our findings support that BCG should be administered early in life.

Increasing evidence supports that BCG, aside from the specific effects, also have NSEs[6,21]. Self-selection to BCG vaccination could create a healthy vaccinee bias, where early BCG is associated with lower mortality because the healthiest children receive BCG early, thereby, creating a beneficial effect of BCG due to healthy children being vaccinated rather than BCG causing healthy children. Adjusting for available potential confounders did not alter the conclusions, nor did the sensitivity analyses conducted. Thus, self-selection for vaccination is unlikely to explain the all of the effect. Since we find marked effects of neonatal BCG on all-cause mortality in both TB-exposed and TB-unexposed children, our results support that the effect of neonatal BCG is not merely due to protection against TB.

Most studies assessing the effect of BCG on all-cause mortality compared BCG-vaccinated children with un-vaccinated children[6,22,23] and randomised trials of BCG-Denmark have found beneficial effects in the neonatal period[8]. It should be noted that two recent trials testing the effect of BCG-Russia in India found no effect[24]; the different results may be related to different BCG strain[24,25]. A previous study from the 1990s in Guinea-Bissau found that BCG was associated with stronger beneficial effects if provided in the first week of life[26]. Our study supported that the timing of BCG is important, not only because children vaccinated later are deprived of beneficial effects early in life, but also that there are benefits beyond the initial early phase.

In line with our study, data from the same setting between 1996 and 1998 indicate that TB exposure was associated with higher mortality in children aged 0-5 years[27]. We also found that TB exposure was associated with higher mortality, but not among children with neonatal BCG, due to no excess mortality among boys with neonatal BCG. It is generally accepted that TB-exposure can result in increased mortality[9,28], and it is recommended to provide preventive TB treatment for children younger than 5 years of age[29], however, a policy-practice gap remains, and the policy is rarely

implemented[30]. A recent modelling study estimated that more than 80,000 TB deaths could be averted globally in children younger than 5 years by altering coverage of household contact management from zero to full coverage[31]. Our data suggest that part of this effect (in boys) may also be obtained by emphasising BCG at birth.

Interpretation and implications

Our results were robust to sensitivity analyses and adjusting for available potential confounders. However, if BCG vaccination was delayed mainly for frail children, this could bias our results. To adjust for indicators of frailty, we conducted sensitivity analyses excluding twins, and stratifying by LBW status. Excluding twins or stratifying by LBW status did not alter the main conclusions. Assessing the effect of neonatal BCG by LBW limited the analysis to the 63% of the TB-exposed children and 68% of the TB-unexposed children with information on birthweight, and should thus be interpreted with caution. As a whole, the sensitivity analyses did not suggest that frailty in children with no neonatal BCG explained our results.

Currently, timing of BCG is not captured as part of the WHO/UNICEF coverage estimates[5], and although BCG is planned to be given at birth in many countries, BCG is often delayed[4,5]. Our results support that timing of BCG is important. WHO already recommends that BCG should be provided as soon as possible after birth[1], but we need more focus on barriers for neonatal BCG vaccination. Since policies are frequently implemented according to donor's priorities, vaccine donors should be involved in creating incentives for neonatal BCG vaccination. Currently, vaccine donors evaluate performance of vaccine programmes by 12 months coverage estimates and vaccine wastage targets[32]. Including neonatal BCG vaccination as a performance indicator in addition to 12 months coverage would provide countries with an incentive to strive for timely vaccination.

Conclusion

Neonatal BCG was associated with reduced mortality in TB-exposed and TB-unexposed children, supporting that timing of BCG is important for all-cause mortality. Neonatal BCG should be emphasised and barriers for neonatal BCG vaccination should be removed.

Authors' contributions

SMT and ABF conceived the idea for the study and planned the analyses. CB, AR, PA and ABF supervised the demographic surveillance data collection, AR and ABF supervised and cleaned the PPD data, VFG, FR and CW supervised the data registration of TB patients. SMT analysed the data, and wrote the first manuscript draft with input from PK, PA and ABF. All authors received and approved the final manuscript.

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Conflicts of interest

All authors have no interests to declare.

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Figure 1: Timeline of study period

Main analysis

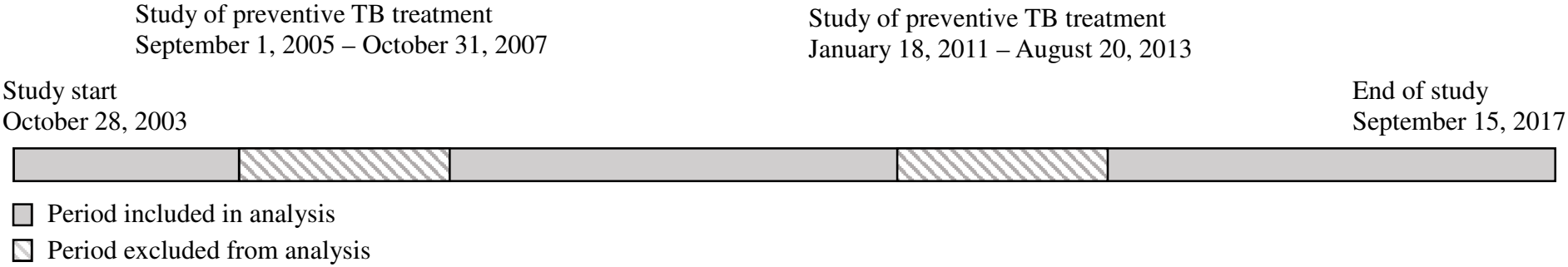


Table 1. Baseline characteristics of children with and without neonatal BCG by registered TB-exposure status.

TB-exposed children are only represented in the TB-exposed group despite some children also contribute observation time as not TB-exposed to avoid comparing a child with itself.

	TB-exposed children				TB-unexposed children			
	Total	Neonatal BCG (n %)	No neonatal BCG (n %)	p-value	Total	Neonatal BCG (n %)	No neonatal BCG (n %)	p-value
Number	2964	2566 (86.6%)	398 (13.4%)		36141	30372 (84.0%)	5769 (16.0%)	
Sex[1]				0.258				.268
Male	1538	1321 (51.5%)	217 (54.5%)		18299	15417 (50.8%)	2882 (50.0%)	
Female	1426	1245 (48.5%)	181 (45.5%)		17841	14955 (49.2%)	2886 (50.0%)	
Twin[2]				<0.0001				<0.0001
Yes	98	71 (2.8%)	27 (6.8%)		1216	811 (2.7%)	405 (7.0%)	
No	2864	2494 (97.2%)	370 (93.2%)		34873	29524 (97.3%)	5349 (93.0%)	
Season of birth				0.583				.026
Rainy season	1438	1250 (48.7%)	188 (47.2%)		17693	14946 (49.2%)	2747 (47.6%)	
Dry season	1526	1316 (51.3%)	210 (52.8%)		18448	15426 (50.8%)	3022 (52.4%)	
Birth location[3]				<0.0001				<0.0001
Home	878	711 (27.8%)	167 (42.2%)		9944	7421 (24.5%)	2523 (44.0%)	
Health centre	1312	1246 (48.6%)	66 (16.7%)		16065	15193 (50.1%)	872 (15.2%)	
Hospital	628	496 (19.4%)	132 (33.3%)		7856	5974 (19.7%)	1882 (32.9%)	
Other	140	109 (4.3%)	31 (7.8%)		2181	1729 (5.7%)	452 (7.9%)	
Year of birth				.466				.001
2000-2006	952	828 (32.3%)	124 (31.2%)		11146	9266 (30.5%)	1880 (32.6%)	
2007-2011	1000	855 (33.3%)	145 (36.4%)		12653	10626 (35.0%)	2027 (35.1%)	
2012-2017	1012	883 (34.4%)	129 (32.4%)		12342	10480 (34.5%)	1862 (32.3%)	
Number of pregnancies[4]				.596				<0.0001
1	824	716 (27.9%)	108 (27.1%)		10877	9140 (30.1%)	1737 (30.1%)	
2 - 3	1213	1056 (41.2%)	157 (39.4%)		15221	12924 (42.6%)	2297 (39.9%)	
4 or more	925	792 (30.9%)	133 (33.4%)		10028	8298 (27.3%)	1730 (30.0%)	
Maternal age[5]	2889	26.6 (6.26)	25.7 (6.41)	.013	34521	26.2 (6.22)	25.4 (6.37)	<0.0001
Education of caretaker[6]				<0.0001				<0.0001
0 year	762	636 (26.6%)	126 (36.1%)		9625	7565 (26.4%)	2060 (39.6%)	

1-4 years	379	328 (13.7%)	51 (14.6%)		4491	3664 (12.8%)	827 (15.9%)	
+4 years	1602	1430 (59.7%)	172 (49.3%)		19778	17463 (60.9%)	2315 (44.5%)	
Suburb[7]				.179				<0.0001
Bandim	1409	1204 (46.9%)	205 (51.5%)		15919	13134 (43.3%)	2785 (48.3%)	
Belem / Mindara	488	432 (16.8%)	56 (14.1%)		5530	4863 (16.0%)	667 (11.6%)	
Cuntum	1067	930 (36.2%)	137 (34.4%)		14682	12369 (40.7%)	2313 (40.1%)	
Ethnicity[8]				.003				<0.0001
Pepel	807	675 (26.7%)	132 (33.3%)		9946	8129 (27.0%)	1817 (31.7%)	
Balanta	278	235 (9.3%)	43 (10.9%)		2932	2443 (8.1%)	489 (8.5%)	
Mandinga / Fula	837	718 (28.4%)	119 (30.1%)		10860	8979 (29.8%)	1881 (32.8%)	
Manjaco / Mancanha	587	528 (20.9%)	59 (14.9%)		6424	5663 (18.8%)	761 (13.3%)	
Others	413	370 (14.6%)	43 (10.9%)		5684	4902 (16.3%)	782 (13.6%)	
Socioeconomic factors								
Type of roof[9]				.488				<0.0001
Straw	95	80 (3.1%)	15 (3.8%)		903	716 (2.4%)	187 (3.3%)	
Hard	2859	2478 (96.9%)	381 (96.2%)		35183	29621 (97.6%)	5562 (96.7%)	
Toilet[10]				.002				<0.0001
None	10	6 (0.2%)	4 (1.0%)		93	73 (0.2%)	20 (0.3%)	
Latrine	2565	2206 (86.3%)	359 (90.7%)		29982	24823 (81.9%)	5159 (89.7%)	
Inside	377	344 (13.5%)	33 (8.3%)		5981	5408 (17.8%)	573 (10.0%)	
Electricity[11]				<0.0001				<0.0001
Yes	941	851 (33.2%)	90 (22.7%)		12787	11397 (37.6%)	1390 (24.2%)	
No	2018	1711 (66.8%)	307 (77.3%)		23302	18941 (62.4%)	4361 (75.8%)	

[1] Missing information for 0 TB-exposed children and 1 TB-unexposed child.
[2] Missing information for 1 TB-exposed children and 52 TB-unexposed children.
[3] Missing information for 6 TB-exposed children and 95 TB-unexposed children.
[4] Missing information for 2 TB-exposed children and 15 TB-unexposed children.
[5] Missing information for 75 TB-exposed children and 1620 TB-unexposed children.
[6] Missing information for 221 TB-exposed children and 2247 TB-unexposed children.
[7] Missing information for 0 TB-exposed children and 10 TB-unexposed children.
[8] Missing information for 42 TB-exposed children and 295 TB-unexposed children.
[9] Missing information for 10 TB-exposed children and 55 TB-unexposed children.
[10] Missing information for 12 TB-exposed children and 85 TB-unexposed children.
[11] Missing information for 5 TB-exposed children and 52 TB-unexposed children.

Table 2. Baseline characteristics of children registered as exposed to and not exposed to TB

TB-exposed children are only represented in the TB-exposed group despite some children also contribute observation time as not TB-exposed to avoid comparing a child with itself.

	TB-exposed children versus TB-unexposed children			
	Total	TB-exposed (n %)	TB-unexposed (n %)	p-value
Number	39105	2964 (7.6%)	36141 (92.4%)	
Sex[1]				.189
Male	19837	1538 (51.9%)	18299 (50.6%)	
Female	19267	1426 (48.1%)	17841 (49.4%)	
Twin[2]				.86
Yes	1314	98 (3.3%)	1216 (3.4%)	
No	37737	2864 (96.7%)	34873 (96.6%)	
Season of birth				.645
Rainy season	19131	1438 (48.5%)	17693 (49.0%)	
Dry season	19974	1526 (51.5%)	18448 (51.0%)	
Birth location[3]				.005
Home	10822	878 (29.7%)	9944 (27.6%)	
Health centre	17377	1312 (44.4%)	16065 (44.6%)	
Hospital	8484	628 (21.2%)	7856 (21.8%)	
Other	2321	140 (4.7%)	2181 (6.1%)	
Year of birth				.257
2000-2006	12098	952 (32.1%)	11146 (30.6%)	
2007-2011	13653	1000 (33.7%)	12653 (34.7%)	
2012-2017	13354	1012 (34.1%)	12342 (33.9%)	
Number of pregnancies[4]				0
1	11701	824 (27.8%)	10877 (30.1%)	
2 - 3	16434	1213 (41.0%)	15221 (42.1%)	
4 or more	10953	925 (31.2%)	10028 (27.8%)	
Maternal age[5]	37410	26.5 (6.29)	26.1 (6.25)	.002
Education of caretaker[6]				.62
0 year	10387	762 (27.8%)	9625 (28.4%)	

1-4 years	4870	379 (13.8%)	4491 (13.3%)	
+4 years	21380	1602 (58.4%)	19778 (58.4%)	
Suburb[7]				<0.0001
Bandim	17328	1409 (47.5%)	15919 (44.1%)	
Belem / Mindara	6018	488 (16.5%)	5530 (15.3%)	
Cuntum	15749	1067 (36.0%)	14682 (40.6%)	
Ethnicity[8]				0
Pepel	10753	807 (27.6%)	9946 (27.7%)	
Balanta	3210	278 (9.5%)	2,932 (8.2%)	
Mandinga / Fula	11697	837 (28.6%)	10860 (30.3%)	
Manjaco / Mancanha	7011	587 (20.1%)	6424 (17.9%)	
Others	6097	413 (14.1%)	5684 (15.9%)	
Socioeconomic factors				
Type of roof[9]				.018
Straw	998	95 (3.2%)	903 (2.5%)	
Hard	38042	2859 (96.8%)	35183 (97.5%)	
Toilet[10]				<0.0001
None	103	10 (0.3%)	93 (0.3%)	
Latrine	32547	2565 (86.9%)	29982 (83.2%)	
Inside	6358	377 (12.8%)	5981 (16.6%)	
Electricity[11]				0.001
Yes	13728	941 (31.8%)	12787 (35.4%)	
No	25320	2018 (68.2%)	23302 (64.6%)	

[1] Missing information for 1 child
[2] Missing information for 54 children
[3] Missing information for 101 children
[4] Missing information for 17 children
[5] Missing information for 1695 children
[6] Missing information for 2468 children
[7] Missing information for 10 children
[8] Missing information for 337 children
[9] Missing information for 65 children
[10] Missing information for 97 children
[11] Missing information for 57 children

Table 3. Mortality of children with and without neonatal BCG by TB-exposure status.

	Number of children	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed				
Neonatal BCG	2566	11.4 (27/2,377)	0.28 (0.13-0.57)	0.35 (0.17-0.71)[1]
No neonatal BCG	398	33.9 (12/354)	Ref	Ref
TB-unexposed				
Neonatal BCG	31836	13.1 (498/37,943)	0.55 (0.46-0.67)	0.55 (0.46-0.67)[2]
No neonatal BCG	5988	22.7 (148/6,530)	Ref	Ref
Combined TB-exposed and TB-unexposed				
Neonatal BCG	34402	13.0 (525/40,320)	0.53 (0.44-0.64)	0.53 (0.44-0.64)[1]
No neonatal BCG	6386	23.2 (160/6,884)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.

Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.

[1] Adjusted HR calculated adjusting for twin status and year of birth among TB-exposed children, excluding 2 children from the analysis.

[2] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Table 4. Mortality of children with and without neonatal BCG by TB-exposure status and sex.

	Number of children	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed boys				
Neonatal BCG	1321	7.3 (9/1,233)	0.19 (0.07-0.56)	0.20 (0.07-0.58)[1]
No neonatal BCG	217	30.7 (6/195)	Ref	Ref
TB-unexposed boys				
Neonatal BCG	16179	13.3 (257/19,343)	0.56 (0.43-0.73)	0.56 (0.43-0.73)[2]
No neonatal BCG	3016	23.0 (76/3,305)	Ref	Ref
TB-exposed girls				
Neonatal BCG	1245	15.7 (18/1,144)	0.33 (0.13-0.86)	0.45 (0.17-1.18)[1]
No neonatal BCG	181	37.7 (6/159)	Ref	Ref
TB-unexposed girls				
Neonatal BCG	15657	13.0 (241/18,599)	0.55 (0.42-0.72)	0.55 (0.42-0.72)[2]
No neonatal BCG	2971	22.3 (72/3,224)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status and year of birth among TB-exposed children, excluding 2 children from the analysis.
[2] Adjusted HR yielded the crude analysis, and no child was excluded.

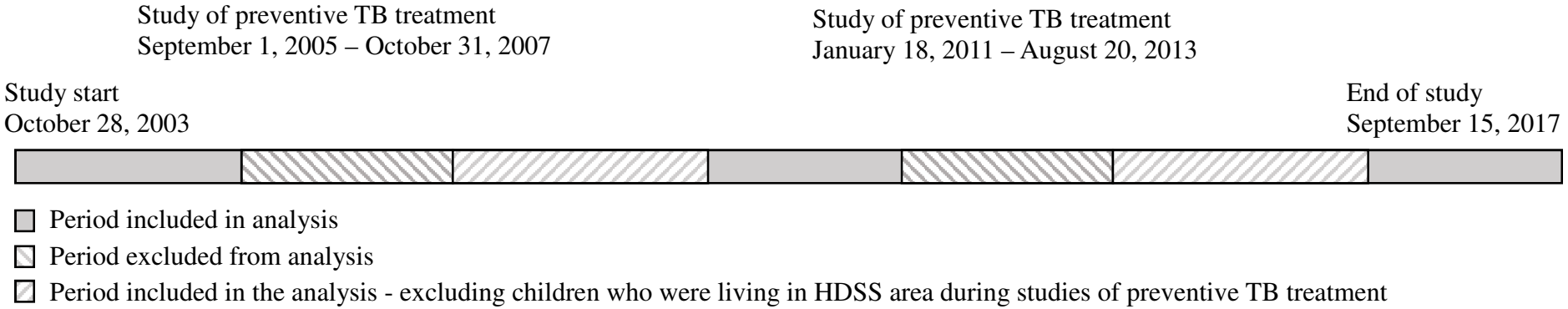
Table 5. Mortality of TB-exposed children compared with TB-unexposed children by timing of BCG and sex.

	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)
All children		
Neonatal BCG		
TB-exposed	11.4 (27/2,377)	1.09 (0.74-1.60)
TB-unexposed	13.1 (498/37,943)	Ref
No neonatal BCG		
TB-exposed	33.9 (12/354)	1.92 (1.06-3.46)
TB-unexposed	22.7 (148/6,530)	Ref
Boys		
Neonatal BCG		
TB-exposed	7.3 (9/1,233)	0.69 (0.36-1.35)
TB-unexposed	13.3 (257/19,343)	Ref
No neonatal BCG		
TB-exposed	30.7 (6/195)	1.75 (0.76-4.01)
TB-unexposed	23.0 (76/3,305)	Ref
Girls		
Neonatal BCG		
TB-exposed	15.7 (18/1,144)	1.53 (0.95-2.47)
TB-unexposed	13.0 (241/18,599)	Ref
No neonatal BCG		
TB-exposed	37.7 (6/159)	2.13 (0.92-4.89)
TB-unexposed	22.3 (72/3,224)	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
Adjusted HR are not presented as no baseline factor changed the estimates by more than 5%.

Supplementary material for "Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children"

Supplementary Figure 1: Timeline of study period; sensitivity analysis excluding children potentially eligible for studies of preventive TB treatment



Supplementary table 1. Mortality of children with early neonatal BCG, late neonatal BCG and no neonatal BCG by TB-exposure status.

	Number of children	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed children				
Early neonatal BCG (Day 0-7)	1697	10.4 (16/1,540)	0.19 (0.08-0.44)	0.24 (0.10-0.57)[1]
Late neonatal BCG (Day 8-28)	869	13.1 (11/837)	0.42 (0.18-0.97)	0.53 (0.22-1.26)[1]
No neonatal BCG	398	33.9 (12/354)	Ref	Ref
TB-unexposed children				
Early neonatal BCG (Day 0-7)	21170	13.6 (336/24,789)	0.58 (0.47-0.73)	0.58 (0.47-0.73)[2]
Late neonatal BCG (Day 8-28)	10666	12.3 (162/13,154)	0.53 (0.42-0.66)	0.53 (0.42-0.66)[2]
No neonatal BCG	5988	22.7 (148/6,530)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.

Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.

[1] Adjusted HR calculated adjusting for twin status and year of birth among TB-exposed children, excluding 2 children from the analysis.

[2] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Supplementary table 2. Sensitivity analyses comparing mortality of children with and without neonatal BCG for TB-exposed and TB-unexposed children.

	Number of children	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
Extended follow-up: Mortality between 28 days and 5 years of life[1]				
TB-exposed				
Neonatal BCG	2705	10.9 (32/2,928)	0.28 (0.14-0.55)	0.36 (0.18-0.70)[5]
No neonatal BCG	423	31.2 (14/448)	Ref	Ref
TB-unexposed				
Neonatal BCG	31900	12.9 (533/41,181)	0.56 (0.46-0.67)	0.56 (0.46-0.67)[6]
No neonatal BCG	5990	22.2 (161/7,239)	Ref	Ref
Only children with a registered BCG vaccine are included in the analysis[2]				
TB-exposed				
Neonatal BCG	2566	11.4 (27/2,377)	0.29 (0.13-0.62)	0.35 (0.16-0.76)[5]
No neonatal BCG	340	32.6 (10/306)	Ref	Ref
TB-unexposed				
Neonatal BCG	31836	13.1 (498/37,943)	0.61 (0.49-0.75)	0.61 (0.49-0.75)[6]
No neonatal BCG	5158	20.3 (117/5,758)	Ref	Ref
Excluding children who were twins[3]				
TB-exposed				
Neonatal BCG	2494	10.8 (25/2,304)	0.32 (0.15-0.70)	0.37 (0.17-0.80)[5]
No neonatal BCG	370	30.3 (10/331)	Ref	Ref
TB-unexposed				
Neonatal BCG	30949	12.8 (471/36,848)	0.57 (0.46-0.70)	0.57 (0.46-0.70)[6]
No neonatal BCG	5553	21.5 (130/6,054)	Ref	Ref
Excluding children potentially eligible for studies with preventive TB treatment for children[4] (Supplementary Figure 1)				
TB-exposed				
Neonatal BCG	1405	15.4 (20/1,297)	0.50 (0.18-1.44)	0.60 (0.21-1.72)[5]
No neonatal BCG	242	24.5 (5/204)	Ref	Ref
TB-unexposed				
Neonatal BCG	22286	14.9 (383/25,669)	0.55 (0.44-0.68)	0.55 (0.44-0.68)[6]
No neonatal BCG	4463	25.7 (120/4,674)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.

Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.

[1] Extending follow-up to 5 years included 164 additional TB-exposed children (7 deaths) and 66 additional TB-unexposed children (48 deaths) in the analysis.

[2] Limiting the analysis to children with a registered BCG vaccine excluded 58 TB-exposed children (2 deaths) and 830 TB-unexposed children (31 deaths) from the analysis.

[3] Limiting the analysis to children who are not twins excluded 100 TB-exposed children (4 deaths) and 1322 TB-unexposed children (45 deaths) from the analysis.

[4] Limiting the analysis to children who had not been eligible for studies of preventive TB treatment (living in the study area and below 5 years of age in the study period) excluded 1299 TB-exposed children (14 deaths) and 10516 TB-unexposed children (143 deaths) from the analysis (Supplementary Figure 1).

[5] Adjusted HR calculated adjusting for twin status and year of birth among TB-exposed children, excluding 2 children from the analysis.

[6] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Supplementary table 3. Mortality of children with and without neonatal BCG by TB-exposure status and low-birth-weight status.

	Number of children	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed				
Low-birth-weight children				
Neonatal BCG	120	8.5 (1/117)	0.08 (0.01-0.62)	0.09 (0.01-0.77)[1]
No neonatal BCG	54	154.6 (7/45)	Ref	Ref
Normal-birth-weight children				
Neonatal BCG	1603	9.5 (14/1,479)	N/A	N/A
No neonatal BCG	101	0.0 (0/102)	Ref	Ref
TB-unexposed				
Low-birth-weight children				
Neonatal BCG	1328	23.8 (36/1,514)	0.66 (0.41-1.05)	0.66 (0.41-1.05)[2]
No neonatal BCG	889	34.0 (35/1,029)	Ref	Ref
Normal-birth-weight children				
Neonatal BCG	21005	12.0 (303/25,340)	0.75 (0.49-1.15)	0.75 (0.49-1.15)[2]
No neonatal BCG	1506	14.0 (25/1,782)	Ref	Ref

Limiting the analysis to children with information on birthweight excluded 1086 TB-exposed children (17 deaths) and 13096 TB-unexposed children (247 deaths) from the analysis.
All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status and year of birth among TB-exposed children, excluding 1 child from the analysis.
[2] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Supplementary table 4. PPD reactions at 6 and 12 months of age of children with and without neonatal BCG by TB-exposure status.

	Number of children	Median PPD response in mm (interquartile range)	Positive PPD 10mm cut-off	Prevalence ratio (95% CI) 10mm cut-off	Positive PPD 15mm cut-off	Prevalence ratio (95% CI) 15mm cut-off
PPD response at 6 months of age						
TB-exposed children						
Neonatal BCG	53	0 (0-4.5)	4 (7.5%)	NA	0 (0.0%)	NA
No neonatal BCG	4	0 (0-3.3)	0 (0.0%)	Ref	0 (0.0%)	Ref
TB-unexposed children						
Neonatal BCG	1243	0 (0-4.5)	93 (7.5%)	0.93 (0.51-1.68)	23 (1.9%)	1.26 (0.30-5.28)
No neonatal BCG	136	0 (0-4.5)	11 (8.1%)	Ref	2 (1.5%)	Ref
PPD response at 12 months of age						
TB-exposed children						
Neonatal BCG	5	0 (0-0)	(0.0%)	NA	0 (0.0%)	NA
No neonatal BCG	2	6 (5.5-6.5)	(0.0%)	Ref	0 (0.0%)	Ref
TB-unexposed children						
Neonatal BCG	297	0 (0-0)	21 (7.1%)	0.90 (0.28-2.86)	6 (2.0%)	0.26 (0.07-0.98)
No neonatal BCG	38	0 (0-2.5)	3 (7.9%)	Ref	3 (7.9%)	Ref

Paper V

Disregarding the restrictive vial-opening policy for BCG vaccine in Guinea-Bissau: impact and cost-effectiveness for tuberculosis mortality and all-cause mortality in children aged 0-4 years

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(manuscript)

Disregarding the restrictive vial-opening policy for BCG vaccine in Guinea-Bissau: impact and cost-effectiveness for tuberculosis mortality and all-cause mortality in children aged 0-4 years

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Abstract

Background

BCG vaccination is often delayed in low-income countries. A major reason for delay is the restrictive vial-opening policy where a vial of BCG vaccine is not opened for few children. During delay, children are unprotected against tuberculosis (TB) and deprived of beneficial non-specific effects of BCG. We assessed the effect and cost-effectiveness of disregarding the restrictive vial-opening policy on TB and all-cause mortality in children aged 0-4 years in Guinea-Bissau.

Methods

Using a static mathematical model, we estimated the absolute and percentage change in TB and all-cause deaths between the baseline scenario with the current BCG vaccine policy, and a non-restrictive policy scenario where all children were vaccinated at the first health-facility contact. The incremental cost-effectiveness was measured by integrating vaccine and treatment costs into the model.

Results

Disregarding the restrictive BCG vial-opening policy was estimated to reduce TB deaths by 11·0% (95% Uncertainty Range (UR): 0·5-28·8%), corresponding to 4 (UR: 0-15) TB deaths per birth cohort in Guinea-Bissau, resulting in an incremental cost-effectiveness of USD 900 per discounted life year gained (LYG) (UR: 142-9,085). For all-cause deaths, the estimated reduction was 8·1% (UR: 3·3-12·7%) corresponding to 392 (UR:158-624) fewer all-cause deaths and an incremental cost-effectiveness of USD 9 per discounted LYG (UR: 5-23).

Conclusion

Disregarding the restrictive BCG vial-opening policy was associated with reductions in TB-deaths and all-cause deaths as well as low cost-effectiveness ratios in Guinea-Bissau. The restrictive vial-opening policy should be removed. Other settings with similar practice are likely also to gain from disregarding this policy.

Research in context

Evidence before this study

Previous studies have demonstrated that BCG vaccination is often delayed in low-income countries. In Guinea-Bissau, a major cause of delays in BCG vaccination is the restrictive vial-opening policy, where vials of BCG are not opened unless 10-12 children are present for vaccination. BCG is recommended to protect against TB, but increasing evidence supports that, aside from the TB-specific effects, BCG also may have beneficial non-specific effects reducing all-cause mortality. We searched PubMed for English literature published until December 2018 using the search terms "BCG vaccine", "child mortality", "children", "mortality", "tuberculosis" and "vaccine delays", and reviewed the literature used for the recent WHO report and recommendations on BCG vaccine of February 2018. We identified one mathematical modelling study that estimated the global impact of different BCG coverage scenarios on TB mortality in children aged 0-14, which concluded that increasing timeliness of BCG vaccination at birth was associated with reductions in TB deaths. We found no studies estimating the health economic impact of increasing timeliness, the impact of increasing BCG timeliness on all-cause mortality, nor any studies assessing the impact at the country level.

Added value of this study

In this study, we provide the first country-level estimates of the impact of change in BCG coverage. We estimated that disregarding the restrictive vial-opening policy and vaccinating every child at the first contact with a health facility could reduce TB mortality by 11%, corresponding to four TB deaths averted per birth cohort in Guinea-Bissau, and all-cause mortality reduced by 8%, equivalent to 392 all-cause deaths averted per birth cohort. Incremental costs per discounted life year gained (LYG) was estimated as USD 900 per discounted LYG when accounting for TB-specific effects only, and USD 9 per discounted LYG when including all-cause effects.

Our study provides the first effect and cost-effectiveness estimates for this easily implementable intervention of disregarding the restrictive vial-opening policy, and our estimates show that it is both effective and highly cost-effective to disregard the restrictive vial-opening policy.

Implications of all available evidence

Existing empirical evidence has demonstrated that BCG is important for TB control and may reduce all-cause mortality. Both available modelling studies provide estimates to support the importance of early BCG vaccination. Specifically, our study strongly supports disregarding the restrictive vial-opening policy as a route to improving timeliness in Guinea-Bissau, as we find that this would reduce child mortality and be highly cost-effective. Since Guinea-Bissau is not the only setting with this restrictive vial-opening policy and delays in BCG vaccination, other settings might also be able to gain important benefits by disregarding the restrictive vial-opening policy.

Introduction

Bacillus Calmette-Guérin (BCG) vaccine is one of the oldest and most widely used vaccines. BCG was developed to protect against tuberculosis (TB), and is currently the only approved TB vaccine[1]. Efficacy and effectiveness of BCG vaccine against TB varies considerably between studies and populations[2], however in studies of neonates BCG has consistently been associated with reduced TB[2].

TB is estimated to be among the top ten causes of death worldwide[3]. In 2015, the global paediatric all-cause mortality was estimated to 5·9 million deaths[4], and a modelling study estimated that TB caused 191,000 (95% CI: 132,000-257,000) of these[5]. However, TB is difficult to diagnose, especially in children, and many TB cases are not diagnosed in low-resource settings. Thus, particularly in settings with high TB burden and few resources, prevention may be a more feasible strategy than cure. Vaccination against TB is part of the strategy of reducing TB deaths by 95% between 2015 and 2035[1,6]. In 2018, the WHO reiterated the recommendation of BCG vaccination at birth in countries with high TB burden[1], partly informed by the work by Roy and colleagues who estimated that increasing global BCG coverage at birth to 92% could reduce TB deaths in children <15 years by 5,449 per birth cohort (95% uncertainty range: 218-15,071)[7].

In addition to the protection against TB, increasing evidence supports that BCG has beneficial non-specific effects (NSE) protecting against disease unrelated to TB[8-11]. In 2014, a WHO-commissioned review acknowledged that BCG may have beneficial NSEs reducing all-cause child mortality[12]. In a meta-analysis of three randomised trials among low-weight children in Guinea-Bissau, BCG-at-birth was associated with 38% (95% CI: 17-54%) lower neonatal mortality than the usual delayed vaccination of this population[8]. Thus, delays in BCG vaccination may be important for both TB-specific mortality and all-cause mortality.

Administrative BCG vaccination coverage reported at 12 months of age is high in most countries with a policy of neonatal vaccination, with a global estimate of 88%[13]. However, a 12 months coverage estimate does not reveal delays in BCG vaccination. To meet vaccine wastage targets, local practices of not opening a vial of BCG vaccine unless a sufficient number of children are present for vaccination has arisen (restrictive vial-opening policy) and BCG is consequently delayed in many low-income countries[14-16]. In Guinea-Bissau, a 20-dose vial of BCG vaccine is usually not opened unless 10-12 children are present for vaccination, leading to many missed vaccination opportunities[14]. The impact of this restrictive BCG vial-opening policy has not been examined.

The restricted vial-opening policy is imposed with the goal of using scarce resources effectively. However, this perceived saving may come at a cost. The objective of this study was to estimate the epidemiological impact and cost-effectiveness of disregarding the restrictive BCG vaccine vial-opening policy in Guinea-Bissau, considering both the TB-specific and all-cause effects of BCG.

Methods

To model the effect of disregarding the restrictive vial-opening policy on TB deaths and all-cause deaths, we developed a static mathematical model of TB and all-cause deaths. The model was based on the one developed by Roy and colleagues[7], but assuming leaky BCG-vaccine efficacy. The primary outcomes were absolute and percentage change in the number of TB deaths and all-cause

deaths averted among children <5 years per birth cohort due to disregard of the restricted BCG vial-opening policy.

The baseline scenario was defined as the current situation with restrictive BCG vial-opening policy, and the non-restrictive scenario as a potential scenario in which every child was BCG vaccinated at the first registered health facility contact.

Data inputs and assumptions

Where data were available, we used country-specific estimates for Guinea-Bissau. Data input, model assumptions, and data sources for the TB model, the all-cause model, and the cost-effectiveness analyses are summarised in Table 1.

Population estimates

The Bandim Health Project (BHP) runs urban and rural Health and Demographic Surveillance System (HDSS) sites in Guinea-Bissau covering all health regions in Guinea-Bissau. Using routine surveillance data from the HDSS sites between 2012-2017, we estimated the daily individual risk of all-cause mortality and all-cause hospital admission in children aged 0-4 years (Details are described in appendix A). We used WHO/UNICEF birth cohort estimates (Appendix A) and World Bank estimate of 57 years life-expectancy in Guinea-Bissau in 2017[17].

TB incidence and mortality

For TB incidence and TB mortality, we used the Global Burden of Disease[18] (GBD) estimates from 2016 for Guinea-Bissau (Table 1). Unlike the WHO TB estimates[19], the GBD reported country-level estimates of TB incidence and TB mortality in children aged 0-4 years. According to the GBD estimates, Guinea-Bissau had 33 paediatric TB deaths and 163 paediatric TB cases in 2016[18]. Using this information, we calculated a TB case fatality rate (CFR) of 0·21.

BCG vaccination coverage estimates

Using BHP routine data, which includes inspection of paediatric vaccination cards at household visits (urban: every 3 months, rural: every 6 months), we estimated BCG coverage in the baseline scenario and in the non-restrictive scenario (See appendix A for details). In the baseline scenario, 59% of children were BCG vaccinated at 1 month of age and 93% at 12 months of age (Figure 1). In the non-restrictive scenario, estimated BCG vaccination coverage was 73% at 1 month of age, and 99% at 12 months.

Efficacy of BCG vaccine against death

In the TB model, we used a BCG vaccine efficacy on TB deaths of 66% (95% CI: 8-88%), obtained from a meta-analysis of five randomised trials in neonates[20]. We assumed that vaccine efficacy was constant regardless of age of administration[7]. Protection of BCG vaccine against TB wanes over time[1], but evidence suggest that the effect may last for up to 15 years[20]. Hence, we assumed no waning of protection between 0-4 years of age. As paediatric TB is minimally infectious[21], we assumed that disregarding the restrictive BCG vial-opening policy would not affect the transmission of TB.

For BCG vaccine efficacy against all-cause mortality, we undertook a meta-analysis of seven studies from Guinea-Bissau (Appendix A), which provided a vaccine efficacy of 42% (95% CI: 19-58%) (Figure 2). The vaccine-efficacy against all-cause hospital admission was obtained from a combined estimate from three trials in low-weight children in Guinea-Bissau of 3% (95% CI: -31-28%)[22]. We assumed that the vaccine efficacies of BCG on all-cause death and all-cause hospital admissions were constant regardless of age at administration and that protection did not wane before 5 years of age. We assumed that BCG vaccine would not prevent disease transmission.

Costs

The incremental cost-effectiveness ratio (ICER) of disregarding the restrictive vial-opening policy was calculated from a societal perspective using 2017 values. We included household costs of seeking BCG vaccination (USD 1·92 per child [23]), costs incurred by the health system per hospital bed day (USD 15·58[24]), and the value of time spent by the mother accompanying her child to the hospital (USD 2·98 per hospital bed day[25], Appendix A). Since hospital admissions are free for children below 5 years of age in Guinea-Bissau[26], we did not include household payments for hospital admissions. We assumed 50% BCG vaccine wastage in the baseline scenario, as a 20-dose vial of BCG is opened if 10 children are present for vaccination, and 95% BCG vaccine wastage, as this would be the consequence if there was always only one child present for vaccination.

Mathematical model

Choosing the leaky vaccine efficacy approach, we assumed that the individual risk of TB death and all-cause death among vaccinated children was equivalent to the individual risk of TB death and all-cause death among unvaccinated children multiplied by (1- BCG vaccine efficacy).

TB model

We estimated the daily risk of TB death in vaccinated and unvaccinated children in the baseline scenario, based on the total number of TB deaths (0-4 years)[18], the BCG vaccine efficacy[20] and the number of children BCG vaccinated and not vaccinated by age (see Appendix A for details on data input and Appendix B1 for model equations). The daily risk of TB was assumed to be constant between 0 and 4 years of age. Applying these daily risks to the number of vaccinated and unvaccinated children allowed calculation of the total number of TB deaths per birth cohort in the first 5 years of life in the baseline and non-restrictive scenarios as a summation of daily TB deaths in BCG-vaccinated and BCG-unvaccinated children between age 0 and age 1826 days. In the TB model, the population at risk was adjusted for all-cause deaths on a daily basis; assuming BCG had no effect on all-cause mortality. Finally, we calculated the absolute and relative difference in TB deaths between the scenarios.

The number of TB cases per birth cohort in the first 5 years of life in each scenario was estimated by dividing the number of TB deaths by the CFR, calculated based on the GBD estimates of TB deaths and cases. We assumed that all identified paediatric TB cases in Guinea-Bissau are hospitalised [personal communication: Victor Gomes, Programmatic Manager of MDR-TB, National TB Programme]. As the GBD estimate of TB cases include undiagnosed TB cases, we calculated the proportion of the GBD estimated TB cases who were hospitalised based on the number of identified TB cases in Guinea-Bissau in 2017. This proportion was applied to the model

estimate of the number of TB cases in each scenario to estimate the number of TB hospital admissions.

Model equations and detailed methods are described in Appendix B1.

All-cause deaths model

In the model of all-cause deaths, we estimated daily, age-specific risk of all-cause death using the BHP routine all-cause mortality data (Appendix A). Based on this daily risk of all-cause death, our BCG vaccine efficacy meta-estimate (Appendix A) and baseline data on BCG coverage by age, we calculated the daily risk of all-cause death in BCG-vaccinated and BCG-unvaccinated children, respectively.

We applied the daily risk of all-cause mortality to the birth cohort estimate of 2017 and estimated daily and cumulative number of deaths between 0-4 years in that cohort under the baseline and non-restrictive policy scenarios (Appendix B2). Using the same approach, we calculated the absolute and relative difference in all-cause hospital admissions between scenarios (Described in detail in Appendix B3).

Increasing evidence suggests that vaccines interact and therefore the NSEs may depend on the last vaccine received[12,27]. We therefore conducted a secondary analysis assessing only the effect in a limited period from birth to six weeks of age, when the next vaccines are scheduled to be given (Appendix C).

Cost-effectiveness analyses

Vaccination costs, including injection supply cost, for each scenario accounted for differences in number of BCG doses delivered (based on BCG coverage), and vaccine wastage (calculated based on conservative assumptions of 50% vaccine wastage in the baseline scenario, and 95% vaccine wastage in the non-restrictive scenario).

We summarised the number of deaths averted per year between 0 and 5 years of age. Life years gained (LYG) by disregarding the restrictive vial-opening policy were calculated by multiplying the number of TB deaths and all-cause deaths averted per year of life by the remaining life expectancy[17]. As recommended by WHO[28], we discounted future life years by 3% per year.

Details on costs and cost-effectiveness equations are available in Appendix B4 and B5.

Urban versus rural sub-analyses

Vaccination opportunities, healthcare-seeking behaviour, and number of children present at health centres are likely to differ between the urban and rural population resulting in different effects of disregarding the vial-opening policy. We estimated the impact and cost-effectiveness of disregarding the restrictive vial-opening policy according to TB-specific effects and all-cause effects separately for urban and rural Guinea-Bissau. We used the same approaches as described above, but substituted risk of all-cause mortality, risk of all-cause hospital admission, BCG coverage, and birth cohort estimates by regional estimates. See Appendix D1 and D2 for BCG vaccination coverage estimates for the urban and the rural population.

Uncertainty and sensitivity analyses

We performed a probabilistic uncertainty analysis using Oracle® Crystal Ball (Release 11.1.2.4.850, Oracle Corporation, USA), where a statistical distribution for each parameter with a reported uncertainty range was set based upon the reported or assumed underlying distributions (Table 1). Location and scale parameters were estimated using the 2·5%, 50% and 97·5% percentiles in the rriskdistributions package in R. Parameters with fixed values were not considered uncertain. A total of 100,000 parameter sets and model outputs were generated through Monte-Carlo simulations. Median and 95% uncertainty ranges for each outcome were calculated from the 100,000 model outputs.

In the baseline analysis, we assumed that female and male estimates were perfectly correlated for TB incidence, and TB mortality. We furthermore assumed that BHP mortality estimates by age were perfectly correlated. To assess the impact of these correlations, we ran a sensitivity analysis assuming no correlation.

In other sensitivity analyses, we assessed the impact of choices for methodological structure and model calibration data. We assessed the impact of assuming leaky BCG vaccine efficacy by adapting the model to all-or-nothing BCG vaccine efficacy, as in previous models[7,29]. For methodological details, see appendix B6. We evaluated the impact of using GBD estimates by performing two sensitivity analyses, the first using TB incidence from WHO[19] combined with estimates of CFR from Jenkins et al[30], and a second using modelled estimates of TB mortality from Dodd and colleagues[5].

Results

Effects of disregarding BCG vial-opening policy on TB-specific outcomes

Disregarding the restrictive vial opening policy was estimated to reduce TB deaths, admissions, and cases by 11·0% (95% Uncertainty Range (UR): 0·5-28·8%). The number of TB deaths were 33 (UR: 13-89) per birth cohort in the baseline scenario and 29 (UR: 11-79) in the non-restrictive scenario, therefore averting 4 (UR: 0-15) TB deaths per birth cohort in the first 5 years of life. TB cases were reduced from 162 (UR: 96-273) to 142 (UR: 82-245), and admissions from 46 to 41 (UR: 33-46) (Table 2).

Effects of disregarding BCG vial-opening policy on all-cause outcomes

Disregarding the restrictive vial opening policy was estimated to reduce all-cause mortality by 8·1% (UR: 3·3-12·7%), from 4,820 (UR: 4,309-5,425) all-cause deaths in the baseline scenario to 4,429 (UR: 3,920-5,028) all-cause deaths in the non-restrictive scenario (Table 2), therefore averting 392 (UR: 158-624) all-cause deaths per birth cohort in the first 5 years of life. There was an estimated 0·4% (UR: -2·6-2·8%) increase in all-cause hospital admissions, from 5,926 (UR: 5,538-6,346) all-cause hospital admissions in the baseline scenario to 5,940 (UR: 5532-6380) in the non-restrictive scenario, due to more children surviving to be admitted.

Costs and cost-effectiveness of disregarding the restrictive BCG vial-opening policy

Disregarding the vial-opening policy resulted in 99 discounted LYG when accounting only for the TB-specific effects of BCG, resulting in an ICER of USD 900 (UR: 142-9,085) per discounted LYG and USD 24,269 (UR: 3,827-244,869) per TB death averted (Table 3).

Including all-cause effects of BCG vaccination, the number of discounted LYG were 10,687, resulting in an ICER of USD 9 (UR: 5-23) per discounted LYG and USD 249 (UR: 144-615) per all-cause death averted (Table 3).

Effects of disregarding BCG vial-opening policy in urban and rural health regions

Disregarding the vial-opening policy in urban Guinea-Bissau was estimated to have little effect on TB mortality, changing the number of TB deaths by 2·6% (0·1-8·3%), corresponding to less than 1 fewer TB deaths per birth cohort (Table 4). The estimated reduction in all-cause mortality in the urban population was 10·4% (UR: 4·5-15·3%), reducing the number of deaths by 111 (UR: 47-172) per birth cohort in the first 5 years of life (Table 4) and resulting in ICERs of USD 10 (UR: 6-23) per discounted LYG and USD 273 (UR: 168-635) per all-cause death averted (Appendix D3).

In the rural population, disregarding the restrictive vial-opening policy was estimated to reduce TB deaths by 16·4% (0·8-38·6%), corresponding to 4 (UR: 0-17) fewer TB deaths. The resulting ICERs were USD 799 (UR: 127-8,088) per discounted LYG and USD 21,528 (UR: 3,430-217,936) per TB death averted. The estimated reduction in all-cause mortality was 8·4% (3·3-13·5%) corresponding to 319 (UR: 124-527) fewer all-cause deaths (Table 4). The resulting ICERs were USD 11 (UR: 6-27) per discounted LYG and USD 288 (UR: 165-742) per all-cause death averted (Appendix D3).

Sensitivity analyses

Structural

Changing the model structure from a model assuming leaky BCG vaccine efficacy (main analysis) to a model assuming all-or-nothing BCG vaccine efficacy did not alter the results (Table 4, Appendix C). Similarly, assuming no correlations between mortality estimates yielded similar results as the main analyses (Appendix D4).

Source of TB data

Using the WHO TB incidence[19] and Jenkins CFR[30] estimates caused an estimated 231 (UR: 196-272) TB deaths per birth cohort in the baseline scenario, and 204 (UR: 156-252) TB deaths in the non-restrictive scenario, therefore averting 25 (UR: 1-68) TB deaths per birth cohort in the first 5 years of life. The estimates by Dodd and colleagues[5] gave an estimated 238 (110-519) TB deaths per birth cohort in the baseline scenario, and 209 (94-463) TB deaths in the non-restrictive scenario, therefore averting 25 (UR: 1-94) TB deaths per birth cohort. While the percentage change in TB deaths between scenarios was the same for all data sources, there were large differences in the absolute change in TB deaths (Table 4).

Discussion

Main findings

Disregarding the restrictive BCG vial-opening policy was estimated to reduce TB deaths by 11·0% (UR: 0·5-28·8%) corresponding to 4 (UR: 0-15) TB deaths averted per birth cohort. All-cause deaths were reduced by 8·1% (UR: 3·3-12·7%) corresponding to 392 (UR:158-624) all-cause deaths per birth cohort. Thus, despite larger percentage change when considering only the TB-specific effect, the absolute change was much larger accounting for all-cause effects of BCG. This

was also reflected in the ICERs: The ICER of disregarding the restrictive vial-opening policy was estimated as USD 900 (UR: 142-9,085) per discounted LYG by averting TB deaths and USD 9 (UR: 5-23) per discounted LYG by averting all-cause deaths. This large difference demonstrates the potential improvement in cost-effectiveness by including all-cause deaths in such analyses, if supported by ongoing research, including all-cause effects in evaluation of policy changes may be very important.

Our results were robust to the sensitivity analyses conducted, however, the TB data sensitivity shows that the main analysis may be a conservative estimate of TB deaths averted by policy change, as using other data sources resulted in 25 TB deaths averted per birth cohort.

Strengths and limitations

This is the first model to assess effects and cost-effectiveness of a change in BCG vaccination vial-opening policy on all-cause mortality, and the first country-level model to assess effects and cost-effectiveness of the restrictive BCG vial-opening policy on any outcome. The BHP HDSS data allowed for country-representative age-specific estimates of BCG coverage, daily mortality risk, and daily risk of hospital admission based on individual level data. Thus, the estimates were more accurate than aggregated country-level data usually available.

Increasing vaccination coverage to a certain threshold is generally resource demanding, and may not be realistic in low-resource settings. In this study, we used the first registered health-facility contact as a proxy for a possible BCG vaccination contact. Thus, the intervention assessed would be easy to implement and our results do not rely on additional initiatives to obtain the coverage in the non-restrictive scenario. Moreover, since only registered health facility contacts are included, our estimates of BCG coverage in the non-restrictive scenario are likely to be underestimated and the impact conservative.

TB surveillance in Guinea-Bissau is limited and many TB cases are likely undiagnosed. Estimates of TB incidence and TB mortality are surrounded by much uncertainty; we therefore used three sources of TB mortality estimates, using different methodology. The GBD estimates were the lowest estimate of the three data sources used, and thus, likely conservative. The efficacy estimates of BCG against tuberculosis have varied between studies and populations[2,20], and thus the meta-analysed estimates have wide uncertainty ranges. We assumed that TB mortality was constant between 0 and 4 years of age, since age-stratified TB data were not available. The estimates of TB cases and TB hospital admissions should be interpreted with caution as they are calculated based on different data sources assuming comparability (Appendix C).

We did not take into consideration that different strains of BCG may have different effects on TB mortality and all-cause mortality[31]. The vaccine efficacy on all-cause deaths was estimated from trials in low-weight children and observational studies. It would be useful with more data to allow for more certain conclusions for normal birth weight children. We did not account for waning or reduced disease transmission due to BCG's all-cause effects. Similar estimates has been found across studies at different ages early in life[8,9]. However, data are not sufficient to conclude on the duration of NSEs. If BCG reduce disease transmission, not considering this would make our estimates conservative.

All cost estimates were derived from Guinea-Bissau. Household costs of seeking BCG vaccination and costs per hospital bed day incurred by the health system were obtained from recently conducted studies. Out-of-pocket payments for treatment were not included, but as hospital admission for children <5 years is free in Guinea-Bissau, we expect out-of-pocket payments to be few. We assumed 50% vaccine wastage in the baseline scenario, and 95% vaccine wastage in the non-restrictive scenario, thereby obtaining conservative estimates, as wastage of BCG would be less than 95% if more than one child were BCG vaccinated per vial.

Comparison with other studies and future perspectives

Previously, Roy and colleagues have estimated the global impact of different BCG timeliness scenarios on TB mortality, and found that increasing BCG coverage to 92% at birth globally could reduce TB deaths by 2·8%[7]. We found a greater percentage reduction in TB deaths (11·0% (UR: 0·5-28·8%)). The difference could be due to more delay in BCG vaccination or higher risk of TB in Guinea-Bissau, but more precise estimates of BCG coverage and delays would also affect the estimate.

In recent years, manufacturing problems have created a shortage of BCG vaccine, which was estimated to be associated with 7,433 (UR: 320-19,477) excess TB deaths per global birth cohort in children aged 0-14 years[29]. The study did not include potential excess all-cause deaths. Our results suggest that if all-cause deaths had been estimated, the increase would have been substantially higher.

Most studies assessing vaccine effectiveness compare the vaccine with a placebo or no vaccine. Rarely, vaccination programme implementations are studied. We evaluated the effect of a potential policy change. Modelling studies require sufficient data input, fortunately, we could use country and age-specific estimates for the all-cause effects, but the TB-specific estimates carries much uncertainty, and better data should be collected. Our results emphasize the importance of including effects on all-cause mortality to inform policy makers of the full impact and cost-effectiveness of a policy change.

We modelled the effect of disregarding the restrictive vial-opening policy in Guinea-Bissau. However, the restrictive vial-opening policy is not limited to Guinea-Bissau[15,32], and thus, important gains might be possible in other settings with restrictive vial opening policy. Our results support that disregarding the policy is cost-effective both in urban areas and rural areas, even with the reduced existing delays in urban areas. The exact impact and cost-effectiveness estimates are not directly transferable to other countries, as BCG coverage and mortality patterns will differ between countries. However, the conclusions of the study are likely to be transferable to other countries with restrictive vial-opening policies.

Conclusion

Disregarding the restrictive vial-opening policy in Guinea-Bissau was estimated to avert 4 (UR: 0-15) TB deaths per birth cohort at an ICER of USD 900 (UR: 142-9,085) per discounted LYG, or 392 (UR: 158-624) per all-cause death averted and USD 9 (UR: 5-23) per discounted LYG.

Thus, our results support that the restrictive vial-opening policy should be disregarded. Other settings with the restrictive vial-opening policy are likely to be able to gain important benefits from a similar policy change.

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Figure 1. BCG coverage estimates in Guinea-Bissau in the baseline scenario and when disregarding the restrictive BCG vial-opening policy

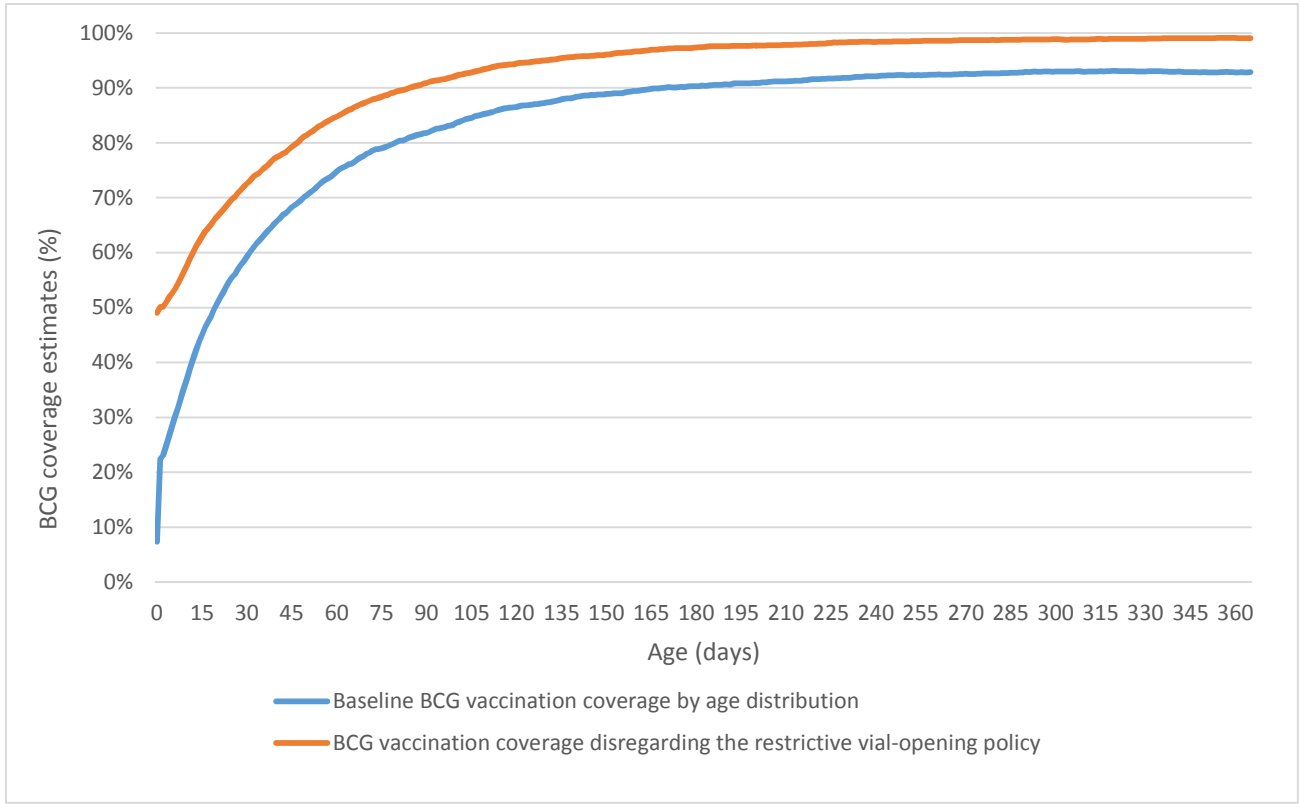

Figure 2. Meta-analysis of the effect of BCG on all-cause mortality in Guinea-Bissau (Estimate (ES): 0.58 (0.42-0.81))

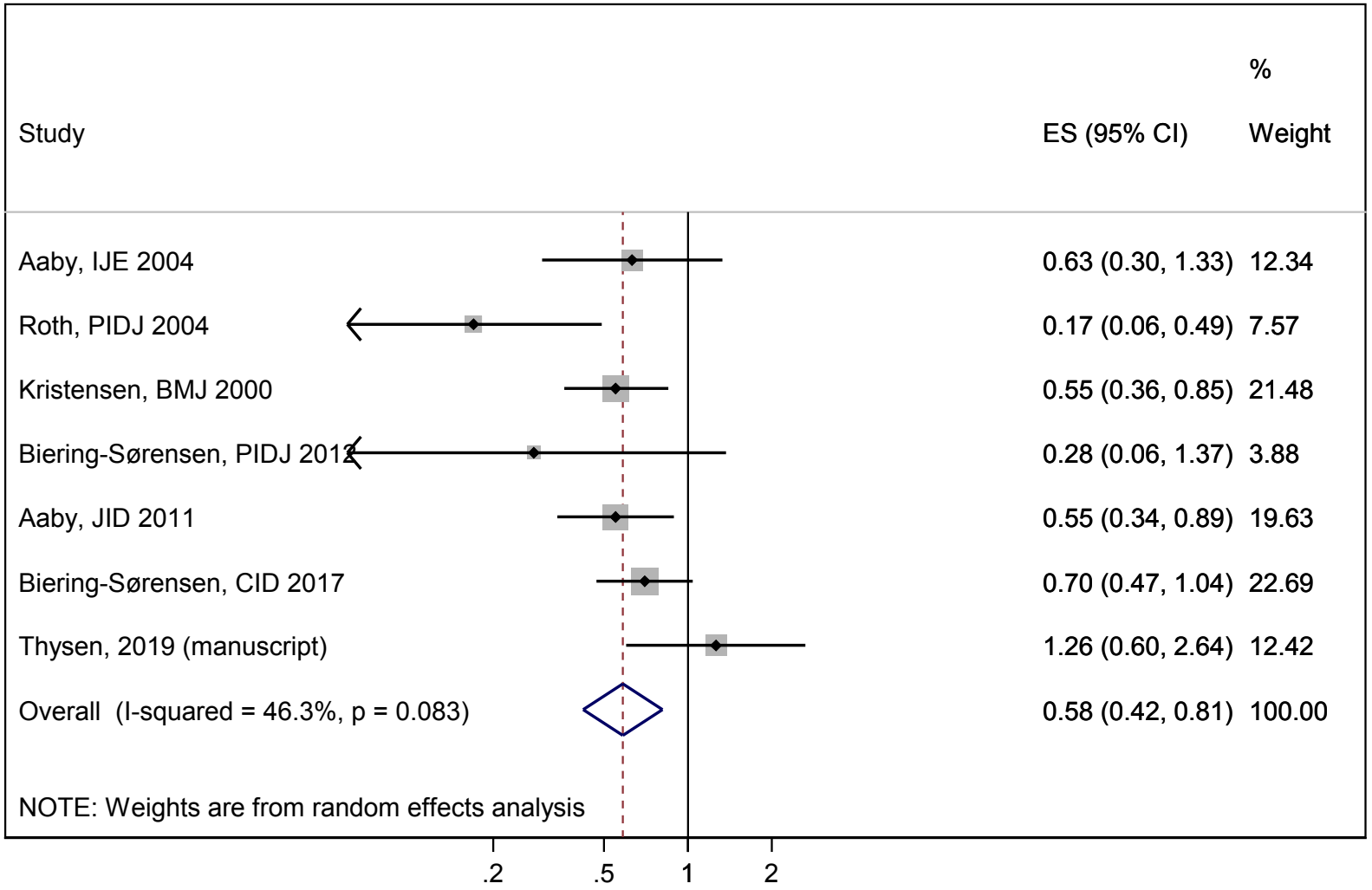

Table 1: Data inputs and assumptions

Variable	Estimate	Source	Distribution
Population characteristics			
Population at risk - birth cohort (2017)	69,212	Joint Reporting Form data from Guinea-Bissau[1]	Fixed
Life expectancy at birth	57 years	World Bank 2017[2]	Fixed
Estimated number of TB cases in males aged 0-4 in 2016	74.02 (36.81-121.37)	Global Burden of Disease Results Tool[3]	Log-normal
Estimated number of TB cases in females aged 0-4 in 2016	88.59 (46.77-145.08)	Global Burden of Disease Results Tool[3]	Log-normal
Estimated number of TB deaths in males aged 0-4 in 2016	16.34 (7.95-35.51)	Global Burden of Disease Results Tool[3]	Log-normal
Estimated number of TB deaths in females aged 0-4 in 2016	17.03 (5.14-51.97)	Global Burden of Disease Results Tool[3]	Log-normal
Individual daily all-cause mortality risk			
Day 0	0.014184 (0.012901-0.015632)	BHP HDSS routine data[4]	Log-normal
Day 1	0.003370 (0.002757-0.004165)	BHP HDSS routine data[4]	Log-normal
Day 2	0.002100 (0.001646-0.002724)	BHP HDSS routine data[4]	Log-normal
Day 3	0.001090 (0.000772-0.001592)	BHP HDSS routine data[4]	Log-normal
Day 4	0.001123 (0.000799-0.001630)	BHP HDSS routine data[4]	Log-normal
Day 5	0.000787 (0.000518-0.001259)	BHP HDSS routine data[4]	Log-normal
Day 6	0.001110 (0.000785-0.001624)	BHP HDSS routine data[4]	Log-normal
Day 7	0.000547 (0.000319-0.001024)	BHP HDSS routine data[4]	Log-normal
Day 8-28	0.000142 (0.000115-0.000179)	BHP HDSS routine data[4]	Log-normal
Day 29-365	0.000054 (0.000049-0.000058)	BHP HDSS routine data[4]	Log-normal
Day 366-1826	0.000018 (0.000017-0.000020)	BHP HDSS routine data[4]	Log-normal
Individual daily risk of all-cause hospital admission			
Day 0-28	0.000083 (0.000065-0.000107)	BHP HDSS routine data[4]	Log-normal
Day 29-365	0.000080 (0.000074-0.000085)	BHP HDSS routine data[4]	Log-normal
Day 366-1826	0.000042 (0.000039-0.000044)	BHP HDSS routine data[4]	Log-normal
BCG coverage distribution in baseline scenario	Figure 1	BHP HDSS routine data[4]	Fixed
BCG coverage distribution disregarding the vial-opening policy	Figure 1	BHP HDSS routine data[4]	Fixed
Vaccine characteristics TB-specific			
Risk ratio of BCG on TB deaths	0.34 (0.12-0.92)	Abubakar, Health Technol Assess, 2013[5]	Log-normal
Duration of protection	> 5 years	Abubakar, Health Technol Assess, 2013[5]	Fixed
Waning of protection	None	Assumption	Fixed
Prevention of transmission by BCG	None	Assumption	Fixed
Vaccine characteristics all-cause effects			
Risk ratio of BCG on all-cause deaths	0.58 (0.42-0.81)	Meta-estimate of studies from Guinea-Bissau[6]	Log-normal
Risk ratio of BCG on all-cause hospital admissions	0.97 (0.72-1.30)	Schaltz-Buchholzer, JID, 2018[7]	Log-normal
Duration of protection	> 5 years	Assumption	Fixed
Waning of protection	None	Assumption	Fixed
Prevention of transmission by BCG	None	Assumption	Fixed
Cost estimates			

BCG vaccine price per dose incl. freight	0.20 USD	National department of the Expanded Programme on Immunization[1]	Fixed
Application syringe incl. freight	0.05 USD	National department of the Expanded Programme on Immunization[1]	Fixed
Mixing syringe incl. freight	0.03 USD	National department of the Expanded Programme on Immunization[1]	Fixed
Safety Box	0.67 USD	National department of the Expanded Programme on Immunization[1]	Fixed
Costs per hospital bed day incurred by health system	15.58 USD	Enemark, manuscript, 2019[8]	Fixed
Household costs per bed day hospital admission	2.98 USD	Knight, PNAS, 2014[9]	Fixed
Household costs of seeking BCG vaccination	1.92 USD	Thysen, manuscript, 2019[10]	Fixed

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Table 2. Effects of disregarding the restrictive BCG vial-opening policy in Guinea-Bissau

	Baseline scenario	**Non-restrictive scenario**	**Absolute change**	**Percentage change**
TB-specific effects	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)
Total number of paediatric TB deaths	33 (13 to 89)	29 (11 to 79)	-4 (-15 to 0)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB cases	162 (96 to 273)	142 (82 to 245)	-18 (-54 to -1)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB hospital admissions	46 (46 to 46)	41 (33 to 46)	-5 (-13 to 0)	-11.0% (-28.8% to -0.5%)
All-cause effects				
Total number of all-cause deaths	4,820 (4,309 to 5,425)	4,429 (3,920 to 5,028)	-392 (-624 to -158)	-8.1% (-12.7% to -3.3%)
Total number of all-cause hospital admissions	5,926 (5,538 to 6,346)	5,940 (5,532 to 6,380)	18 (-125 to 133)	0.4% (-2.6% to 2.8%)

Table 3. Cost-effectiveness of disregarding the restrictive BCG vial-opening policy

	Baseline scenaio	**Non-restrictive scenario**
2017 birth cohort	69,212	69,212
Number of children born in health facilities	33,354	33,354
Number of children BCG-vaccinated at birth	13,438	33,354
Number of children not BCG-vaccinated at birth	55,774	35,858
Total times seeking BCG vaccination	1.26	1.00
Vaccine wastage	50 %	95 %
BCG coverage at 12 months of age	93 %	99 %
Total household costs of seeking BCG vaccination	134,631 USD	68,694 USD
Total BCG vaccine costs	26,221 USD	279,769 USD
Total injection supply costs	3,909 USD	6,640 USD
Cost per bed day incurred by the health system	15.58 USD	15.58 USD
Household costs of accompanying a child per bed day hospital admission	2.98 USD	2.98 USD
TB-specific effects only	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)
Total number of paediatric TB deaths	33 (13 to 89)	29 (11 to 79)
Total LYG		203
Total LYG discounted		99
Total number of paediatric TB hospital admissions	46 (46 to 46)	41 (33 to 46)
Median bed day per TB hospital admission	60 days	60 days
Total costs of TB hospital admissions	51,207 USD	45,557 USD
Incremental costs of disregarding the restrictive vial-opening policy (including vaccination costs, household costs and costs averted by TB hospital admissions)		184,692 USD
ICER (USD/LYG)		440 (69 to 4,440)
ICER (USD/discounted LYG)		900 (142 to 9,085)
ICER (USD/TB death averted)		24,269 (3,827 to 244,869)
All-cause effects		
Total number of all-cause deaths	4,820 (4,309 to 5,425)	4,429 (3,920 to 5,028)
Total LYG		22,212
Total LYG discounted		10,687
Total number of all-cause hospital admissions	5,926 (5,538 to 6,346)	5,940 (5,532 to 6,380)
Median bed day per all-cause hospital admission	5 days	5 days
Total costs of all-cause hospital admissions	549,815 USD	551,478 USD
Incremental costs of disregarding the restrictive vial-opening policy (including vaccination costs, household costs and costs averted by hospital admissions)		192,005 USD
ICER (USD/LYG)		4 (3 to 11)
ICER (USD/discounted LYG)		9 (5 to 23)
ICER (USD/all-cause death averted)		249 (144 to 615)

USD: US Dollar 2017 value, LYG: Life year gained, ICER: Incremental cost-effectiveness ratio

Table 4. Effects of disregarding the restrictive BCG vial-opening policy in Guinea-Bissau – sensitivity analyses

	Baseline scenario	**Non-restrictive scenario**	**Absolute differences**	**Percentage change**
TB-specific effects	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)
Total number of paediatric TB deaths - main analysis	33 (13 to 89)	29 (11 to 79)	-4 (-15 to 0)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB deaths - WHO data	231 (196 to 272)	204 (156 to 252)	-25 (-68 to -1)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB deaths - P.Dodd data	238 (110 to 519)	209 (94 to 463)	-25 (-94 to -1)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB deaths - All-or-nothing BCG effectiveness	33 (13 to 89)	29 (11 to 79)	-4 (-15 to 0)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB deaths - urban data	8 (3 to 22)	8 (3 to 21)	0 (-1 to 0)	-2.6% (-8.3% to -0.1%)
Total number of paediatric TB deaths - rural data	26 (10 to 69)	21 (8 to 57)	-4 (-17 to 0)	-16.4% (-38.6% to -0.8%)
All-cause effects				
Total number of all-cause deaths day - main analysis	4,820 (4,309 to 5,425)	4,429 (3,920 to 5,028)	-392 (-624 to -158)	-8.1% (-12.7% to -3.3%)
Total number of all-cause deaths day 0-42	1,922 (1,612 to 2,318)	1,648 (1,352 to 2,028)	-277 (-415 to -120)	-14.5% (-20.7% to -6.3%)
Total number of all-cause deaths day 1-1826	3,838 (3,417 to 4,345)	3,621 (3,208 to 4,112)	-216 (-373 to -80)	-5.6% (-9.5% to -2.1%)
Total number of all-cause deaths urban data	1,071 (897 to 1,302)	961 (795 to 1,180)	-111 (-172 to -47)	-10.4% (-15.3% to -4.5%)
Total number of all-cause deaths rural data	3,787 (3,303 to 4,386)	3,467 (2,992 to 4,048)	-319 (-527 to -124)	-8.4% (-13.5% to -3.3%)
Total number of all-cause deaths urban day 1-1826	716 (583 to 899)	691 (565 to 865)	-24 (-46 to -9)	-3.4% (-5.8% to -1.3%)
Total number of all-cause deaths rural day 1-1826	3,193 (2,788 to 3,703)	2,951 (2,551 to 3,445)	-240 (-414 to -89)	-7.5% (-12.6% to -2.8%)

Appendix A. Details on data derived from the BHP's urban and rural HDSS sites

Population estimates

We used data from the WHO/UNICEF Joint Reporting Forms 2017 to obtain the annual birth cohort in Guinea-Bissau [personal communication: Carlitos Bale, Director of the Vaccination Programme in Guinea-Bissau].

The daily individual risk of all-cause mortality was estimated based on data from 2012-2017. In survival models with age as underlying time scale, we assessed the daily risk of dying between 0-4 years of age using Kaplan-Meier estimates. Since mortality declines rapidly early in life, we estimated the risk of dying on a daily basis in the first week (day 0-day 7), and in intervals: day 8 to 28, day 29 to 365, and finally day 366 to 1826. Children entered the analysis on January 1, 2012, registration in the HDSS, or birth, whichever came last. Children were followed until death, 5 years of age, or December 31, 2017, whichever came first.

Likewise, we estimated the individual risk of all-cause hospital admission on data from 2012-2017. The risk of hospital admission was estimated in survival models with age as underlying time scale in the following intervals: day 0 to 28, day 29 to 365, and day 366 to 1826. Children entered the analysis on January 1, 2012, registration in the HDSS, or birth, whichever came last. Children were followed until death, migration, 5 years of age, or December 31, 2017, whichever came first. After a hospital admission, children re-entered the analysis, and were able to contribute with more than one event.

BHP covers a random sample of villages/towns in each region, but the regional sample is not weighted according to the region's population. All estimates were computed by region. The regional estimates were weighted according to the sample representation of the total population in the region according to data from the WHO/UNICEF Joint Reporting Forms.

BCG coverage

The coverage was estimated as daily BCG vaccination coverage until 365 days of life (Figure 1) using information collected in the year following the coverage age, e.g. BCG coverage at day 1 was estimated using vaccination information collected between day 1 and day 366. Only those with assessed vaccination status in the relevant period contributed to the BCG coverage estimate at a certain age. BCG is typically not provided after 1 year of age, and we thus assumed that the BCG coverage was constant from 1 year of age. Using known prior contact with a health facility (reported to be born at a health facility or registration of other vaccines according to the vaccination card); we calculated the possible BCG coverage by age in the non-restrictive scenario, if all children were vaccinated at their first registered contact with a health facility.

Meta-analysis of BCG studies

To assess the effect of BCG on all-cause mortality, we conducted a meta-analysis of all studies assessing the effect of BCG versus no BCG in Guinea-Bissau. We identified six published studies[1-6], and one manuscript under preparation[7]. Of the six studies, three were randomised trials conducted in low-weight infants, where children were randomised to BCG-at-birth or the usually delayed BCG. We used the effect of BCG in the neonatal period, since few children in the control group had received BCG at this age[6]. BCG-at-birth was associated with 72% lower mortality in trial I (Hazard

Ratio (HR): 0·28 (0·06-1·37)[5], 45% lower mortality in trial II (HR:0·55 (0·34-0·89)[4], 11% of children in the control group had received BCG at 28 days), and 30% lower mortality in trial III (HR: 0·70 (0·47-1·04)[6], 17% of children in the control group had received BCG at 28 days). The four other studies included in the meta-analysis were observational studies, one of which was conducting in low-weight children[2]. All observational studies compared mortality of BCG-vaccinated children with mortality of BCG-unvaccinated children[1-3,7]. In the meta-analysis, BCG was associated with 42% (19-58%) lower all-cause mortality (Figure 2).

In the three trials mentioned above, the effect of BCG on hospital admissions was also assessed. A combined estimate of the three trials found that BCG was associated with no effect on hospital admissions (Incidence Rate Ratio (IRR): 0·97 (0·72-1·31))[8]. We used the estimate at 28 days, as few children in the control group had received BCG at this age.

Vaccination costs

We obtained costs of BCG vaccines, syringes, safety boxes and freight from the national department of the Expanded Programme on Immunizations (EPI) [personal communication: Carlitos Bale, Director of the Vaccination Programme in Guinea-Bissau] (Table 1). We assumed that there would be no other additional costs related with disregarding the vial-opening policy. We thus assumed that the extra time spent by health staff to perform BCG vaccination would correspond to the time used explaining the mothers to return for vaccination another day and providing the vaccine to the majority of these children on a later occasion.

Currently a vial of BCG is opened if 10-12 children are present for vaccination. We therefore assumed 50% wastage of BCG vaccine in the baseline scenario. When disregarding the restrictive vial-opening policy, a vial of BCG should be opened even if only one child is present. We therefore assumed 95% wastage of BCG when disregarding the vial-opening policy (non-restrictive scenario). In both scenarios, we assumed 5% wastage of syringes and safety boxes.

Household costs of seeking BCG

We have previously estimated the household costs of seeking BCG vaccination in rural Guinea-Bissau to 1·89 USD (2016 value) per BCG vaccinated child[9]. Using the World Bank Consumer Price Index (CPI)[10], we updated this value to 1·92 USD in 2017 values. We found that mothers on average went to the health facility 1·26 times before succeeding in getting their child vaccinated[9]. Children born in health facilities can be BCG-vaccinated without actively seeking BCG-vaccination. We therefore estimated the proportion of children born in health facilities, and the proportion of these vaccinated at birth according to BHP data, and applied these proportions to the WHO/UNICEF 2017-birth cohort estimate (Table 3). For all children not vaccinated at birth, we assumed that their mother would seek vaccination 1·26 times in order to get the child vaccinated in the baseline scenario. In the non-restrictive scenario, we assumed that children born in health facilities would be vaccinated at birth and that all children would be vaccinated at the first contact with a health facility. Thus, mothers would bring their child for BCG vaccination only once.

We did not have corresponding information for the urban population, and we therefore used the household costs of seeking BCG vaccination obtained in the rural area for the whole population.

Costs of hospital admissions

Using data from the rural HDSS and from the National Hospital Simão Mendes, we assessed the median number of bed days for all-cause hospital admissions of children less than 5 years old. In both settings, the median number of bed days per hospital admission was 5 days.

We have estimated the cost incurred by the health system per hospital bed day as 14·92 USD in a micro-costing study from Guinea-Bissau in 2014[11], corresponding to a 2017-value of 15·58 USD using the World Bank CPI[10]. Hospital admissions are free for all children below 5 years of age in Guinea-Bissau[12]. We therefore assumed that the costs incurred by the household per hospital bed day only consisted of the value of the mothers time spent. We have previously estimated the value of a mother's time per day to 2·80 USD[13] (2·98 2017-USD) based on a regression model by Knight and colleagues estimating average monthly earnings in 2011[14].

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Appendix B. Details on model equations

The static mathematical model used for the present study was developed based on a model by Roy and colleagues[1]. Since we, in addition to the TB-specific effect, estimate the effect of BCG on all-cause deaths, we assumed leaky BCG vaccine efficacy as it is not plausible to assume that BCG-vaccinated children cannot die from any disease.

Appendix B1. TB model

The percentage change in TB deaths, $pTBD$, was calculated based on the estimated number of TB deaths in the non-restrictive scenario with no restrictive vial-opening policy, $nTBD_{non-restrictive}$, and the number of TB deaths in the current baseline situation, $nTBD_{baseline}$. The baseline scenario was calibrated to the 2016 Global Burden of Disease (GBD) estimate of paediatric TB deaths using the daily risk of TB death in children aged 0-4 years:

$$pTBD = ((nTBD_{non-restrictive} - nTBD_{baseline})/nTBD_{baseline}) \times 100 \quad \text{Equation 1}$$

$$nTBD_{baseline} = \sum_{t=0}^{t=1826} n_t \times R_{0-4} \quad \text{Equation 2}$$

Where n_t was the sum of children alive at age t, and R_{0-4} was the daily risk of TB death in children aged 0-4. The daily risk of TB death is the risk per person per day. The population at risk was adjusted for all-cause deaths on a daily basis; assuming BCG had no effect on all-cause mortality.

We estimated the number of TB deaths in children aged 0-4 in the non-restrictive scenario using:

$$nTBD = (\sum_{t=0}^{t=1826} V_t \times RV_{0-4}) + (\sum_{t=0}^{t=1826} U_t \times RU_{0-4}) \quad \text{Equation 3}$$

Where V_t was the number of BCG-vaccinated children at time t, $RV_{0-4}$was the daily individual risk of TB death in BCG-vaccinated children aged 0-4, U_t was the number of BCG-unvaccinated children at time t, and RU_{0-4} was the daily risk of TB death in BCG-unvaccinated children aged 0-4.

The individual daily risk of TB death in unvaccinated children, $RU_{0-4,}$ was estimated using

$$nTBD = (\sum_{t=0}^{t=1826} V_t \times RU_{0-4} \times RR) + (\sum_{t=0}^{t=1826} U_t \times RU_{0-4})$$

$$RU_{0-4} = \frac{nTBD}{(\sum_{t=0}^{t=1826} V_t \times RR) + (\sum_{t=0}^{t=1826} U_t)} \quad \text{Equation 4}$$

Where RR was the risk-ratio of TB death in BCG-vaccinated children compared with BCG-unvaccinated children.

The individual daily risk of TB death among BCG-vaccinated children, $RV_{0-4,}$ was calculated using

$$RV_{0-4} = RR \times RU_{0-4} \quad \text{Equation 5}$$

We estimated the number of TB deaths at time t in the non-restrictive scenario for BCG-vaccinated ($nTBDv_{tNR}$) and BCG-unvaccinated children ($nTBDu_{tNR}$), respectively:

$$nTBDv_{tNR} = RV_{0-4} \times V_{tNR} \quad \text{Equation 6}$$

$$nTBDu_{tNR} = RU_{0-4} \times U_{tNR} \quad \text{Equation 7}$$

Where RV_{0-4} was the daily individual risk of TB death among BCG-vaccinated children aged 0-4 (calculated based on the baseline scenario – Equation 5), V_{tNR} was the number of BCG-vaccinated

children at time *t* in the non-restrictive scenario, $RU_{0\text{-}4}$ was the daily individual risk of TB death among BCG-unvaccinated children aged 0-4 (calculated based on the baseline scenario – Equation 4), and U_{tNR} was the number of BCG-unvaccinated children at time *t* in the non-restrictive scenario.

We then calculated the total number of TB deaths in children aged 0-4 in the non-restrictive scenario.

$$nTBD_{0-4} = \sum_{t=0}^{t=1826} nTBDv_{tNR} + \sum_{t=0}^{t=1826} nTBDu_{tNR} \quad \text{Equation 8}$$

Using the case-fatality ratio, CFR, we estimated the number of TB cases:

$$nTB_{0-4} = {}^{nTBD_{0-4}}/_{CFR} \quad \text{Equation 9}$$

Appendix B2. All-cause deaths model

The effect of disregarding the restrictive BCG vial-opening policy on all-cause deaths was estimated using the same approach as for TB deaths, but with minor adjustments as we had more precise data for all-cause deaths allowing for age-specific mortality estimates (Table 1).

The percentage change in all-cause deaths, $pACD$, was calculated based on number of all-cause deaths in the non-restrictive scenario, $nACD_{non-restrictive}$, and the number of all-cause deaths in the baseline scenario, $nACD_{baseline}$. In the baseline scenario, we used paediatric mortality estimates obtained from the Bandim Health Project 2012-2017 (Appendix A), using the daily risk of all-cause death per person at age *t*.

$$pACD = ((nACD_{non-restrictive} - nACD_{baseline})/nACD_{baseline}) \times 100 \quad \text{Equation 10*}$$

$$nACD_t = n_t \times R_t \quad \text{Equation 11}$$

Where n_t was the sum of children alive at age *t*, and R_t was the daily risk of all-cause death at age *t*.

Leading to the total number of all-cause deaths in children aged 0-4, $nACD_{0-4baseline}$:

$$nACD_{0-4baseline} = \sum_{t=0}^{t=1826} nACD_{tbaseline} \quad \text{Equation 12}$$

We estimated the number of all-cause deaths at age *t* in the non-restrictive scenario using:

$$nACD_t = (V_t \times RV_t) + (U_t \times RU_t) \quad \text{Equation 13}$$

Where V_t was the number of BCG-vaccinated children at time *t*, RV_t was the daily individual risk of all-cause death in BCG-vaccinated children at age *t*, U_t was the number of BCG-unvaccinated children at time *t*, and RU_t was the daily risk of all-cause death in BCG-unvaccinated children at age *t*.

Given $RV_t = RU_t \times RR$, the individual daily risk of all-cause death in unvaccinated children at age *t*, RU_t, was estimated using

$$nACD_t = (V_t \times RU_t \times RR) + (U_t \times RU_t)$$

$$RU_t = \frac{nACD_t}{(V_t \times RR) + (U_t)} \quad \text{Equation 14}$$

Where RR was the risk-ratio of all-cause death in BCG-vaccinated children compared with BCG-unvaccinated children.

The individual daily risk of all-cause death at time *t* among BCG-vaccinated children, RV_t, was calculated using

$$RV_t = RR \times RU_t \quad \text{Equation 15*}$$

We estimated the number of all-cause deaths at time *t* in the non-restrictive scenario for BCG-vaccinated ($nACDv_{tNR}$) and BCG-unvaccinated children ($nACDu_{tNR}$), respectively:

$$nACDv_{tNR} = RV_t \times V_{tNR} \quad \text{Equation 16*}$$

$$nACDu_{tNR} = RU_t \times U_{tNR} \quad \text{Equation 17*}$$

Where RV_t was the daily individual risk of all-cause death at time *t* among BCG-vaccinated children (calculated based on the baseline scenario – Equation 15), V_{tNR} was the number of BCG-vaccinated children at time *t* in the non-restrictive scenario, RU_t was the daily individual risk of all-cause death at time *t* among BCG-unvaccinated children (calculated based on the baseline scenario – Equation 14), and U_{tNR} was the number of BCG-unvaccinated children at time *t* in the non-restrictive scenario.

We then calculated the total number of all-cause deaths in children aged 0-4 in the non-restrictive scenario.

$$nACD_{0-4} = \sum_{t=0}^{t=1826} nACDv_t + \sum_{t=0}^{t=1826} nACDu_t \quad \text{Equation 18*}$$

Appendix B3. Details on all-cause hospital admission model

The daily age-specific risk of all-cause hospital admission (risk of all-cause hospital admission per person per day) was estimated using the BHP age-stratified hospital admission data (Methods explained in Appendix A). Based on the daily risk of all-cause hospital admission and the meta-estimate of the effect of BCG on all-cause hospital admission (Appendix A), we calculated the daily risk of all-cause hospital admission in BCG-vaccinated and BCG-unvaccinated children, respectively.

The daily risk of all-cause hospital admission was applied to the WHO/UNICEF birth cohort estimate of 2017 [personal communication: Carlitos Bale, Director of the Vaccination Programme in Guinea-Bissau]. We calculated the percentage change in all-cause hospital admission, $pACH$, based on number of all-cause hospital admissions in the non-restrictive scenario, $nACH_{non-restrictive}$, and the number of all-cause hospital admissions in the baseline scenario, $nACH_{baseline}$. We used paediatric hospital admissions estimates obtained from the BHP surveillance data in 2012-2017 (Appendix A) to obtain the daily risk of all-cause hospital admission at age *t*.

$$pACH = ((nACH_{non-restrictive} - nACH_{baseline})/nACH_{baseline}) \times 100 \quad \text{Equation 19}$$

$$nACH_t = n_t \times R_t \quad \text{Equation 20}$$

Where n_t was the sum of children alive at age t, and R_t was the daily risk of all-cause hospital admission at age t.

Leading to the total number of all-cause hospital admissions in children aged 0-4, $nACH_{0-4baseline}$:

$$nACH_{0-4baseline} = \sum_{t=0}^{t=1826} nACH_{tbaseline} \quad \text{Equation 21}$$

We estimated the number of all-cause hospital admissions at age t in the non-restrictive scenario using:

$$nACH_t = V_t \times RV_t + U_t \times RU_t \quad \text{Equation 22}$$

Where V_t was the number of BCG-vaccinated children at time t, RV_t was the daily individual risk of all-cause hospital admission in BCG-vaccinated children at age t, U_t was the number of BCG-unvaccinated children at time t, and RU_t was the daily risk of all-cause hospital admission in BCG-unvaccinated children at age t.

The individual daily risk of all-cause hospital admission in unvaccinated children at age t, RU_t, was estimated using

$$nACH_t = (V_t \times RU_t \times RR) + (U_t \times RU_t)$$

$$RU_t = \frac{nACH_t}{(V_t \times RR) + (U_t)} \quad \text{Equation 23}$$

Where RR was the risk-ratio of all-cause hospital admission in BCG-vaccinated children compared with BCG-unvaccinated children.

The individual daily risk of all-cause hospital admission at time t among BCG-vaccinated children, RV_t, was calculated using

$$RV_t = RR \times RU_t \quad \text{Equation 24}$$

We estimated the number of all-cause hospital admissions at time t in the non-restrictive scenario for BCG-vaccinated ($nACHv_{tNR}$) and BCG-unvaccinated children ($nACHu_{tNR}$), respectively:

$$nACHv_{tNR} = RV_t \times V_{ta} \quad \text{Equation 25}$$

$$nACHu_{tNR} = RU_t \times U_{ta} \quad \text{Equation 26}$$

Where RV_t was the daily individual risk of all-cause hospital admission at time t among BCG-vaccinated children (calculated based on the baseline scenario – Equation 24), V_{tNR} was the number of BCG-vaccinated children at time t in the non-restrictive scenario, RU_t was the daily individual risk of all-cause hospital admission at time t among BCG-unvaccinated children (calculated based on the baseline scenario – Equation 23), and U_{tNR} was the number of BCG-unvaccinated children at time t in the non-restrictive scenario.

We then calculated the total number of all-cause hospital admissions in children aged 0-4 in the non-restrictive scenario.

$$nACH_{0-4} = \sum_{t=0}^{t=1826} nACHv_t + \sum_{t=0}^{t=1826} nACHu_t \quad \text{Equation 27}$$

Appendix B4. Details on incremental costs of disregarding the restrictive vial-opening policy

We estimated the vaccine costs in each scenario based on the number of children vaccinated by 12 months of age, $nBCG_{12}$, the vaccine wastage factor, *WF*, and the price per dose including freight costs, $price_{BCG}$:

$$vaccine_{costs} = nBCG_{12} \times WF \times price_{BCG} \quad \text{Equation 28}$$

The wastage factor was based on the vaccine wastage assumed in each scenario. In the baseline scenario, we assumed 50% vaccine wastage corresponding to a wastage factor of 2. In the non-restrictive scenario, we assumed 95% vaccine wastage corresponding to a wastage factor of 20.

We calculated the injection supply costs as a summation of the costs of application syringe costs, mixing syringe costs and safety box cost:

$$injection\ supply_{costs} = application\ syringe_{costs} + mixing\ syringe_{costs} + safety\ box_{costs} \quad \text{Equation 29}$$

For each item, we calculated the costs by multiplying the number of items used by the item unit price including freight:

$$cost_{item} = n_{item} \times price_{item} \quad \text{Equation 30}$$

Number of items used were calculated by equations 31-33 assuming 5% wastage of each item, resulting in a wastage factor of 1·05:

$$n_{app.syr} = nBCG_{12} \times WF \quad \text{Equation 31}$$

$$n_{mix.syr} = \frac{nBCG_{12} \times WF_{BCG}}{vial\ size} \times WF_{mix.syr} \quad \text{Equation 32}$$

$$n_{safety\ box} = \frac{n_{app.syr} + n_{mix.syr}}{safety\ box_{capacity}} \times WF_{safety\ box} \quad \text{Equation 33}$$

Where vial size was assumed to be 20 dose vials and safety box capacity was assumed to be 100 syringes as previously used[2].

We calculated the incremental costs of averted/additional TB hospital admissions and all-cause hospital admissions:

$$costs_{hosp} = n_{hosp} \times n_{bedday} \times (cost_{bedday} + cost_{mother}) \quad \text{Equation 34}$$

Where n_{hosp} was the number of additional admissions (if negative, number of averted admissions) in the non-restrictive scenario, n_{bedday} was 60 days for TB hospital admissions and 5 days for all-cause hospital admissions, $cost_{bedday}$ was the cost incurred by the health system per bed day and $cost_{mother}$ was the cost incurred by the household per bed day.

We calculated the household costs of seeking BCG vaccination. The number of children vaccinated at birth in the baseline scenario was estimated based on the proportion of children born in health

facilities multiplied by the proportion of these vaccinated at birth. In the non-restrictive scenario, it was assumed that all children born in health facilities were vaccinated at birth.

$$n_{noBCG} = n_{total} - n_{total} \times p_{HF} \times p_{BCG} \quad \text{Equation 35}$$

Where n_{total} was the birth cohort of 2017, p_{HF} was the proportion born in health facilities and p_{BCG} was the proportion of children vaccinated at birth in the health facility, and n_{noBCG} was the number of children not BCG vaccinated at birth, and for whom the mother should seek vaccination. As we have previously found that mothers on average bring their child for vaccination 1·26 times before obtaining BCG vaccination at cost of USD 1·92 per time, we used this information to calculate the household costs of seeking BCG vaccination in each scenario:

$$HHcost_{BCG} = n_{noBCG} \times 1.26 \times 1.92\ USD \quad \text{Equation 36}$$

The incremental household costs of seeking BCG when disregarding the restrictive vial-opening policy was obtained by subtracting the household costs in the non-restrictive scenario from the household costs in the baseline scenario.

Finally, we summed the incremental vaccination costs, the incremental hospital admission costs and incremental household costs of seeking BCG vaccination, and obtained the total incremental costs of disregarding the restrictive vial-opening policy.

Appendix B5. Details on cost-effectiveness estimates

To obtain an estimate of the cost-effectiveness of disregarding the restrictive vial-opening policy, we initially calculated life years gained (LYG) from disregarding the restrictive vial-opening policy accounting for TB-specific and all-cause effects of BCG vaccine.

Using the estimated number of TB deaths, *TBD,* and all-cause deaths, *ACD,* averted, and the World Bank estimate of a life expectancy, *LE*, of 57 years at birth in Guinea-Bissau in 2017[3], we calculated the number of LYG per year accounting for TB-specific, LYG_{yTB}, and all-cause effects, LYG_{yAC}, respectively:

$$LYG_{yTB} = TBD \times (LE - y) \quad \text{Equation 37}$$

$$LYG_{yAC} = ACD \times (LE - y) \quad \text{Equation 38}$$

Thus, the number of TB deaths/all-cause deaths averted per year of age was multiplied by the remaining life expectancy.

In the base case analysis, we discounted future life years by 3% per year as recommended by WHO[4]:

$$LYG_{disc} = (D_{averted}/0.03) \times (1 - e^{-0.03 \times (LE-y)}) \quad \text{Equation 39}$$

The incremental cost-effectiveness ratio (ICER) of disregarding the restrictive vial opening policy were calculated accounting for TB-specific and all-cause effects, respectively, using the same approach, described by equation 40-42:

$$ICER_{LYG} = \frac{Total\ incremental\ costs}{LYG_{non-restrictive}}$$ Equation 40

$$ICER_{LYGdisc} = \frac{Total\ incremental\ costs}{LYGdisc_{non-restrictive}}$$ Equation 41

$$ICER_{Daverted} = \frac{Total\ incremental\ costs}{Daverted_{non-restrictive}}$$ Equation 42

Appendix B6. Details on TB model assuming all-or-nothing BCG vaccine efficacy

As a sensitivity analyses, we changed the model structure from assuming leaky BCG vaccine efficacy to a model structure assuming all-or-nothing BCG vaccine efficacy, as in the original model developed by Roy and colleagues[1]. We calculated the absolute and percentage change in number of TB deaths per birth cohort during the first 5 years of life in the non-restrictive scenario compared with the baseline scenario. The percentage change in TB deaths, $pTBD$, was calculated based on number of TB deaths in the non-restrictive scenario, $nTBD_{non-restrictive}$, and the number of TB deaths in the baseline scenario, $nTBD_{baseline}$. As in the main analysis, the baseline scenario was calibrated to the 2016 Global Burden of Disease estimates[5] of paediatric TB deaths using the daily risk of TB death in unprotected children aged 0-4.

$$pTBD = ((nTBD_{non-restrictive} - nTBD_{baseline})/nTBD_{baseline}) \times 100$$ Equation 43

The number of paediatric TB deaths per birth cohort in the first 5 years of life (*nTBD*) was estimated to be:

$$nTBD = \sum_{t=0}^{t=1826} nUP_t \times R_{0-4}$$ Equation 44

Where *t* was the age in days; nUP_t was the number of unprotected children at age *t*; R_{0-4} was the daily individual risk of TB death in unprotected children aged 0-4 years.

The number of unprotected children at age *t* (nUP_t) was estimated to be the number of unvaccinated children at age *t* plus the number of vaccinated children with insufficient immune response to prevent TB death at age *t*:

$$nUP_t = U_t + \left(V_t \times (1 - VE)\right)$$ Equation 45

Where U_t was the number of unvaccinated children at age *t*, V_t was the number of vaccinated children at age *t*, and *VE* was the vaccine efficacy (proportion), and:

$$U_t = \left((BC - D_t) \times (1 - Cov_t)\right)$$ Equation 46

$$V_t = (BC - D_t) \times Cov_t$$ Equation 47

$$VE = 1 - RR$$ Equation 48

Where, *BC* was the annual number of live births in Guinea-Bissau in 2017, D_t was the number of all-cause deaths at age *t*, Cov_t was the proportion of BCG-vaccinated children at *t*, and *RR* was the rate ratio of TB deaths in BCG-vaccinated children compared with BCG-unvaccinated children.

The daily individual risk of TB death in unprotected children aged 0-4 years (R_{0-4}) was:

$$R_{0-4} = Mort_{0-4} / \sum n_{0-4}$$ Equation 49

Where $Mort_{0-4}$ was the number of TB deaths in each age group, and $\sum n_{0-4}$ was the sum of unprotected person days.

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Appendix C. Additional results and discussion

Leaky versus all-or-nothing BCG vaccine efficacy

For the effect of disregarding the BCG vial opening policy on TB-specific deaths, we assessed the impact of changing the model structure from a model assuming a leaky BCG vaccine efficacy as in the main analyses to a model assuming an all-or-nothing vaccine efficacy. Using an all-or-nothing vaccine efficacy, the number of TB deaths were 33 (UR: 13-89) in the baseline scenario, and 29 (UR: 11-79) when disregarding the vial opening policy. Thus, disregarding the vial-opening policy was associated with 11.0% (0.5-28.8%) fewer TB deaths, which yields the same results as the main analysis (Table 4).

All-cause deaths between day 0 and 42

Disregarding the restrictive vial-opening policy was estimated to reduce all-cause mortality 14.5% (6.3-20.7%) between day 0 and day 42, from 1,922 (UR: 1,612-2,318) all-cause deaths in the baseline scenario to 1,648 (UR: 1,352-2,028) (Table 4).

As we were not able to account for interaction and sequence of vaccines in the model, we assessed the impact of disregarding the vial-opening policy in a limited period between birth and 42 days of life to exclude possible interaction with other vaccines. We found that 277 (UR: 120-415) all-cause deaths could be averted in this early period by disregarding the vial-opening policy. More data are needed in order to make reasonable and data driven assumptions to account for the impact of interactions of vaccines for use in future models.

Supplementary strengths and limitations

The estimate of efficacy of BCG against all-cause mortality was based on available studies from Guinea-Bissau. The three randomised trials enrolled only low-weight children for whom BCG is normally delayed. We are currently conducting a randomised trial enrolling both low-birth-weight children and normal-birth-weight children in rural Guinea-Bissau[1], so future analyses could be based upon data from normal birth weight children also.

BCG is recommended at birth for children without symptoms of an immunodeficiency[2], since BCG is associated with severe adverse events in children with severe immunodeficiencies[2]. We did not account for the HIV prevalence and contradiction in this group at birth, as in Guinea-Bissau, the data on HIV prevalence in children is scarce, and few children present symptoms of immunodeficiency at birth so almost all receive BCG.

Due to lack of data on TB hospital admissions, we used identified TB cases in Guinea-Bissau as a proxy for TB hospital admissions in the baseline scenario. The estimates of TB cases and TB hospital admissions in each scenario were calculated using a CFR obtained from the GBD estimates of TB incidence and TB mortality, and thus, the results must be interpreted with caution as they are surrounded by much uncertainty.

We used the household costs of seeking BCG calculated from a recent study conducted in rural Guinea-Bissau. The availability of data on household costs of seeking BCG vaccination was a strength to the study; however, since we did not have corresponding estimates from urban Guinea-Bissau, we applied the estimates from the rural area for all children. The household costs of seeking

BCG may differ between the urban and the rural population. We did not include out-of-pocket medical expenses incurred by the household in relation to hospital admissions. Hospital admissions in Guinea-Bissau are free for children below 5 years of age[3], thus, we expect the out-of-pocket payments to be few.

References

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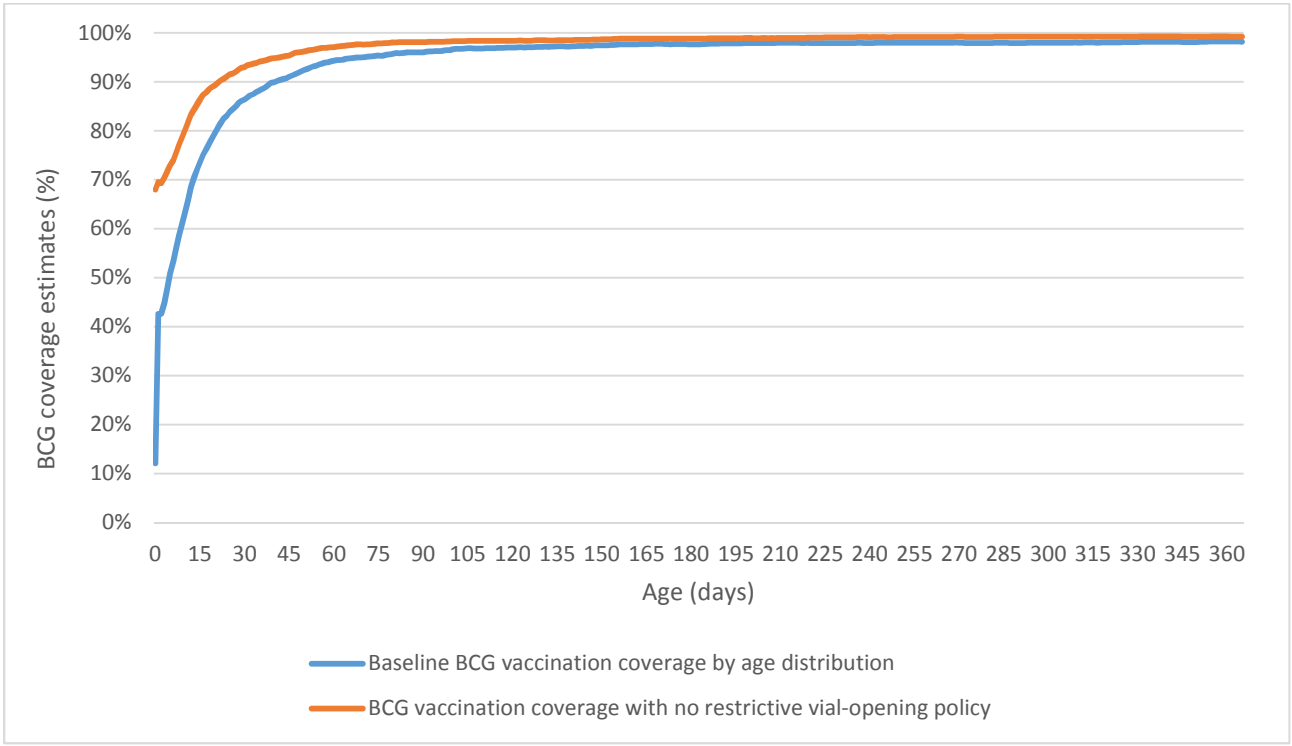
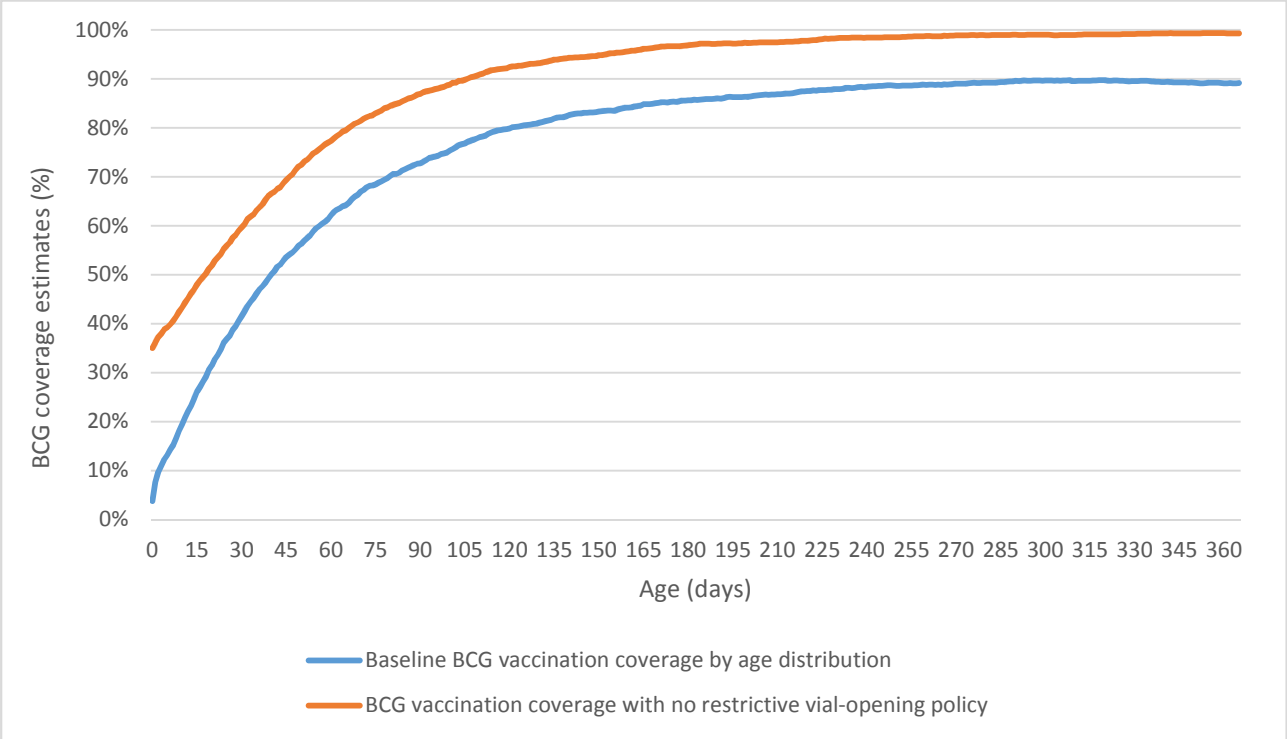
Appendix D. Supplementary Figures and tables

Appendix D1. BCG coverage estimates in urban Guinea-Bissau in the baseline scenario and the non-restrictive scenario



Appendix D2. BCG coverage estimates in rural Guinea-Bissau in the baseline scenario and the non-restrictive scenario



Appendix D3. Cost-effectiveness analyses of disregarding the restrictive BCG vial-opening policy by urban and rural population

	URBAN POPULATION		RURAL POPULATION	
	Baseline scenario	**Non-restrictive scenario**	**Baseline scenario**	**Non-restrictive scenario**
Birth population of 2017	19,018	19,018	50,194	50,194
Total number of children born in health facilities	12,791	12,791	7,791	7,791
Number of children BCG-vaccinated at birth	7,987	12,791	1,940	7,791
Number of children not BCG-vaccinated at birth	11,031	6,227	17,078	11,227
Total times seeking BCG vaccination	1.26	1.00	1.26	1.00
Vaccine wastage	50 %	95 %	50 %	95 %
BCG coverage at 12 months of age	98 %	99 %	89 %	99 %
Total household costs of seeking BCG vaccination	26,626 USD	11,929 USD	41,224 USD	21,508 USD
Total BCG vaccine costs	7,621 USD	77,008 USD	18,257 USD	203,330 USD
Total Injection supply costs	1,136 USD	1,828 USD	2,722 USD	4,826 USD
Cost per bed day incurred by the health system	15.58 USD	15.58 USD	15.58 USD	15.58 USD
Household costs of accompanying a child per bed day hospital admission	2.98 USD	2.98 USD	2.98 USD	2.98 USD
TB-specific effects only	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)
Total number of paediatric TB deaths	8 (3 to 22)	8 (3 to 21)	26 (10 to 69)	21 (8 to 57)
Total LYG		12		234
Total LYG discounted		6		115
Total number of paediatric TB hospital admissions	11	11	36	30
Median bed day per TB hospital admission	60 days	60 days	60 days	60 days
Total costs of TB hospital admissions	12,638 USD	12,314 USD	39,740 USD	33,218 USD
Incremental costs of disregarding the restrictive vial-opening policy (including vaccination costs, household costs and costs averted by TB hospital admissions)		55,058 USD		160,939 USD
ICER (USD/LYG)		2,473 (423 to 23,733)		391 (62 to 3,955)
ICER (USD/discounted LYG)		5,081 (869 to 48,759)		799 (127 to 8,088)
ICER (USD/TB death averted)		137,303 (23,493 to 1,317,677)		21,528 (3,430 to 217,936)
All-cause effects				
Total number of all-cause deaths	1,071 (897 to 1,302)	961 (795 to 1,180)	3,787 (3,303 to 4,386)	3,467 (2,992 to 4,048)
Total LYG		6,375		17,927
Total LYG discounted		3,054		8,656
Total number of all-cause hospital admissions	1,806	1,816	4,004	4,012
Median bed day per all-cause hospital admission	5 days	5 days	5 days	5 days
Total costs of all-cause hospital admissions	167,510 USD	168,459 USD	371,471 USD	372,135 USD
Incremental costs of disregarding the restrictive vial-opening policy (including vaccination costs, household costs and costs averted by hospital admissions)		56,331 USD		168,126 USD
ICER (USD/LYG)		5 (3 to 11)		6 (3 to 14)
ICER (USD/discounted LYG)		10 (6 to 23)		11 (6 to 27)
ICER (USD/all-cause death averted)		273 (168 to 635)		288 (165 to 742)

USD: US Dollar 2017 value, LYG: Life year gained, ICER: Incremental cost-effectiveness ratio

Appendix D4. Main results of disregarding the restrictive vial-opening policy with and without assumed correlation of mortality estimates and TB incidence estimates

	Baseline scenario	**Non-restrictive scenario**	**Absolute change**	**Percentage change**
TB-specific effects	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)	Median (95% Uncertainty Range)
Total number of paediatric TB deaths[1]	33 (13 to 89)	29 (11 to 79)	-4 (-15 to 0)	-11.0% (-28.8% to -0.5%)
Total number of paediatric TB deaths - assuming no correlations[2]	35 (18 to 74)	31 (15 to 66)	-4 (-13 to 0)	-11.0% (-28.8% to -0.5%)
All-cause effects				
Total number of all-cause deaths[1]	4,820 (4,309 to 5,425)	4,429 (3,920 to 5,028)	-392 (-624 to -158)	-8.1% (-12.7% to -3.3%)
Total number of all-cause deaths - assuming no correlations[3]	4,833 (4,632 to 5,041)	4,440 (4,155 to 4,743)	-394 (-615 to -160)	-8.1% (-12.7% to -3.3%)

[1] Assuming perfect correlation between male and female TB incidence, and perfect correlation between male and female TB mortality

[2] Assuming no correlations between data input

[3] Assuming perfect correlation between all-cause mortality by age