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Can earlier BCG-Japan and OPV vaccination reduce early infant mortality? A cluster-randomised trial in Guinea-Bissau

Sanne Marie Thysen ,[1,2] Igualdino da Silva Borges,[1] Jailson Martins,[1] Alexander Dahl Stjernholm,[1] Jesper Sloth Hansen,[1] Leontino Manuel Vieira da Silva,[1] Justiniano Sebastião Durga Martins,[1] Aksel Jensen,[1,3] Amabelia Rodrigues,[1] Peter Aaby,[1,2] Christine Stabell Benn,[1,2,4] Ane Baerent Fisker [1,2]

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For numbered affiliations see end of article.

Correspondence to
Dr Sanne Marie Thysen;
s.thysen@bandim.org

ABSTRACT

Objective To assess the effect of providing BCG and oral polio vaccine (OPV) at an early home visit after delivery.

Design Cluster-randomised trial, randomising 92 geographically defined clusters 1:1 to intervention/control arms.

Setting Bandim Health Project Health and Demographic Surveillance System, Guinea-Bissau.

Participants 2226 newborns enrolled between July 2016 and August 2019.

Interventions In both arms, newborns received a home visit within 72 hours after birth. In intervention clusters (n=46), BCG and OPV were provided at the home visit.

Main outcome measure Rates of non-accidental mortality were compared in Cox proportional hazards models from (last of) day 1 or enrolment, until (first of) day 60 or registration of non-trial vaccines.

Results A total of 35 deaths (intervention: 7, control: 28) were registered during the trial. Providing BCG and OPV reduced non-accidental early infant mortality by 59% (8–82%). The intervention also reduced non-accidental hospital admissions. The intervention had little impact on growth and BCG scarring and tended to increase the risk of consultations.

Conclusions The trial was stopped early due to lower-than-expected enrolment and event rates when 33% of the planned number of newborns had been enrolled. Despite the small size of the trial, the results support that early BCG and OPV vaccinations are beneficial and reduce early child mortality and morbidity.

Trial registration number ClinicalTrials.gov Registry (NCT02504203).

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Vaccines play a major role in reducing the risk of infections and mortality worldwide.
⇒ The vaccines scheduled at birth, BCG vaccine and the oral polio vaccine (OPV), may have reduced early infant mortality from causes other than tuberculosis and polio.
⇒ The WHO-recommended home visits are currently not used as an opportunity to provide vaccines at birth, and vaccines scheduled at birth are often delayed.

WHAT THIS STUDY ADDS

⇒ This study demonstrated that providing BCG and OPV shortly after birth reduced early infant non-accidental mortality and hospital admissions considerably.
⇒ The trial also indicated that implementing home visits with vaccination may not be a feasible way to ensure early vaccination in Guinea-Bissau, as a large number of newborns were not reached within 72 hours of delivery.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study highlights the importance of early vaccination; thus, local practices such as restrictive vial opening should be removed, so vaccines are provided as early as possible.
⇒ The WHO recommendation of providing home visits after birth should be revised to include provision of BCG and OPV wherever possible.

INTRODUCTION

Despite a global child mortality decline of 59% between 1990 and 2019, neonatal mortality remains high.[1] It is estimated that 47% of the 5.2 million under-5 deaths occur within the first month of life.[1] To reduce the high neonatal mortality, the WHO recommends three home visits after birth,[2] including an assessment of the baby, promotion of exclusive breast feeding and cord care.[2] The recommendation is based on nine trials, which found that home visits reduced neonatal mortality by 24% (relative risk (RR)=0.76,

95% CI 0.62 to 0.92).[2] Providing vaccines is not part of the recommendations.[2] Vaccines have contributed significantly to the decline in child mortality. There is accumulating evidence supporting that the impact of vaccines is broader than the anticipated effect from reduction of target diseases. The BCG vaccine is recommended at birth to protect against tuberculosis. Randomised trials[3 4] and immunological studies[5] support that the BCG vaccine has non-specific effects and can reduce mortality from non-tuberculosis infections. Thus, current evidence indicates a broader benefit of giving BCG at birth.

Despite BCG being recommended at birth, BCG vaccination is often delayed in low-income countries.[6] A major reason for this delay is the focus on reducing vaccine wastage. Freeze-dried vaccines like BCG must be used within 6 hours after reconstitution with their diluent. The BCG vaccine contains 20 infant doses, and a vial of BCG vaccine is often not opened unless 10–12 children are present for vaccination. Hence, there are many missed vaccination opportunities. In 2017, only half the children in rural Guinea-Bissau were vaccinated during the first 2 weeks of life.[7] As 73% of neonatal deaths occur in the first week of life,[8] much could potentially be gained by early vaccination of all neonates. In addition to the BCG vaccine, the oral polio vaccine (OPV) is also recommended at birth.[9] OPV at birth (OPV0) is part of the strategy for eradicating polio.[10] As for the BCG vaccine, increasing evidence supports that OPV has beneficial non-specific effects.[11] A trial comparing BCG only versus BCG+OPV0 in neonates found that OPV0 reduced infant mortality by 32% (95% CI: 0% to 55%) before the next OPV campaign.[12]

In the present trial, we aimed to test the impact of combining the WHO-recommended home visits with provision of the vaccines recommended at birth (BCG and OPV0). We hypothesised that a single home visit after birth with provision of BCG and OPV0 could reduce early infant non-accidental mortality by 40%. In the trial, we assessed the effect of BCG and OPV provided at a single home visit within 72 hours after birth on early infant mortality and morbidity. The trial was stopped early for futility due to low recruitment numbers and low mortality.

METHODS

Study setting

This cluster-randomised trial was conducted in Guinea-Bissau, where the WHO-recommended home visits have not yet been implemented. It was implemented within the Bandim Health Project's (BHP) urban and rural Health and Demographic Surveillance System (HDSS) sites.[13 14] The HDSS sites follow women and children through regular household visits. In addition to the HDSS set-up, we implemented a platform with community key informants (CKIs) and study nurses. The trial was initiated in the rural health regions in July 2016 before being expanded to the urban study area in September 2017. Due to lower-than-expected enrolment rates, enrolments were suspended in the Cacheu region and in the urban study area in March 2019 and the trial was later stopped in the remaining regions in August 2019. Details on decision to expand and stop can be found in online supplemental file 1. The trial and the HDSS sites are described in further detail in the published trial protocol.[15]

In the rural study area, study nurses were selected among nurses working at a health centre with a catchment area corresponding to the study clusters. Hence, they were able to conduct home visits in addition to their normal routines. For the urban study area, the BHP employed a study nurse full-time.

In the rural areas, CKIs ensured timely information about deliveries. The CKIs were selected among residents in each rural village and collected information about pregnancies and deliveries. In the urban area, we took advantage of the close follow-up by the field assistants, who circulate the area daily.

Randomisation and blinding

The trial was a non-blinded cluster-randomised trial, where 92 clusters were randomised 1:1 to two different treatment groups, stratified by region and pre-trial mortality level (high/low, split at median). Clusters were defined as village clusters in the rural area and as subdistrict zones in the urban area. Clusters were randomised prior to study start using computer-generated random numbers.[15]

In both trial arms, newborns were visited as soon as possible after birth. In intervention clusters, the study nurses provided standard care (umbilical cord and skin care, encourage skin-to-skin contact, examine and weigh the newborn) and vaccines (BCG, OPV0) to children at the home visits; in control clusters, children only received standard care and were informed about vaccination recommendations, but no vaccines were administered.

Safety

The trial is registered at ClinicalTrials.gov (NCT02504203).

A Data Safety and Monitoring Board (DSMB), consisting of a paediatrician (Anja Poulsen, Rigshospitalet, Denmark), a statistician (Morten Frydenberg, Aarhus University, Denmark) and an epidemiologist (Torben Sigsgaard, Aarhus University, Denmark), was overseeing the trial and was involved in the discussions of ending the trial before target sample size was reached.

Participants

Inclusion criteria

All children registered during pregnancy were eligible for the study. Children were only enrolled if they were visited within 72 hours after birth.

Exclusion criteria

Children were excluded if they:

- Were already BCG vaccinated.

- Were moribund (expected to not survive the next 24 hours, as evaluated by the study nurse at the enrolment visit).
- Lived in a rural village where the BHP mobile teams coincidentally visited the same day (and vaccinated all children).

Informed consent

At the registration of a new pregnancy, the trial was explained and oral and written consent was obtained by the BHP nurse at the two monthly household visits in the rural area and by the fieldwork assistant at monthly visits in the urban area.[15] At the time of the home visit, confirmation of the consent was obtained by the study nurse. If women were not able to show the written consent form, a new written consent form was made.

Enrolment

All newborns received a home visit by a study nurse shortly after the birth was reported to the study staff, if possible, on the same day. Newborns born in healthcare facilities were enrolled at home after they had been discharged. At the home visit, the nurse obtained confirmation of consent for participation before revealing the randomisation. For all newborns, the nurse examined and weighed the newborn, encouraged skin-to-skin contact and if necessary performed umbilical cord care.[15]

Procedures

Intervention

For children in the control clusters, the nurse informed about vaccination opportunities (vaccination at closest health centre or vaccination by BHP nurse at subsequent village visit). For children in the intervention clusters, the nurse administered BCG and OPV0.

The BCG vaccine was administered by intradermal injection of 0.05 mL vaccine. All nurses were trained in correct vaccination technique prior to trial start and supervised intensively during the trial. The Tokyo 172 BCG strain from BCG-Japan was used for the study (batch numbers: 1578, 1599, 1621, 1624 and 1630). The vaccine is prequalified by the WHO.

The OPV0 was administered as two oral drops. The OPV was supplied from the national vaccination programme and thus strain, manufacturer and batch varied during the course of the trial (Polio Sabin from GlaxoSmith-Kline, Belgium; BioPolio from Bharat Biotech, India; and Bivalen from Biofarma, Indonesia).

Follow-up

Children were followed up until age 60 days at which age most children in the control group would also be vaccinated. All children enrolled in the trial were visited and an interview was conducted as early as possible after this age. In the rural area, the interviews were carried out in connection with the bimonthly HDSS visits. At follow-up visits, information on vital status, breastfeeding status, supplementary feeding, mid-upper arm circumference (MUAC), vaccinations, hospital admissions and whether the child had received interventions provided in campaigns was collected. For hospital admissions, information on cause, including accidents, was obtained. For all deaths, at a subsequent visit, a verbal autopsy was conducted, where information on cause of death was obtained.

Outcomes

The primary outcome was 'non-accidental mortality' during follow-up. The follow-up began at the time of the home visit or at 24 hours after birth whichever came last, thereby excluding deaths on the day of birth. Follow-up ended at the subsequent follow-up visit by the BHP (at that time point, all unvaccinated children were offered BCG/OPV), the date when the first non-trial vaccine was registered by BHP, migration, death due to accident or 60 days of age, whichever came first. All children living in intervention and control clusters were followed through the HDSS routines with collection of information on vital status for all children (figure 1).

Secondary outcomes

Prespecified secondary outcomes were 'non-accidental hospital admission', 'severe morbidity' (composite outcome of non-accidental mortality and non-accidental hospital admissions) and 'consultations' during the same follow-up period. Furthermore, secondary outcomes were 'MUAC' and 'weight-for-age z-score' (measured at the first follow-up visit), and 'BCG scarring' (measured at 6–12 months of age).

Sample size

The target sample size for the trial was based on previous data from the rural HDSS with an estimated proportion of events of 2.4%. The trial was sized to obtain 80% power to detect a reduction in early infant severe morbidity if the true effect of the intervention was larger than 40%. The target sample size was 6666 children. Details on sample size calculation can be found in the published protocol.[15] The trial was stopped before the target sample size was reached due to low recruitment and low mortality, and the trial population corresponds to only 33% of the target sample size.

Statistical analyses

General analysis principles can be found in online supplemental file 2. In Cox proportional hazards models with age as underlying time scale, we assessed the effect of providing BCG and OPV at a single home visit in a per-protocol analysis. We stratified for factors used in the randomisation (region and cluster-level pre-trial mortality (high/low)) and sex. To account for within-village clustering, cluster-robust variance estimates were employed on all analyses. Children entered the analysis on the date of enrolment or 24 hours of age, whichever came last, and remained in the analysis until whatever came first: the first subsequent visit by the BHP team, date of registering first non-trial vaccine after enrolment, death, migration or 60 days of life. Proportional hazards

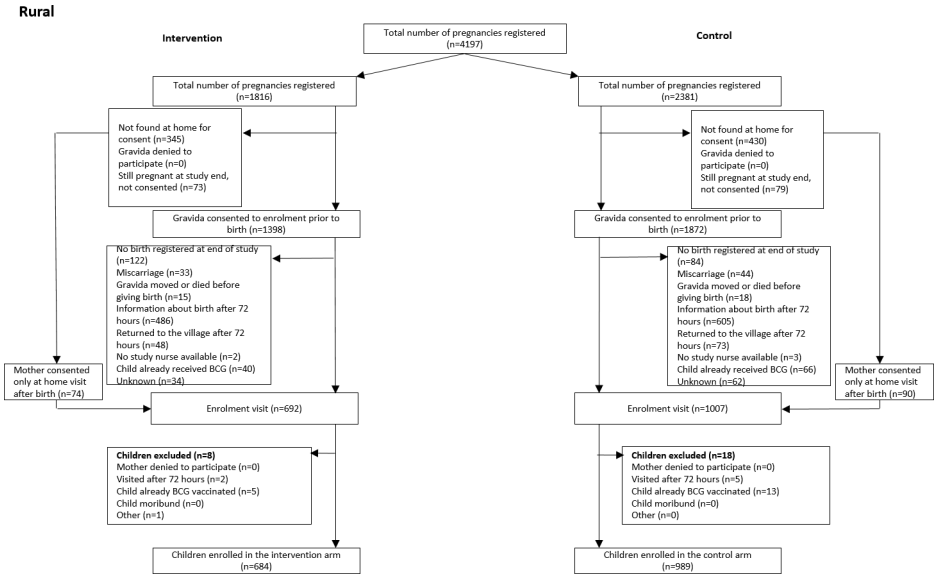

Figure 1 Flow chart of participants in the rural area.

assumptions were evaluated using Schoenfeld residuals significance test, log–log survival curves and by checking whether the effect interacted with the underlying time scale using the time-varying covariates (tvc) options. Further details are provided in the prespecified analysis plan (online supplemental file 3).[15]

Analyses of secondary outcomes are described in more detail in online supplemental file 3. In brief, the effect of providing BCG and OPV on non-accidental hospital admissions and severe morbidity (composite outcome of non-accidental mortality and non-accidental hospital admissions) was assessed in Cox proportional hazards models. The effect of providing BCG and OPV on all-cause consultations and BCG scarring was assessed in log-binomial regression models. The effect of providing BCG and OPV on MUAC and weight-for-age z-score between enrolment and first follow-up visit within 6 months of age was assessed in linear regression models.

In the analysis plan, a cost-effectiveness analysis was specified. As the trial was stopped prematurely, we did not perform a cost-effectiveness analysis.

Sensitivity analyses

In planned sensitivity analyses, we assessed the robustness of the main results by (1) censoring follow-up at general health intervention campaigns (a list of all health intervention campaigns in the period can be found in the online supplemental file 1), and (2) using an intention-to-treat approach, including all children, who had a home visit by a study nurse. We furthermore assessed the effect of the intervention on repeated hospital admissions.

Exploratory analyses

In an exploratory analysis, we assessed whether censoring follow-up time at deaths, hospital admissions and registration of non-trial vaccines affected the risk of consultations by further adjusting the consultation analysis for the length of follow-up time in the main analysis.

Patient and public involvement

The communities were involved in locating households, when the HDSS was set up and contributed information allowing tracing of internal migrants between villages throughout the study period. No participants were involved in setting the research question or the outcome measure, nor were they involved in developing plans for recruitment, design or implementation of the study. Local CKIs were selected to ensure timely information about birth. Nurses from local health centres were involved in the trial, which ensured a close collaboration with the local health system. No participant was asked to advise on interpretation or writing up the results. The results were disseminated to the national public health institute. There are no plans to directly disseminate the results of the research to study participants. However, all women in the HDSS are informed about the importance of early vaccinations during BHP village visits. Study results will be disseminated to local nurses involved in the study. The collaboration between local and international researchers is described in the author reflexivity statement (online supplemental file 1).

RESULTS

During the trial period (between 28 July 2016 and 31 August 2019), a total of 8811 pregnancies were registered in the BHP HDSS, of these 48% (4197) were registered in the rural HDSS, while 52% (4614) were registered in the urban HDSS.

In the rural area, 78% of pregnant women provided consent prior to giving birth (77% in intervention clusters/79% in control clusters). The main reason for not giving consent was that the pregnant woman was not found at home during village visits before delivery. Of those giving consent, 52% (49% in intervention clusters/54% in control clusters) received a home visit by a study nurse. Of the 1699 newborns receiving a home

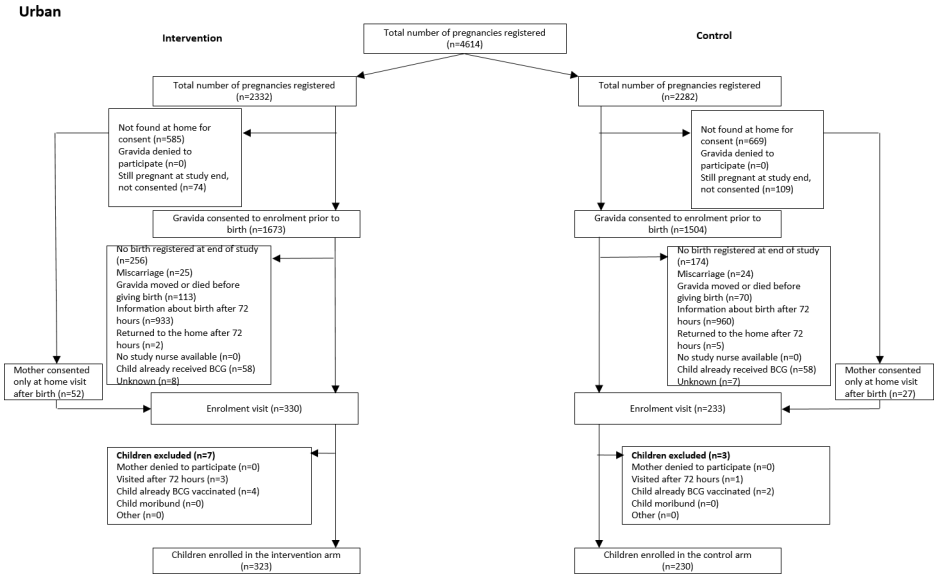

Figure 2 Flow chart of participants in the urban area.

visit, 1673 newborns were enrolled. In total, 26 newborns were excluded (7 newborns were visited later than 72 hours after birth, 18 newborns had already received BCG vaccine, 1 newborn was excluded by mistake, no newborns were excluded because the mother denied participation or because the newborn was considered moribund) (figure 1).

In the urban area, 69% (72% in intervention clusters/66% in control clusters) of pregnant women provided consent prior to giving birth. Again, the main reason for not giving consent was that the pregnant woman was not found at home during visits before delivery. In the urban area, more children were born at the national hospital, where BHP provides BCG and OPV0 vaccination before discharge[16] and were therefore not eligible for enrolment. Consequently, only 18% (20% in intervention clusters/15% in control clusters) received a home visit by a study nurse. Of the 563 newborns receiving a home visit, 553 newborns were enrolled. In total, 10 newborns were excluded (4 newborns were visited later than 72 hours after birth, 6 newborns had already received BCG vaccine, no newborns were excluded because the mother denied participation or because the newborn was considered moribund) (figure 2). Thus, the resulting sample size was 2226 newborns.

Most newborns were enrolled within 24 hours after birth (54% in intervention clusters and 57% in control clusters). Newborns from intervention and control clusters had similar distribution of background characteristics and most statistically significant differences reflected small absolute differences (table 1). However, in the Oio health region, more newborns were enrolled in control clusters. This was mainly driven by few large clusters with many enrolments. In the urban area, more newborns in the intervention group were born at health centres, whereas more children in the control group were born either at home or in the hospital (table 1).

Main outcome: non-accidental mortality

Among the 2226 newborns, 2212 entered the analyses (13 were visited by the mobile team on/before enrolment and 1 moved at the date of enrolment before being 24 hours old). In total, 35 children died during follow-up and the mortality rate was 66.5/1000 person-years (PYRS) (7 deaths during 105 PYRS) in the intervention group and 276.5/1000 PYRS (28 deaths/101 PYRS) in the control group. The resulting HR was 0.41 (95% CI: 0.18 to 0.92) stratified for region, cluster-level pre-trial mortality and sex (table 2). Thus, providing BCG and OPV reduced non-accidental early infant mortality by 59% (95% CI: 8% to 82%). The Schoenfeld residuals test did not indicate a violation of the proportional hazard assumptions (p=0.16) nor did the log–log curves, but the HR was not constant over time when tested with the tvc options (p=0.02). Inspecting follow-up of events revealed that all events in the intervention group occurred before age 10 days. Before day 10, the HR was 0.66 (95% CI: 0.26 to 1.66); after day 10, the HR was undefined but the rates were significantly different (online supplemental table 1).

Almost all deaths (34) occurred in the rural area. Stratifying on study area resulted in an HR of 0.37 (0.15 to 0.87) in the rural area, while it was not possible to calculate an HR for the urban area, as there was only one death in the intervention group and no deaths in the control group.

The effect of providing BCG and OPV at home visits was not modified by sex (p=0.40), maternal BCG scar (p=0.81), birth weight (p=0.97) or season (p=0.18) (table 2). One-third of the deaths were classified as due to sepsis, and effects were similar across all categories (online supplemental table 2). Sensitivity analyses did not alter conclusion (online supplemental tables 3–6).

Table 1 Baseline characteristics of trial participants in the intervention and control clusters

	All		Rural		Urban	
	Intervention	Control	Intervention	Control	Intervention	Control
n (%)	1007 (45)	1219 (55)	684 (41)	989 (59)	323 (58)	230 (42)
Males, n (%)	514 (51)	612 (50)	344 (50)	485 (49)	170 (53)	127 (55)
Age at enrolment, n (%)						
<24 hours	541 (54)	689 (57)	403 (59)	598 (60)	138 (43)	91 (40)
24–47 hours	237 (24)	274 (22)	73 (11)	147 (15)	164 (51)	127 (55)
48–71 hours	229 (23)	256 (21)	208 (30)	244 (25)	21 (7)	12 (5)
Region, n (%)						
Oio	346 (34)	619 (51)	346 (51)	619 (63)	–	–
Biombo	178 (18)	206 (17)	178 (26)	206 (21)	–	–
Cacheu	160 (16)	164 (13)	160 (23)	164 (17)	–	–
Bissau	323 (32)	230 (19)	–	–	323 (100)	230 (100)
Weight at enrolment*, mean (SD)/kg	2.94 (0.45)	2.88 (0.46)	2.92 (0.45)	2.85 (0.45)	2.99 (0.44)	2.98 (0.45)
Low birth weight, n (%)						
Yes	145 (14)	220 (18)	105 (15)	185 (19)	40 (12)	35 (15)
No	859 (85)	988 (81)	576 (84)	793 (80)	283 (88)	195 (85)
Temperature at enrolment†, mean (SD)/°C	36.7 (0.8)	36.7 (0.8)	36.6 (0.8)	36.7 (0.8)	36.8 (0.6)	36.7 (0.6)
Mid-upper arm circumference at enrolment‡, mean (SD)/mm	98 (9)	97 (10)	97 (9)	97 (10)	98 (8)	99 (11)
Head circumference at enrolment§, mean (SD)/cm	34 (1)	34 (2)	34 (1)	34 (2)	34 (1)	34 (1)
Place of birth¶, n (%)						
Home	571 (57)	801 (66)	474 (69)	694 (70)	97 (30)	107 (47)
Health centre	306 (30)	247 (20)	144 (21)	204 (21)	162 (50)	43 (19)
Hospital	80 (8)	122 (10)	46 (7)	75 (8)	34 (11)	47 (20)
Other	48 (5)	47 (4)	19 (3)	15 (2)	29 (9)	32 (14)
Season, n (%)						
Rainy	490 (49)	482 (40)	319 (47)	381 (39)	171 (53)	101 (44)
Dry	516 (51)	737 (60)	365 (53)	608 (61)	151 (47)	129 (56)
Maternal education**, n (%)						
No schooling	357 (35)	503 (41)	255 (37)	428 (43)	102 (32)	75 (33)
1–4 years	206 (20)	291 (24)	178 (26)	272 (28)	28 (9)	19 (8)
5–7 years	213 (21)	215 (18)	158 (23)	161 (16)	55 (17)	54 (23)
>7 years	165 (16)	123 (10)	34 (5)	46 (5)	131 (41)	77 (33)
Maternal BCG scar††, n (%)						
Yes	606 (60)	705 (58)	388 (57)	572 (58)	218 (67)	133 (58)
No	304 (30)	377 (31)	221 (32)	309 (31)	83 (26)	68 (30)
Toilet‡‡, n (%)						
No toilet	224 (22)	202 (17)	221 (32)	202 (20)	3 (1)	0 (0)
Latrine	736 (73)	974 (80)	457 (67)	778 (79)	279 (86)	196 (85)
Inside the house	38 (4)	37 (3)	0 (0)	5 (1)	38 (12)	32 (14)
Roof§§, n (%)						
Straw	130 (13)	138 (11)	130 (19)	138 (14)	320 (99)	229 (100)
Hard	870 (86)	1075 (88)	550 (80)	846 (86)	–	–
Electricity¶¶, n (%)						

Continued

Table 1 Continued

	All		Rural		Urban	
	Intervention	Control	Intervention	Control	Intervention	Control
Yes	527 (52)	594 (49)	340 (50)	477 (48)	187 (58)	117 (51)
No	468 (46)	613 (50)	336 (49)	501 (51)	132 (41)	112 (49)

*14 newborns have missing information on weight.
†13 newborns have missing information on temperature.
‡2 newborns have missing information on mid-upper arm circumference.
§6 newborns have missing information on head circumference.
¶4 newborns have missing information on place of birth.
**153 newborns have missing information on maternal education.
††234 newborns have missing information on maternal BCG scar.
‡‡15 newborns have missing information on toilet.
§§13 newborns have missing information on roofing.
¶¶24 newborns have missing information on electricity.

Non-accidental hospital admission

The rate of hospital admission was 66.8 (7 hospital admissions during 105 PYRS) in the intervention group and 149.2 (15 hospital admissions during 101 PYRS) in the control group. The intervention group thus had 67% fewer hospital admissions (HR: 0.33 (95% CI: 0.13 to 0.84)) (table 3). Schoenfeld residuals (p=0.005), log–log plots and the tvc option (p=0.04) all indicated that the proportional hazards assumption was violated. Splitting follow-up after day 10 resulted in an HR of 0.06 (95% CI: 0.01 to 0.39) for the early period and 1.60 (95% CI: 0.28 to 9.11) in the late period (data not shown). In the rural area, the intervention resulted in an HR of 0.49 (95% CI: 0.15 to 1.59), while the corresponding estimate in the urban area was 0.21 (95% CI: 0.05 to 0.86). There were no repeated hospital admissions during follow-up.

Severe morbidity

Combining mortality and hospital admissions in a composite outcome of severe morbidity resulted in an event rate of 133.6/1000 PYRS (14 events during 105 PYRS) in the intervention group and 358.0/1000 PYRS (36 events during 101 PYRS) in the control group. Thus, the intervention resulted in 58% fewer events (HR: 0.42 (95% CI: 0.22 to 0.81)). The intervention tended to reduce the risk of events in both the rural (HR: 0.49 (95% CI: 0.23 to 1.04)) and the urban area (HR: 0.28 (95% CI: 0.08 to 0.94)) (table 3).

Outpatient consultations

Among the 2208 newborns entering the main analysis, 177 (101 in intervention and 76 in control clusters) received a consultation during follow-up. Providing BCG and OPV0 at home visits tended to increase the risk of consultation by 32% (RR=1.32 (95% CI: 0.92 to 1.88), adjusted for sex, region and cluster-level pre-trial mortality). Newborns in the urban area were more likely to have a consultation during follow-up, but the effect of BCG and OPV0 was similar in the rural (RR=1.29 (95% CI: 0.73 to 2.26)) and the urban areas (RR=1.42 (95% CI: 0.94 to 2.15)) (table 4). In a post hoc analysis, adjusting for the (differential) length of follow-up, the RR was 1.08 (95% CI: 0.74 to 1.58).

BCG scarring

Among the 2208 newborns, 1753 children were present at an HDSS visit between 6 and 12 months of age and had their arm inspected for a BCG scar. Most of these children had developed a BCG scar (95% in the intervention group and 92% in the control group). In the rural area, the intervention had little impact on BCG scarring (RR=1.03 (95% CI: 0.99 to 1.07)) (table 4). Only three children had not developed a scar in the urban area.

Growth

The intervention had no effect on growth (difference in MUAC was 0.82 mm (95% CI: −0.39 to 2.03) and difference in weight z-score was 0.04 (95% CI: −0.06 to 0.13)) (online supplemental table 7).

Adverse events

During the trial, three cases of lymphadenitis were identified in the intervention group and no adverse events in the control group (online supplemental table 8).

DISCUSSION

Providing BCG and OPV0 vaccinations at a single home visit within 72 hours after birth reduced early infant mortality (HR: 0.41 (95% CI: 0.18 to 0.92)), hospital admissions (HR: 0.33 (95% CI: 0.13 to 0.84)) and event-free survival for either of the outcomes (HR: 0.42 (95% CI: 0.22 to 0.81)). The intervention was not associated with growth and BCG scarring and tended to increase the risk of consultations during follow-up. The trial was stopped early due to low enrolment rates and low mortality.

We conducted the trial in a setting where the WHO-recommended home visits are not yet implemented. We created new vaccination opportunities at birth to be able to test the effect of providing vaccines recommended at birth with the home visits as this is not currently

Table 2 Effect of providing BCG and OPV at a home visit on non-accidental mortality and potential effect modifiers

	n	MR/1000 PYRS (deaths/PYRS)	HR (95% CI)
Main outcome: non-accidental mortality			
Intervention	1006	66.5 (7/105)	0.41 (0.18 to 0.92)
Control	1206	276.5 (28/101)	Reference
Rural			
Intervention	683	95.3 (6/63)	0.37 (0.15 to 0.87)
Control	976	328.9 (28/85)	Reference
Urban			
Intervention	323	23.7 (1/42)	NA
Control	230	0.0 (0/16)	Reference
Effect modifiers			
Sex			
Male, intervention	514	76.1 (4/53)	0.59 (0.20 to 1.76)
Male, control	603	233.0 (12/51)	Reference
Female, intervention	492	57.0 (3/53)	0.28 (0.08 to 0.98)
Female, control	603	321.5 (16/50)	Reference
Maternal BCG scar			
Maternal BCG scar, intervention	605	61.7 (4/65)	0.45 (0.15 to 1.36)
Maternal BCG scar, control	702	234.7 (14/60)	Reference
No maternal BCG scar, intervention	304	63.1 (2/32)	0.35 (0.08 to 1.57)
No maternal BCG scar, control	371	284.5 (9/32)	Reference
Birth weight			
Low birth weight, intervention	145	211.8 (3/14)	0.44 (0.11 to 1.78)
Low birth weight, control	216	811.1 (15/18)	Reference
Normal birth weight, intervention	858	44.1 (4/91)	0.46 (0.16 to 1.32)
Normal birth weight, control	979	159.0 (13/82)	Reference
Season			
Rainy season, intervention	490	77.5 (4/52)	0.85 (0.22 to 3.30)
Rainy season, control	476	148.7 (6/40)	Reference
Dry season, intervention	516	56.0 (3/54)	0.26 (0.08 to 0.80)
Dry season, control	730	361.2 (22/61)	Reference

MR, mortality rate; NA, not applicable; OPV, oral polio vaccine; PYRS, person-years.

part of the WHO recommendation. To do so, we set up a CKI system to allow for timely information about births. This mimics the community health worker system implemented in many low-income countries, and thus strengthens the ability to transfer trial results to future policy. The close collaboration with the national health system was also reflected in the recruitment of study nurses, whom we trained on home visit routines and vaccination technique. In addition to the close collaboration with the national health system, the trial set-up benefited from the implementation within a research platform with extensive trial experience. By nesting the trial within an HDSS, it became feasible to identify pregnancies and ensure that all pregnant women in a defined area were invited to participate in the trial, and that no trial participants were lost to follow-up. Furthermore, nurses and data collectors were intensively supervised. A major limitation was that the trial was ended early as there were lower-than-expected enrolment rates and few deaths and hospital admissions. This was mainly due to difficulties in obtaining timely information about births. Thus, the trial only included 33% of the planned sample size. However, no interim analyses were performed: analyses of the trial were only conducted after the trial had ended, and the results of the trial were therefore unknown when deciding to end the trial. The decision to end the trial was made in collaboration with the DSMB based on enrolment rates and the overall event rates. As the trial was stopped early, the results of the trial are likely to be more imprecise leading to an increased risk

Table 3 Effect of providing BCG and OPV at a home visit on non-accidental hospital admission and severe morbidity (composite outcome of non-accidental mortality and non-accidental hospital admission)

	n	MR/1000 PYRS (deaths/PYRS)	HR (95% CI)
Non-accidental hospitalisation			
Intervention	1006	66.8 (7/105)	0.33 (0.13 to 0.84)
Control	1206	149.2 (15/101)	Reference
Rural			
Intervention	683	63.7 (4/63)	0.49 (0.15 to 1.59)
Control	976	106.0 (9/85)	Reference
Urban			
Intervention	323	71.4 (3/42)	0.21 (0.05 to 0.86)
Control	230	384.2 (6/16)	Reference
Composite outcome: non-accidental hospitalisation and non-accidental mortality*			
Intervention	1006	133.6 (14/105)	0.42 (0.22 to 0.81)
Control	1206	358.0 (36/101)	Reference
Rural			
Intervention	683	159.2 (10/63)	0.49 (0.23 to 1.04)
Control	976	353.2 (30/85)	Reference
Urban			
Intervention	323	95.3 (4/42)	0.28 (0.08 to 0.94)
Control	230	384.2 (6/16)	Reference

Two newborns with missing information on whether hospital admission was caused by accident.
*In the intervention group, there was no overlap between events (death and hospital admission), whereas seven newborns in the control group were first admitted to hospital and later died.
MR, mortality rate; OPV, oral polio vaccine; PYRS, person-years.

of dismissing a beneficial or harmful effect of the intervention. The trial set-up was very resource demanding in a setting where the WHO-recommended home visits are not implemented.

Historically, low birthweight infants were recommended delayed BCG vaccination. This made it possible and ethically justified to study the impact of early BCG vaccination in randomised trials among low birthweight infants. Therefore, most randomised trials assessing the impact of BCG vaccination on mortality early in life have been done in low birthweight infants. Three trials from Guinea-Bissau in low birthweight infants found that BCG+OPV versus just receiving OPV reduced neonatal mortality by 38% (95% CI: 17% to 54%).[3]

In the present trial, we took advantage of the common delays in vaccination,[7 17] and were therefore able to enrol normal birthweight newborns, still ensuring that no newborn received the BCG and OPV0 vaccines later than had the trial not been carried out. Since we found a similar benefit of BCG and OPV0 in newborns with low and normal birth weight, the benefits do not appear to be limited to children with low birth weight.

Since the Guinean low birthweight BCG trials, two trials have been conducted in India. These trials provided BCG alone or BCG+OPV0 versus no vaccines to neonates weighing <2000 g. Neither of the trials found any effect on neonatal mortality.[18] It has been proposed that the discrepancy between the trial results may be explained by different BCG strains.[19] The three trials from Guinea-Bissau in low birthweight infants all used BCG-Denmark,[3] whereas the two trials from India used BCG-Russia.[18] The hypothesis on different non-specific effects of different BCG strains was tested in a randomised trial comparing the effect of BCG-Denmark and BCG-Japan with BCG-Russia on hospital admissions (primary outcome) and mortality. The trial found no difference between BCG-Denmark and BCG-Russia for either admission (HR: 1.08 (95% CI: 0.84 to 1.37)) or mortality (HR: 1.15 (95% CI: 0.74 to 1.81)),[16] while the BCG-Japan strain tended to increase hospital admissions compared with BCG-Russia (HR: 1.15 (95% CI: 0.93 to 1.43)) but not mortality (HR: 0.71 (95% CI: 0.43 to 1.19)).[16] In the present trial, BCG-Japan was used in combination with OPV0 vaccines and reduced early infant mortality and the risk of non-accidental hospital admission. The effect of BCG-Denmark on hospital admissions was also assessed in a trial in Denmark, which also found no effect of BCG on hospital admission,[20] but a protective effect against atopic dermatitis,[21] as seen in other high-income settings.[22] A trial from Uganda found BCG-Denmark at birth to be associated with 29% (95% CI: 5% to 47%) lower infectious disease incidence, most pronounced in low birthweight

Table 4 Effect of providing BCG and OPV at a home visit on all-cause outpatient consultations and BCG scarring

		Consultations	Adjusted for region, cluster-level pre-trial mortality and sex	Further adjusted for FU time
	n	n (%)	RR (95% CI)	RR (95% CI)
All-cause consultations				
Intervention	1006	101 (10)	1.32 (0.92 to 1.88)	1.08 (0.74 to 1.58)
Control	1206	76 (6)	Reference	Reference
Rural				
Intervention	683	47 (7)	1.29 (0.73 to 2.26)	1.25 (0.71 to 2.19)
Control	976	50 (5)	Reference	Reference
Urban				
Intervention	323	54 (17)	1.42 (0.94 to 2.15)	0.98 (0.64 to 1.51)
Control	230	26 (11)	Reference	Reference
BCG scarring				
	n	BCG scar n (%)	RR (95% CI)	
Intervention	771	734 (95)	NA	
Control	982	907 (92)	Reference	
Rural				
Intervention	575	539 (94)	1.03 (0.99 to 1.07)	
Control	826	753 (91)	Reference	
Urban				
Intervention	196	195 (99)	NA*	
Control	156	154 (99)	Reference	

*The model could not converge as only three children had no scar in the urban area.
FU, follow-up; NA, not applicable; OPV, oral polio vaccine; RR, risk ratio.

newborns.[4] In our trial, BCG-Japan and OPV0 tended to increase the risk of consultation. However, this could at least partly be ascribed to the longer follow-up period, as the effect disappeared in the urban area when adjusting for follow-up time.

In the Danish trial, stratified analyses suggested that maternal BCG vaccination status modified the effect of BCG vaccination. Among children of BCG-vaccinated mothers, BCG vaccination was associated with 35% (95% CI: 6% to 55%) fewer infectious disease hospital admissions.[23] This was supported by a study from Guinea-Bissau,[24] which also found stronger effects of BCG among infants when the mother had a BCG scar (an indicator of a successful BCG vaccination). Other studies have found maternal BCG scar to be beneficial on its own.[25 26] In the present trial, BCG and OPV were beneficial for both children of mothers with and without a BCG scar.

The trial is to our knowledge the only trial assessing the effect of BCG and OPV vaccination versus no vaccination on early infant mortality in both low birthweight and normal birthweight newborns.

Prior trials conducted in low birthweight infants found that BCG did not reduce the risk of hospital admission, but it reduced the case fatality among hospitalised children, indicating that BCG might not reduce the incidence, but rather the severity of infection.[27] In contrast, the present trial indicates a protective effect of BCG and OPV0 against hospital admissions. We speculate that a potential explanation for this could be different thresholds for hospital admission among children born at the national hospital (previous trials) and families with children who were unvaccinated, when met at home by the study nurse (the present trial). The latter group will likely have a higher threshold for hospitalisation, which would mean that only the most severely ill children would be hospitalised.

The trial was stopped due to low enrolment rates and low mortality. Obtaining information on births within 72 hours was far from always possible, and among those who were registered and consented during pregnancy, 33% provided information on delivery more than 72 hours after birth. Though the vaccine delivery set-up employed in the present trial was not likely to be effective at scale in a setting like the Guinean, vaccine delivery could relatively easily be implemented in settings where the WHO-recommended home visits are implemented or by implementing BCG vaccination at birth facilities. The WHO recommendation of providing home visits after birth should be revised to include provision of BCG and OPV wherever possible. Previous research has shown that

the BCG vaccine is often delayed due to local practices of not opening a vial of BCG for few children. The results of the present trial stress the importance of removing such barriers. With the strong effect indicated in the present trial and in a number of other trials,[3 12 28 29] providing BCG and OPV at birth could be an important contributor to lowering neonatal mortality.

CONCLUSION

Despite of its small size, our trial supports that early vaccination with BCG and OPV0, as recommended in low-income countries, has the potential to lower early child mortality and morbidity. Early vaccination should therefore be of highest priority.

Transparency statement

Sanne Marie Thysen is the guarantor of the trial and affirms that the manuscript is an honest, accurate, and transparent account of the trial; no important aspects of the trial have been omitted; any discrepancies from the original trial protocol have been explained.

Author affiliations
[1]Bandim Health Project, Bissau, Guinea-Bissau
[2]Bandim Health Project, Research unit OPEN, Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[3]Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark
[4]Danish Institute for Advanced Study, University of Southern Denmark, Copenhagen, Denmark

Twitter Sanne Marie Thysen @Sthysen and Ane Baerent Fisker @AneFisker

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Contributors AR, PA and CSB conceived the idea for the trial. SMT, PA, CSB and ABF designed the study. SMT set up the study with help from IdSB, JSH, ADS and ABF. SMT, IdSB, JM, ADS, JSH, LMVdS and ABF supervised data collection. JSDM diagnosed the verbal autopsies. SMT, AJ and ABF planned the data analyses. SMT analysed the data and drafted the manuscript with input from ABF. ABF verified the data analyses. All authors read and approved the final manuscript. SMT is the guarantor of the trial and affirms that the manuscript is an honest, accurate, and transparent account of the trial; no important aspects of the trial have been omitted; any discrepancies from the original trial protocol have been explained.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants and the the trial was approved by the Guinean Ethical Committee (reference number: 0016/CNES/INASA/2015) and the Danish Ethics Committee gave consultative approval. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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ORCID iDs
Sanne Marie Thysen http://orcid.org/0000-0003-4541-3901
Ane Baerent Fisker http://orcid.org/0000-0002-8521-0992

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Can earlier BCG vaccination reduce early infant mortality? A randomised trial

The protocol was last updated on June 30, 2017 – see list of changes to the protocol on page 2

Investigators:

Sanne Marie Thysen (PhD student)

Ane Bærent Fisker (MD, PhD)

Amabelia Rodrigues (PhD)

Christine Stabell Benn (MD, PhD, DMsc)

Peter Aaby (DMsc)

Aksel Karl Georg Jensen (Statistician, PhD)

Study site

55 rural clusters followed through Bandim Health Project's Rural Health and Demographic Surveillance site in Oio, Biombo and Cacheu regions in Guinea-Bissau and 37 urban clusters followed through Bandim Health Project's Urban Health and Demographic System site in Bissau.

Bandim Health Project, Apertado 861, 1004 Bissau Codex, Guinea-Bissau

Protocol changes

May 2015 Original protocol *(Approved by the Guinean ethical committee June 2015)*

May 2016 Protocol adapted to include Tokyo 172 strain from BCG Japan *(Approved by the Guinean ethical committee June 2016)*

May 2017 Definitions of primary and secondary outcomes were clarified. Analyses section was clarified and supplemented by the analysis plan. Sample size considerations were updated.

The Danish BCG strain was removed from the protocol, since this strain was only used during the pilot phase of the trial.

June 2017 Study area was expanded to include BHP's urban study area. The protocol format was changed to comply with the current guidelines of the Ethical Committee in Guinea-Bissau.

INDEX

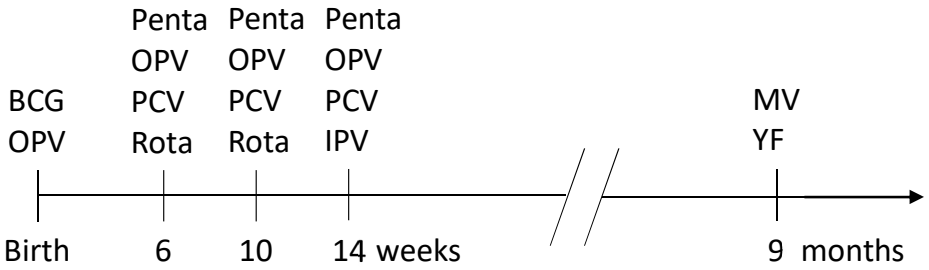
ABBREVIATIONS AND ACRONYMS

BCG	Bacillus Calmette-Guérin vaccine
BHP	Bandim Health Project
CKI	Community key informant
DTP	Diphtheria, tetanus and pertussis vaccine
EPI	Expanded Program on Immunization
LBW	Low-birth weight
MUAC	Mid-upper-arm-circumference
MV	Measles vaccine
NBW	Normal-birth weight
NSE	Non-specific effect
OPV	Oral polio vaccine
RCT	Randomised controlled trial
TB	Tuberculosis

1 INTRODUCTION

1.1 Vaccines and non-specific effects

WHO recommends home visits after birth to reduce neonatal mortality[1]. Guinea-Bissau has not implemented such home visits yet. Vaccinations are normally provided at the health centre, the current vaccination policy is to provide Bacillus Calmette-Guérin vaccine (BCG) and oral polio vaccine (OPV) at birth, pentavalent vaccine (diphtheria-tetanus-pertussis-H. influenzae type B-Hepatitis B vaccine), OPV and pneumococcal vaccine at 6, 10, and 14 weeks, rota-virus vaccine at 6 and 10 weeks, inactivated polio vaccine at 14 weeks, and measles vaccine (MV) and yellow fever vaccines at 9 months (Figure 1).



BCG: Bacillus Calmette-Guérin vaccine, OPV: Oral Polio vaccine
Penta: Diphteria, Pertussis, Tetanus, Haemophilus Influenzae B, Hepatitis B
PCV: Pneumoccocal vaccine, Rota: Rotavirus vaccine, IPV: Inactivated Polio Vaccine
MV: Measles vaccine, YF: Yellow Fever vaccine

Figure 1: Recommended vaccination schedule in Guinea-Bissau

Public health policies in low-income countries are based on a one-disease-one-solution paradigm: for each of the important diseases, a specific vaccine is developed. It is believed that the vaccine prevents only the specific disease and does nothing else. Therefore, most routine vaccinations (including MV, BCG, OPV, Diphtheria-tetanus-pertussis vaccine (DTP)) have not been tested for their effect on overall mortality.

The Bandim Health Project (BHP) has shown in numerous observational studies[2-4] and randomised controlled trials (RCTs)[5-7] in low-income countries that the "one-disease-one-solution" paradigm is wrong. Apart from preventing specific diseases, the vaccines may also have much wider effects on the immune system, which leads to changes in resistance/susceptibility to unrelated infections[8].

1.2 Non-specific effects of BCG

Several observational studies in Africa and other high-mortality areas show that BCG is associated with better child survival[2,9,10], which cannot be explained by prevention of tuberculosis (TB)[11]. WHO recommends BCG vaccine at birth to normal-birth-weight children (NBW) in low-income

countries to prevent TB. For low-birth-weight children (LBW;<2500g) BCG vaccination is often delayed because these children are assumed to be immunologically immature, and therefore they are typically only vaccinated when they have reached 2500g. However, in two RCTs in Guinea-Bissau, BCG-at-birth reduced neonatal mortality in LBW children by 48% (95%CI: 18-67%)[5,6]. TB is very rare in early childhood[12] and these strong beneficial effects can therefore not be ascribed to prevention of TB. Rather the effect is due to a beneficial training of the immune system, preventing or ameliorating other infections, which cause neonatal and childhood death. The effect of BCG-at-birth on neonatal mortality was seen already during the first three days post-vaccination, the reduction in mortality being 58% (8-81%)[6]. This rapid effect suggests that the effect is due to changes in the innate immune system rather than the adaptive immune system.

1.3 Biological mechanism of the effect of BCG

Recent immunological studies have shown that BCG induces epigenetic changes in monocytes, which reprogram the innate immune system to increased pro-inflammatory responses against unrelated pathogens[13,14]. These findings strongly support that BCG may indeed have non-specific beneficial effects, protecting the recipients not only against TB, but against non-targeted infectious diseases[14,15].

2 JUSTIFICATION

BCG is rarely given at birth in rural areas in low-income countries; in rural Guinea-Bissau only 38% are vaccinated with BCG in the first month of life[16]. A major reason for this delay is the immunization programme (EPI)'s focus on reducing wastage of vaccines. Freeze dried vaccines like BCG have to be used within 6 hours after reconstitution with a diluent. Therefore, a vial of BCG vaccine, which contains 20 doses, is not opened unless 10-12 children are present to be vaccinated. Hence, there are many missed vaccination opportunities. This delay in BCG vaccination may be very important. Approximately 75% of neonatal deaths occur within the first week of life[11] and only 11% of the children receive BCG in the first week of life[16]. If BCG vaccine indeed has profound effects on innate immunity and neonatal mortality and morbidity in both LBW and NBW children many lives could be saved if BCG was provided earlier.

3 AIM

In the present randomised trial, we aim to study the effect of BCG and OPV vaccinations provided at a single home visit within 72 hours after birth on early infant mortality and morbidity among infants in a rural and an urban area in Guinea-Bissau.

4 HYPOTHESIS

BCG at birth provided at a single home visit shortly after birth will reduce early infant non-accidental mortality by 40% between the home visit and the subsequent home visit or pentavalent vaccine.

5 METHODS

5.1 Setting

The study will be conducted in Bandim Health Project's Health and Demographic Surveillance System (HDSS) site in rural and urban Guinea-Bissau.

5.1.1 The rural HDSS

The rural HDSS was established in 1990. The BHP teams survey women of fertile age and their children below the age of 5 years in randomly selected clusters of villages in all the nine health regions in the country. Children in the three rural regions closest to Bissau (Oio, Biombo and Cacheu) will be eligible for the study.

The rural clusters are followed with two-monthly visits by the mobile teams. At all visits the women are asked whether they are pregnant. When a pregnancy is registered; the woman's nutritional status is assessed by measurement of a mid-upper-arm-circumference (MUAC); information on antenatal care is collected prior to giving birth, as well as at the first visit after delivery. Socio-economic factors (type of roofing, type of bathroom, possession of a mobile phone, radio and generator) are registered. After the delivery, information on the place of delivery (home, health facility) and who assisted the delivery is collected.

5.1.2 The urban HDSS

The urban HDSS was established in 1978. Fieldwork assistants follow children below the age of 3 years through home visits every third month. Pregnancies are registered every month and followed monthly until birth. When a pregnancy is registered information on ethnic group, gestational age, use of mosquito net is collected. After the delivery, information on place of delivery (home, health facility) is collected. Children in the urban study area will be eligible for the study.

5.1.3 Study nurses

For the present study, we will select study nurses to conduct home visist. The study nurses will be selected in collaboration with the regional health authorities of Oio, Biombo, and Cacheu. They will be selected among nurses working at a health centre with a catchment area, which corresponds to the study cluster in order to allow them to carry out the project visits in addition to their normal routines. For the urban study area, the BHP will employ a study nurse fulltime. The nurses will use

a project motorbike to visit new-borns at home. They will receive a monthly salary subsidy, and a subsidy for each home visit conducted shortly after birth of the child.

5.1.4 Community Key Informants

To ensure that the nurses are informed immediately about deliveries, we will use community key informants (CKI) in the rural area. A CKI will be selected among residents in each rural village to collect information about pregnancies, deliveries and deaths. Where possible, the CKI will be the community health worker (ASB). The CKI will communicate immediately any delivery in the village to the study nurse. In the urban area, we will take advantage of the close follow-up by the fieldwork assistants that circulate the zones each day. To carry out the job, the CKI or fieldwork assistant will receive a subsidy for each timely call about a new-born.

5.2 Study design and randomisation

We will conduct a cluster-randomised trial, randomising clusters to two different treatment groups, stratified by region and pre-trial mortality level (high/low). Pre-trial mortality level is assessed using the BHP rural HDSS data from 2012 to 2015. Pre-trial mortality level in the urban area will be based on the BHP urban HDSS data from 2013 to 2016, to account for a change in the registration system in 2012.

Randomisation of clusters will be by computer generated random numbers. All new-borns will be visited as soon as possible after birth. To children in half of the clusters we will provide standard care and vaccines (BCG, OPV) at the home visits; children in the remaining clusters will only receive standard care but no vaccines (Table 1).

5.3 Sample size considerations

Based on previous data from the rural HDSS in the areas where the current study will be conducted, the expected proportion of events (deaths and hospitalisation) between day 1 and the next home visit or 60 days of age, whichever comes first is 2.4% (unpublished data). We expect the proportion of events to be at least as high in the urban area. A recent trial in Ghana indicated that three home visits during the first week of life to promote essential new-born care practices and to weigh and assess children for danger signs was associated with an 8% (-12 to 25%) reduction in neonatal mortality. Based on pre-trial mortality data from the same rural clusters, we estimate the design effect to be 1.35 (ratio of square of the standard errors for the cluster-adjusted/unadjusted HRs). Thus, in order to obtain 80% power to detect a reduction in early infant severe morbidity if the true reduction of BCG and OPV provided at home visits is larger than 40%, we will need to enrol at least 6666 children.

5.4 Procedures

5.4.1 Enrolment

The study nurse will visit every new-born child shortly after a CKI calls, if possible on the same day. At the home visit immediately after birth, the nurse will ask the mother of the child for confirmation of her consent for participation in the study before revealing the randomisation (for details see section 8.1). The nurse will bring a questionnaire, a study number, sticker with study number and a vaccination card.

5.4.2 Intervention

For all children, the nurse will examine and weigh the child, encourage skin-to-skin contact to keep the new-born warm and if necessary perform umbilical cord care[1]. For children in the control clusters, the nurse will inform about vaccination opportunities (vaccination at closest health centre or vaccination by BHP nurse at next visit), as recommended by WHO[1]. For children in the intervention clusters, the nurse will inform the parents that it is recommended to administer BCG and OPV at birth. If parents accept vaccination, the nurse will administer BCG and OPV, opening a vial of BCG even if there is just one child to be vaccinated (Table 1). Children in the intervention group will be vaccinated with BCG and OPV within 72 hours after birth.

5.4.2.1 BCG vaccine

BCG vaccination is administered by intradermal injection; after vaccination most children develop a scar at the injection site. Among BCG-vaccinated children, having a BCG scar is associated with improved survival[17-19]. The proportion of children developing a scar after BCG vaccination depends on the vaccination technique and strain[20-22]. All nurses will be trained prior to the trial and supervised intensively during the beginning of the trial to ensure correct vaccination technique. The Tokyo 172 BCG strain from BCG Japan will be used for the study. The vaccine is approved by WHO and has previously been used in the national vaccination programme in Guinea-Bissau. We originally planned to use the Danish BCG strain, which has previously been used in randomised trials in Guinea-Bissau[5,6]. Due to manufacturing problems at Statens Serum Institut we were not able to buy the Danish Strain used in the pilot phase of the study. We therefore decided to use the Tokyo 172 strain. The three BCG strains are currently being tested in a randomised trial at the national hospital in Guinea-Bissau.

5.4.2.2 OPV vaccine

The OPV vaccine will be supplied from the national vaccination programme and thus strain, manufacturer and batch might vary. For all vaccines, the manufacturer, batch and expiration date will be registered.

5.4.3 Follow-up

All children enrolled in the study will receive two follow-up visits, and will be followed to 4 months of age.

5.4.3.1 Follow-up in the rural regions

The study participants will be followed through the rural HDSS, where all children below 5 years of age are followed, and information on vital status, breastfeeding status, supplementary feeding, MUAC, vaccinations, hospital admissions and whether the child has received interventions provided in campaigns is collected.

The BHP teams are accompanied by a nurse, who administers vaccines. All children have their vaccination cards examined and those missing one or more routine vaccines according to the schedule are offered these vaccines at the follow-up visits. The BHP nurse will perform the follow-up visits for the present study.

5.4.3.2 Follow-up in the urban region

The study participants will receive two follow-up visits by the study nurse at 2 and 4 months of age. The study nurse will bring BCG and OPV for children in control clusters. For the remaining routine vaccines as part of the national programme, the children will be referred to the closest health care centre. All children below the age of 3 years are followed through home visits every third month, where information on vital status, breastfeeding status, supplementary feeding, MUAC, vaccinations, hospital admissions and vaccination campaigns are collected.

5.4.4 Inclusion criteria

All children registered during pregnancy will be eligible for the study provided they have not yet received BCG at the date of the home visit.

5.4.5 Exclusion criteria

There are very few exclusion criteria, because the study is expected to answer a pragmatic question about the effect of BCG and OPV vaccination at home visits shortly after birth.

- Children born outside the cluster, and returning more than 72 hours after the delivery
- Children that the nurse evaluates to die within the next 24 hours.

5.5 Outcomes

5.5.1 Primary outcome: Non-accidental early infant mortality

The primary outcome is non-accidental mortality between the home visit and, the next follow-up visit by the BHP, when all unvaccinated children, who are home will be offered BCG or the date, where the first non-trial vaccine is registered by BHP. All children living in intervention and control clusters will be followed through the rural or urban HDSS routines, where information on vital

status of all followed children is collected. With declining mortality, we may however not be able to obtain conclusive result. We have therefore defined the sample size for the composite outcome, but retained mortality as the primary outcome.

5.5.2 Secondary outcomes

Through analyses of the secondary outcomes, we will try to gain a better knowledge of the effects of BCG and OPV. We will investigate, whether BCG and OPV affects severe morbidity:

- Non-accidental hospital admission. Hospital admission is defined as an overnight stay in a health facility. At the follow-up visits, the mother/guardian is asked, whether the child has been hospitalised. A special questionnaire is completed for each case of hospital admission to obtain information on timing, symptoms, duration and place of admission.
- Combined non-accidental mortality and non-accidental hospital admissions – severe morbidity defined as a composite outcome of mortality and first hospital admission.

Furthermore, we will assess the effect of BCG and OPV on other child-health-related outcomes:

- All-cause consultations
- Growth
    - Mid-upper-arm circumference
    - Weight-for-age z-score
- BCG scarring
- Cost-effectiveness of providing BCG and OPV at home visits
    - The cost of seeking vaccinations in rural Guinea-Bissau and the costs of consultations and hospitalisations are currently being evaluated in other studies. We will study the cost-effectiveness of providing BCG and OPV at a home visit using the effects on mortality and hospital admission from the present project.

5.6 Data management

Records with registration of all pregnant women will be copied from the data tables of the routine HDSS to the BCG trial data table. A list of future eligible children (pregnant women) will be printed prior to a visit to ensure that all pregnant women are invited to participate in the study. Enrolment forms will be entered to the BCG data table. Inconsistencies between the BCG trial data and the HDSS data will be checked. Main outcome events will be reviewed individually.

5.7 Statistical analyses

All children registered during pregnancy will enter the study when they are visited at home or 24 hours after birth, whichever comes last, thereby excluding deaths and hospital admissions on the day of birth.

5.7.1 Assessment of baseline distribution

Proportion of women, who gave consent during pregnancy, children visited and screened for enrolment criteria, fulfilling enrolment criteria and enrolled by trial arm will be reported. Distribution of background factors will be described by group assignment using proportions, means and medians as appropriate.

5.7.2 Analyses of outcomes

The data will be analysed on individual level data to account for varying cluster sizes. We will use the conventional 5% significance level and 95% confidence interval. Below are a summary of the planned analysis. Further details are provided in the analysis plan in the appendix.

5.7.3 Primary analysis of the primary outcome

In the primary analysis of the primary outcome, we will compare the effect of BCG and OPV on early infant mortality in a Cox proportional hazards model with age as underlying time. The analysis will be performed on a per-protocol population consisting of all BCG-unvaccinated children visited within 72 hours after birth, who received the assigned treatment. Follow-up will be censored at:

- Subsequent visit by the BHP in rural areas or follow-up visits by the study nurse in the urban area, where children in the control group will receive BCG
- Date of registration of first non-trial vaccine
- Death due to accident
- Migration

5.7.4 Primary analyses of secondary outcomes

We will assess the effect of BCG and OPV on severe morbidity (hospital admission and composite outcome of hospital admission and death) using Cox proportional hazards models with age as underlying time scale. We will furthermore assess the effect of BCG and OPV on the above-mentioned other secondary outcomes. The effect on growth measures will be assessed in linear regression models. To assess the effect on BCG scarring and consultations, we will use binary regression models.

5.7.5 Effect modifier analyses of primary outcome

In secondary analysis, we will investigate, whether the effect of BCG+OPV differ by the following potential effect modifiers:

- Low birth weight, defined as a birth weight lower than 2500g.
    - Previous randomised trials have been limited to LBW children for whom vaccination is normally delayed. In the present trial, the majority of the children will be of

normal-birth weight (>2500g, NBW). Hence, we will assess whether LBW modifies the effect of BCG+OPV on early infant mortality and morbidity.

- Sex
    - Since we have shown sex-differential effects of vaccines[4,7,8,23], we will evaluate if sex modifies the effect of BCG+OPV on early infant morbidity and mortality.
- Maternal BCG scarring
    - Previous studies of non-specific effects suggest that maternal priming may be important for the NSEs of vaccines[24]. Therefore, we will assess, whether the effect of OPV and BCG is modified by maternal BCG-scar status.
- Season
    - Guinea-Bissau has two distinct seasons, a dry season from December to May and a rainy season from June to November. Previous trials have indicated that the beneficial effect of BCG was particularly marked during the dry season (unpublished). We will therefore assess whether the effect in the dry season (December-May) is stronger than the effect during the rest of the year.

All analyses will be adjusted for clustering. Further details are provided in the analysis plan in the appendix.

5.7.6 Intention-to-treat analyses of primary outcome

We will also report intention-to-treat analyses including:

- all children, who had a home visit by a study nurse (i.e., including children who did not receive assigned treatment, children who were not enrolled because they were moribund, or who did not accept to participate).

5.7.7 Sensitivity analyses

In sensitivity analyses, we will assess whether the conclusions are robust to censoring at general health intervention campaigns, and whether we can identify if OPV and BCG prevents cause-specific hospitalisations or deaths (for details, see appendix: analysis plan).

6 TIME SCHEDULE AND ECONOMY

The trial will be initiated with a pilot study in Biombo in June 2015. The trial will be scaled up to full study including Oio and Cacheu regions in July 2016. The trial will be expanded to include the urban study area in July 2017. We expect enrolments to end in December 2020. External funding will be sought, but the Research Center for Vitamins and Vaccines, Bandim Health Project and Statens Serum Institut guarantee to cover the project finances.

7 DISSEMINATION OF RESULTS

The findings will be published in international peer-reviewed journals and results with implications for WHO vaccination policy implementation will be communicated to WHOs Strategic Advisory Group of Experts on Immunization (SAGE) and Global Advisory Committee on Vaccine Safety. A report of the study results will be provided to the National Institute of Public Health. The close cooperation between BHP and the Ministry of Health in Guinea-Bissau will ensure that the information gathered will be disseminated to the national primary health programme.

8 ETHICAL CONSIDERATIONS

BCG is recommended at birth but vaccination is often delayed; in rural Guinea-Bissau most children receive BCG when aged >1month[16]. While there may now be increasing acceptance that BCG has beneficial non-specific effects, we are still a long way from altering the implementation of the vaccination programme. The proposal compares two ways of delivering BCG and both will be an improvement relative to the current situation, where less than 40% of all infants get BCG during the first month of life. Hence, no child will receive BCG later than it would have done, had the trial not been carried out.

8.1 Explanation to participants

The pregnant women will receive an oral and a written explanation of the study at the time of registration. It will be explained that normally new-born children should go to the closest health centre to receive their first vaccinations. However, some studies have suggested that it might be beneficial to receive BCG and OPV early and the BHP will therefore like to know whether it is worthwhile to provide the vaccination at home. The study will therefore give BCG+OPV to children in half of the clusters. The study will therefore provide standard care in control clusters and standard care and vaccines in intervention clusters. It will be explained that children in the control clusters can seek vaccination elsewhere and will be offered the BCG vaccine and OPV at the next home visit.

8.2 Informed consent

All pregnant women will receive an oral and a written explanation of the study, and be offered to participate in the study at the time of registration of the pregnancy. In the rural area the explanation will be given by the BHP nurse at the two-monthly visits. In the urban area the explanation will be given by the fieldwork assistant at a monthly visit. If the pregnant woman accepts to take part in the study, they will be asked to sign or fingerprint a consent form. The fingerprint has to be confirmed by an independent witness who also signs the form. At the home visit after birth, the study nurse will explain the study to the mother again and ask for oral confirmation of consent to participate in

the study. If the mother is not able to present the signed consent form, new consent forms will be filled out.

8.3 Safety monitoring

WHO recommends the BCG and OPV vaccines to be given at birth in low-income countries. The safety of the BCG Japan vaccine, which is used for the present study, have been evaluated by WHO, who has approved the vaccine. OPV is provided from the national health programme. Adverse reactions are rare for both BCG and OPV. At the two first visits after enrolment in the study, the BHP nurse will examine the BCG vaccination site and the axillary lymph glands of all children to assess suppurative lymphadenitis as an adverse reaction to the BCG vaccination. Other serious adverse events will be captured through primary and/or secondary outcomes (mortality, hospitalisations and consultations).

A data safety and monitoring board will oversee the trial procedures.

8.4 Potential problems

In case of vaccine shortage of BCG Japan, the study group will consult the Data Safety and Monitoring Board to discuss, whether the study should be discontinued or if the study can proceed with another BCG vaccine.

9 IMPLICATIONS

WHO recommends BCG at birth for normal-birth-weight children. However, due to the focus on not wasting vaccine doses BCG vaccination is delayed in many low-income countries. WHO recommends three home visits after birth, which is resource demanding and not implemented in many low-income countries. It is not recommended that the home visits are utilised for early vaccination. If we can confirm our hypothesis of reduced early infant mortality by providing BCG and OPV at a single home visit, this should be included in the WHO recommendations, and it will be an incentive for countries to introduce a single home visit with vaccines. In countries, where home visits are already in place, vaccines can easily be added to reduce early infant mortality.

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Table 1: Trial design

Timing	**Routine visit before birth**	**Shortly after birth – within 72 hours**		**1st routine visit after birth**	**2nd and 3rd routine visit after birth**
Personel	BHP Field assistant (FA) and Nurse	Community Key Informant (CKI)	Study Nurse	BHP FA and Nurse	BHP FA and Nurse
Control group	Information about trial and invitation to participate. Informed consent.	CKI inform about birth	• Umbilical cord and skin care • Encourage skin-to-skin contact to keep the new-born warm • Examine and Weigh the child • ***Inform about vaccination opportunities***	FA: • Interview on morbidity and mortality Nurse: • Examine the BCG vaccination site and lymph glands • Measure temperature • Weigh the child • ***Provide BCG and OPV***	FA: • Interview on morbidity and mortality Nurse: • Examine the BCG vaccination site and lymph glands • Measure temperature • Weigh the child • Provide routine vaccinations
Intervention group			• Umbilical cord and skin care • Encourage skin-to-skin contact to keep the new-born warm • Examine and weigh the child • ***Administer BCG and OPV***	FA: • Interview on morbidity and mortality Nurse: • Examine the BCG vaccination site and lymph glands • Measure temperature • Weigh the child	

Supplementary material - Can earlier BCG-Japan and OPV vaccination reduce early infant mortality? A cluster-randomised trial in Guinea-Bissau

1 Decision to expand and stop the trial

In 2017, the investigators discussed the lower-than-expected mortality and lower-than-expected enrolment rates with the Data Safety and Monitoring Board. Following this discussion, the investigators decided to expand the trial to include the BHP urban study area. The fieldworkers in the urban HDSS were trained in trial routines and a BHP nurse was trained to conduct home visits. Once the trial routines were in place the trial was expanded to the urban area from September 18, 2017.

In addition to expanding the trial, several efforts were made to increase the enrolment rate:

- Additional village visits with information about the trial and information on who to call
- Posters with trial information was placed in all villages (see supplementary figure 1)
- Community Key Informants were in close contact with the trial supervisor
- Pregnant women, who had consented for trial participation, were reminded of the trial at all subsequent household visits

Despite these efforts to increase enrolment rates, enrolments were progressing much slower than anticipated. The low enrolment rates were mainly due to problems with identifying births in time (before 72 hours after birth). This problem was probably both due to mothers not remembering to contact the BHP team/CKI directly after birth, and that CKIs were not able to/expected to make outreach daily. After renewed discussion with the Data Safety and Monitoring Board, it was decided to discontinue the trial in the urban area: there was only a single death registered in the urban area, which did therefore not add power to the trial. Further, it was decided to discontinue the trial in the Cacheu health region in rural Guinea-Bissau, as enrolments were few and resource demanding, therefore trial resources were focussed on Oio and Biombo health regions. The trial was stopped in the urban area and Cacheu health region on March 15, 2019.

The discussion with the Data Safety and Monitoring Board continued and the investigators decided to end the trial in all regions. The trial was ended on August 31, 2019, due to lower-than-expected mortality and lower-than-expected enrolment rates.

Analyses of the trial was only conducted after the trial was ended, and the results of the trial was therefore unknown at the time of deciding to end the trial. Thus, results did not affect the decision to end the trial.

2 Statistical analyses of secondary outcomes

The complete analysis plan can be found in the published protocol paper[1].

"Non-accidental hospital admission" and "severe morbidity"

The effect of providing BCG and OPV at a single home visit on non-accidental hospital admissions and severe morbidity (composite outcome of non-accidental mortality and non-accidental hospital admissions) was assessed in Cox proportional hazards models with age as underlying timescale. The analyses were stratified for factors used in the randomisation (region and cluster-level pre-trial mortality (high/low)) and sex. Children entered the analyses on the date of enrolment or 24 hours of age, whichever came last, and remained in the analysis until whatever came first: the first subsequent visit by the BHP team, date of registering first non-trial vaccine after enrolment, death, hospital admission, migration, or 60 days of life. Proportional hazards assumptions were evaluated using Schoenfeld residuals significance test, log-log survival curves, and by checking whether the effect interacted with the underlying time scale using the time-varying-covariates (tvc)-options.

"Consultations"

The effect of providing BCG and OPV at a single home visit on all-cause consultations was assessed in log-binomial regressions. The analysis was adjusted for factors used in the randomisation (region and

cluster-level pre-trial mortality (high/low)) and sex. Robust standard errors were used to account for clustering.

"Mid-upper-arm circumference" and "weight for age z-score"

The effect of providing BCG and OPV at a single home visit on mid-upper-arm circumference and weight for age z-score between enrolment and first follow-up visit within 6 months of age was assessed in linear regressions. The analyses were adjusted for factors used in the randomisation (region and cluster-level pre-trial mortality (high/low)) and sex. Robust standard errors were used to account for clustering.

"BCG scarring"

The effect of providing BCG and OPV at a single home visit on BCG scarring at the first visit after 6 months of age was assessed in log-binomial regressions. The analysis was adjusted for factors used in the randomisation (region and cluster-level pre-trial mortality (high/low)) and sex. Robust standard errors were used to account for clustering.

3 List of health intervention campaigns during the trial targeting trial participants

Dates	Target group	Intervention
24-11-2017 to 27-11-2017	0-59 months of age	Oral polio vaccine*
20-04-2018 to 24-04-2018	0-59 months of age	Oral polio vaccine*

* Children aged 6-11 months were also eligible for Vitamin A, and children aged 12-59 months were eligible for both oral polio vaccine, vitamin A and mebendazole. However, trial participants were all aged less than 6 months.

4 Author reflexivity statement

Study conceptualisation

1. How does this study address local research and policy priorities?

   The trial was made to address a local issue with delay in vaccinations recommended at birth, partly due to restricted vial-opening policies.

2. How were local researchers involved in study design?

   AR conceived the idea for the study together with PA and CB. The study was designed by SMT, PA, CB, and ABF, who have all worked in Guinea-Bissau for at least 10 years. The study design was made in close communication with local health authorities, and AR was consulted during this process.

Research management

1. How has funding been used to support the local research team(s)?

   The funding has been used to support the Health and Demographic Surveillance System, which has been in place in Guinea-Bissau since 1978, and was established by PA. Thus, the funding was primarily used for salaries for local staff.

Data acquisition and analysis

1. How are research staff who conducted data collection acknowledged?

   Supervisors of the data collection, both local (IdSB, JaM, and LMVdS) and international (SMT, ADS, JHS, and ABF) are co-authors of the paper. However, further field assistants and nurses were involved in the trial. As they do not fulfill the ICMJE criteria for authorship, they have been acknowledged in the acknowledgement section.

2. How have members of the research partnership been provided with access to study data?

The original data is stored on local servers in Guinea-Bissau and local and international researchers have had access to the trial data.

3. How were data used to develop analytical skills within the partnership?

   The trial has been used to build competencies on data management for IdSB, JaM, and LMVdS. During the trial period JuM initiated a master in public health and development, although this was not based on the present work.

Data interpretation

1. How have research partners collaborated in interpreting study data?

   The results have been discussed with the whole research team, and both local and international researchers have contributed to the interpretation of study data. As not all local researchers are able to read and write English, the paper have been discussed in the local language, and a summary of trial results have been made in Portuguese. Local researchers have adapted the Portuguese summary for communication of trial results to the Ethical Committee in Guinea-Bissau, and to inform local health authorities.

Drafting and revising for intellectual content

1. How were research partners supported to develop writing skills?

   Writing skills in English have not been prioritized as part of this project. However, this is prioritized in other collaborative projects within the group.

2. How will research products be shared to address local needs?

   Research results are shared with local health authorities in Portuguese, and we are currently conducting a follow-up trial in close collaboration with the local health authorities, where the effect of making BCG vaccine available at the first health-facility visit is investigated.

Authorship

1. How is the leadership, contribution and ownership of this work by LMIC researchers recognised within the authorship?

   Local researchers have been deeply involved with setting up and maintaining the trial routines, and have ownership over the trial. Due to not speaking English or having statistics training, most of the local study team has not been able to participate directly in the analyses and writing of the paper, but have rather commented on results. However, all researchers involved in the project (both local and international) have significantly increased the research skills, and it has been a focus throughout the trial to ensure that local researchers (as well as field assistants and nurses) developed new skills during the trial. All LMIC researchers that could be acknowledged as authors have been included as such.

2. How have early career researchers across the partnership been included within the authorship team?

   Early career researchers have been supported throughout the trial and are included as authors.

3. How has gender balance been addressed within the authorship?

   This has not been a particular focus for the present trial. The author group consists of 4 female authors and 8 male authors.

Training

1. How has the project contributed to training of LMIC researchers?

   The trial has trained CKIs and nurses, most of whom had previously not been involved with research, to be able to participate in data collection for research. Further, the trial has trained trial supervisors to be able to take a more leading position in the trial routines. Most trial supervisors were field assistants prior to the initiation of the present trial. JuM has during the trial been supported in their analytical and interpretation skills by some of the international authors, although this project was not the major focus of JuM's work.

Infrastructure

1. How has the project contributed to improvements in local infrastructure?

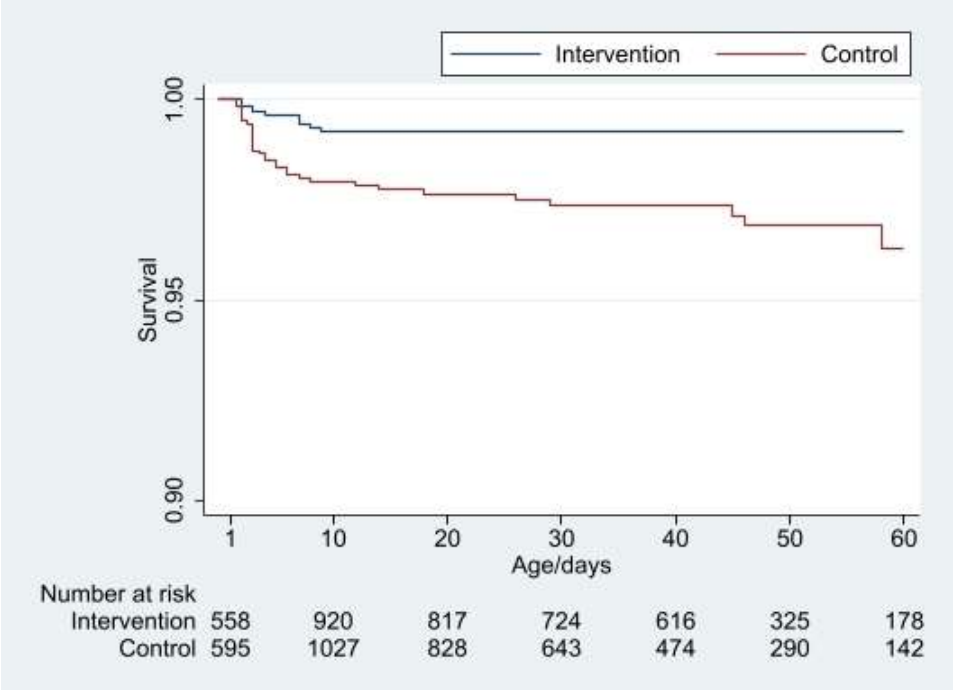
   The trial was conducted within the urban and rural HDSS sites, which have been in place since 1978 and 1990. These sites are the most important research infrastructure in Guinea-Bissau. During the trial, the collaboration with local health authorities have been strengthened and the trial, which is now conducted based on the results of the present trial, takes place at the local health facilities in close collaboration with local health authorities.

Governance

1. What safeguarding procedures were used to protect local study participants and researchers?

   Informed consent processes were implemented and staff were trained on GCP during the trial implementation.

Supplementary Figure 1. Kaplan-Meier survival curve for the primary outcome non-accidental mortality



Appendix 1: General analysis principles

1 Participant population

All main analyses will be completed in a per-protocol (PP) population of all BCG-unvaccinated children, who received a home visit within 72 hours after birth, and who received the assigned treatment. The primary analysis and hence the main conclusion of the trial will be based on the PP analysis. Unless explicitly stated all analyses will be PP.

Since the cluster-size varies, data will be analysed on individual level data. All statistical tests will be 2-tailed and p<=0.05 considered statistically significant for analyses involving the primary outcome.

2 Unadjusted and adjusted analyses

Both unadjusted analyses and analyses adjusted for place of delivery will be reported. Conclusions will be based on the unadjusted analyses.

3 Multiple testing

P-values will not be corrected for multiple testing. Secondary outcomes are tested to observe if the pattern is similar across other health outcomes. Consequently, p<=0.05 will not be employed as a threshold for statistical significance for secondary outcomes. For the sensitivity analyses, we will not consider statistical significance, but rather robustness of the conclusions across different definitions of outcomes and co-variates.

4 Missing data

All analyses will be complete-case analyses.

5 Proportional hazards

To test the proportional hazards assumption, a required assumption of the Cox regression, we will perform formal significance tests based on Schoenfeld residuals. In addition, we will assess proportionality by allowing the hazard ratio to interact with the underlying timescale to identify a possible time trend. Finally, we will assess proportionality graphically via log-log survival curves.

Significance tests based on Schoenfeld residuals will be performed via the stata command *estat phtest, detail* leading to both a global test and a test for each covariate, the latter being relevant only when we study effect modifications. Presentation of log-log survival curves will be undertaken via *stphplot.* Finally, possible interactions between hazard ratios and the underlying time scale will be

further investigated via the *stcox* procedure and the *tvc()* option. For the models including effect modifications we will construct a new interaction variable (i.e., a four-level variable representing the interaction) such that a graphical assessment of proportionality can be undertaken assessing the four-level variable in a log-log survival plot.

If we identify evidence for non-proportionality, we will still report the marginal hazard ratios but supplement this measure by hazard ratios for 2-3 properly selected categorical time-periods identified based on the aforementioned proportionality investigations.

Appendix 2 - Planned analyses

1 Baseline

Descriptive statistics:

For eligible children visited by a study nurse within 72 hours after birth, we will describe reasons for exclusion by group allocation.

Distribution of background factors will be presented by group allocation overall and by sex and region. Background factors will be summarised by counts (percentages), means (standard deviation) or medians (interquartile range) as appropriate. Information on the proportion with missing information will be provided.

Table 1: Summary of background factors by intervention and control group

- Sex
- Age at enrolment
- Region
- Weight at enrolment
- Temperature
- Mid-upper-arm circumference
- Head circumference
- Place of birth
- Socioeconomic factors (maternal education and housing conditions)

2 Primary analysis of primary outcome

The primary analysis of early infant non-accidental mortality will be assessed on a per-protocol (PP) analysis allowing for different baseline hazards according to factors used in the randomization (Region, pre-study mortality level (high/low)) and sex, thus allowing different baseline hazards for boys and girls. To account for clustering we will employ cluster-robust variance estimates.

For the primary outcome, we will use Cox proportional hazards models that allow different baseline hazards according to the above mentioned factors and with age as underlying time-scale. Deaths due to accidents will be censored.

The primary analysis of the primary outcome is described in more detail in table 2.

Table 2: Primary analysis of primary outcome

Population	Children visited within 72 hours after birth are eligible for the study. Exclusion criteria:

	- Children already BCG vaccinated - Moribund children (Expected not to survive the next 24 hours, as evaluated by the health facility nurse at the enrolment visit) - Children in rural villages where the BHP mobile teams coincidentally were in the village the same day (and vaccinated all children)
Observation period	From: Enrolment visit or 24 hours after birth, whichever comes last To: 60 days of life Censoring, first of: - Visit by the BHP - 60 days - Death due to accident - Date of registering first non-trial vaccine given after enrolment - Migration (migration out of the study area as per HDSS definition)
Time scale	Age
Failure definition	Death
Statistical tool	Cox proportional hazards model
Stratification	We will employ models that allow for different baseline hazards according to sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
<u>Outline stata code</u> For analysis: For model checking:	 stset outdate, f(dead==1) enter (max(dob+1, date_enrol) /// exit (min (dnasc+60, date_mobileteam) origin(dob) stcox random, strata(sex reg prmorlev) vce(cl regam) estat phtest, detail† stphplot, strata(random) adj(sex reg prmorlev) stcox random, strata(sex reg prmorlev) vce(cl regam) tvc(random) texp(_t) *reg=region, prmorlev=pre-study mortality level, regam=village cluster

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

3 Effect-modifier analyses of primary outcome

We will assess whether the effect of the intervention on the primary effect measure is modified by the following potential effect modifiers.

Table 3. Sex as a potential effect modifier of the primary outcome

Potential effect modifier	Sex
Design	We will perform the analysis as describe above (for the primary analysis) allowing the effect of the intervention to differ between the sexes.
Reasoning	Previous studies have found sex-differential non-specific effects[1 2], therefore, we will assess the sex-differential effects.
Outline stata code: For analysis: For model checking:	 stcox random#sex sex, strata(sex reg prmorlev) vce(cl regam) contrast random#sex estat phtest, detail† stphplot, strata(random_sex) adj(sex reg prmorlev) stcox random#sex sex, strata(sex reg prmorlev) vce(cl regam) /// tvc(random#sex sex) texp(_t) *reg=region, prmorlev=pre-study mortality level, regam=village cluster, random_sex=a four-level variable based on the four possible combinations of random and sex

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

Table 4. Maternal BCG scar as a potential effect modifier of the primary outcome

Potential effect modifier	Maternal BCG scar (yes/no)
Design	We will perform the analysis as described above (for the primary analysis) allowing the effect of the intervention to differ by maternal BCG-scar status
Reasoning	A recent randomized trial in Denmark found that the effect of BCG varied by whether the mother had received BCG or not[3]. Since, BCG scar is a life-long marker of a successful BCG-vaccination, we will assess whether the effect of BCG differs by maternal BCG-scar status
Outline stata code: For analysis:	 stcox random#mBCGscar mBCGscar, strata(sex reg prmorlev) vce(cl regam)

For model checking:	contrast random#mBCGscar estat phtest, detail† stphplot, strata(random_mBCGscar) adj(sex reg prmorlev) stcox random#mBCGscar mBCGscar, strata(sex reg prmorlev) /// vce(cl regam) tvc(random#mBCGscar mBCGscar) texp(_t) * mBCGscar= maternal BCG scar, reg=region, prmorlev=pre-study mortality level, regam=village cluster, random_mBCGscar=a four-level variable based on the four possible combinations of random and maternal BCG scar

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

Table 5. Low birthweight as a potential effect modifier of the primary outcome

Potential effect modifier	Low birthweight (<2500g: yes/no)
Design	We will perform the analysis as described above (for the primary analysis) allowing the effect of the intervention to differ by birthweight strata
Reasoning	Birthweight is an important risk factor for mortality. Previous randomised trials from Guinea-Bissau assessing the effect of BCG on mortality have been performed among low-birth-weight children.
<u>Outline stata code:</u> For analysis: For model checking:	 stcox random#LBW LBW, strata(sex reg prmorlev) vce(cl regam) contrast random#LBW estat phtest, detail† stphplot, strata(random_LBW) adj(sex reg prmorlev) stcox random#LBW LBW, strata(sex reg prmorlev) vce(cl regam) /// tvc(random#LBW LBW) texp(_t) * LBW=Low birthweight, reg=region, prmorlev=pre-study mortality level, regam=village cluster, random_LBW=a four-level variable based on the four possible combinations of random and low birthweight

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

Table 6. Season as a potential effect modifier of the primary outcome

Potential effect modifier	Season of birth (Dry: December-May/Rainy: June-November)
Design	We will perform the analysis as described above (for the primary analysis) allowing the effect of the intervention to differ by season of birth
Reasoning	Previous studies have found that the effect of some vaccines is stronger in the dry season[4]. Therefore, we would like to assess if the effect of BCG and OPV differs according to season.
Outline stata code: For analysis: For model checking:	 stcox random#season season, strata(sex reg prmorlev) vce(cl regam) contrast random#season estat phtest, detail† stphplot, strata(random_season) adj(sex reg prmorlev) stcox random#season season, strata(sex reg prmorlev) vce(cl regam) /// tvc(random#season season) texp(_t) *reg=region, prmorlev=pre-study mortality level, regam=village cluster, random_season=a four-level variable based on the four possible combinations of random and season

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

4 Primary analyses of secondary outcomes

Non-accident morbidity

Since it can be difficult to distinguish between hospital admissions and outpatient contact through interviews, we have defined hospital admissions as an overnight stay in a health facility. Hospital admissions due to accidents are ignored but the follow-up time is (interval) censored while the child is admitted.

Table 7: Non-accident hospitalisation

Population	Identical to primary analysis of primary outcome
Observation period	From: Enrolment visit or 24 hours after birth, whichever comes last To: 60 days of life Censoring, first of: - Visit by the BHP - Date of registering first non-trial vaccine given after enrolment - Death - Hospital admission due to accident

	- 60 days - Migration (migration out of the study area as per HDSS definition)
Time scale	Age
Failure definition	First hospital admission – only overnight hospitalisations or arrival at the hospital and death within the first day will be considered in this analysis.
Statistical tool	Cox proportional hazards model
Stratification	We will employ models that allow for different baseline hazards according to sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
Outline stata code For analysis: For model checking:	 stset outdate, f(hosp==1) enter (max(dob+1, date_enrol) /// exit (min (dnasc+60, date_mobileteam) origin(dob) stcox random, strata(sex reg prmorlev) vce(cl regam) estat phtest, detail† stphplot, strata(random) adj(sex reg prmorlev) stcox random, strata(sex reg prmorlev) vce(cl regam) tvc(random) texp(_t) *reg=region, prmorlev=pre-study mortality level, regam=village cluster

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

Severe morbidity

We will evaluate the effect on severe morbidity considered as the composite outcome of non-accidental death and non-accidental hospital admission. Since it can be difficult to distinguish between hospital admissions and outpatient contact through interviews, we have defined hospital admissions as an overnight stay in a health facility. Hospital admissions due to accidents are ignored but the follow-up time is (interval) censored while the child is admitted. The potential effect modifiers for the primary outcome specified in tables 3-6 will also be assessed for the composite outcome.

Table 8: Severe morbidity

Population	Identical to primary analysis of primary outcome
Observation period	From: Enrolment visit or 24 hours after birth, whichever comes last

	To: 60 days of life Censoring, first of: - Visit by the BHP - Date of registering first non-trial vaccine given after enrolment - Death/Hospital admission due to accident - 60 days - Migration (migration out of the study area as per HDSS definition)
Time scale	Age
Failure definition	Death or first hospital admission
Statistical tool	Cox proportional hazards model
Stratification	We will employ models that allow for different baseline hazards according to sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
Outline stata code: For analysis For model checking	 stset outdate, f(event==1) enter (max(dob+1, date_enrol) /// exit (min (dnasc+60, date_mobileteam) origin(dob) stcox random, strata(sex reg prmorlev) vce(cl regam) estat phtest, detail† stphplot, strata(random) adj(sex reg prmorlev) stcox random, strata(sex reg prmorlev) vce(cl regam) tvc(random) texp(_t) *reg=region, prmorlev=pre-study mortality level, regam=village cluster

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

Table 9: All-cause consultations

Population	Identical to primary analysis of primary outcome
Observation period	From: Enrolment visit or 24 hours after birth, whichever comes last To: 60 days of life Censoring, first of: - Visit by the BHP - Date of registering first non-trial vaccine given after enrolment - 60 days

	- Migration (migration out of the study area as per HDSS definition) - Death - End of study
Failure definition	An out-patient consultation within the observation period
Statistical tool	Log-binomial regression
Adjustment	We will adjust the analysis for sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
Stata code	Binreg cons random sex b1.reg prmorlev, rr vce(cl regam) *cons=out-patient consultation, reg=Region, prmorlev=Pre-study mortality level, regam=village cluster

Growth

Table 10: Mid-upper-arm circumference (MUAC)

Population	Identical to primary analysis of primary outcome
Observation time point	First visit by the mobile teams
Growth measures	MUAC will be analysed using the measured value
Statistical tool	Linear regression
Adjustment	We will adjust the analysis for MUAC at enrolment, age at MUAC measurement, sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
Stata code	Regress MUAC random sex b1.reg prmorlev MUACenrol MUACage, /// vce(cl regam) *reg=region, prmorlev=pre-study mortality level, MUACenrol=MUAC at enrolment, MUACage= age at MUAC assessment, regam=village cluster

Table 11: Weight-for-age z-score

Population	Identical to primary analysis of primary outcome
Observation time point	First visit by the mobile teams
Growth measures	Weight will be analysed using the WHO weight-for-age z-score
Statistical tool	Linear regression
Adjustment	We will adjust the analysis for weight-for-age at enrolment, sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster

Stata code	Regress w-z-score random w-z-enrol sex b1.reg prmorlev, vce(cl regam) *w-z-score=weight-for-age z-score at first visit by the mobile teams, w-z-enrol=weight-for-age z-score at enrolment, reg=region, prmorlev=pre-study mortality level, regam=village cluster

BCG scarring

Table 12: BCG scarring

Population	Identical to primary analysis of primary outcome
Observation timepoint	First visit after 6 months of age
Failure definition	Scar (yes/no)
Statistical tool	Log-binominal regression
Adjustment	We will adjust the analysis for sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
Stata code	Binreg scar random sex b1.reg prmorlec, rr vce(cl regam) *reg=region, prmorlev=pre-study mortality level, regam=village cluster

Cost-effectiveness of providing BCG and OPV at birth

A cost effectiveness analysis seeking to measure the cost per death averted using a societal perspective will be performed, contrasting the costs of vaccine provision in the present programme and an outreach system as tested in the trial. The costs/savings associated with different rates of consultations and admissions will also be taken into account.

Suppurative lymphadenitis

We will assess the incidence of suppurative lymphadenitis as a reaction to BCG vaccination in the intervention and control clusters. Other serious adverse events to the BCG and OPV vaccine will be captured through the outcome measures (mortality, hospital admission and consultations).

5 Sensitivity analyses to test for robustness of conclusions

Table 13: Cause-specific death

Population	Identical to primary analysis of primary outcome
Observation period	From: Enrolment visit or 24 hours after birth, whichever comes last To: 60 days of life Censoring, first of:

	- Visit by the BHP - 60 days - Death due to accident - Date of registering first non-trial vaccine given after enrolment - Migration (migration out of the study area as per HDSS definition)
Time scale	Age
Failure definition	Death due to: Malaria, Respiratory Infection, Sepsis, Gastrointestinal disease, Other
Statistical tool	Cox proportional hazards model
Stratification	We will employ models that allow for different baseline hazards according to sex, region and pre-study mortality level
Clustering	We will use robust standard errors to account for clustering within village cluster
<u>Outline stata code</u> For analysis: For model checking:	 stset outdate, f(event==1&cause==X) enter (max(dob+1, date_enrol) /// exit (min (dnasc+60, date_mobileteam) origin(dob) stcox random, strata(sex reg prmorlev) vce(cl regam) estat phtest, detail† stphplot, strata(random) adj(sex reg prmorlev) stcox random, strata(sex reg prmorlev) vce(cl regam) tvc(random) texp(_t) *reg=region, prmorlev=pre-study mortality level, regam=village cluster

† If the proportional hazards assumption is not fulfilled we will still report the marginal hazard ratio but it will be supplemented by hazard ratios for two-three categorized time periods.

In sensitivity analyses, we will furthermore, assess whether the conclusions are robust to the following:

- Censoring follow-up at general health intervention campaigns (e.g. OPV campaigns)
- Altering the population to using an intention-to-treat approach, including all children, who had a home visit by a study nurse (i.e., including children who did not receive assigned treatment, children who were not enrolled because they were moribund, or who did not accept to participate).

- Altering the outcome from non-accidental mortality to all-cause mortality including also deaths due to accidents.
- For the non-accident hospitalisations, we will perform the analyses allowing for repeated hospitalisations. A child will return to the at-risk population the day after discharge from the hospital.

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Supplementary table 1. Effect of providing BCG and OPV vaccines at a home visit on non-accidental mortality when follow-up is censored at day 10 of life

	n	MR per 1000 PYRS (deaths/PYRS)	HR (95% CI)
Intervention	1006	313.5 (7/22)	0.66 (0.26 - 1.66)
Control	1206	780.7 (20/26)	Reference
Rural			
Intervention	683	403.2 (6/15)	0.60 (0.23 - 1.61)
Control	976	957.8 (20/21)	Reference
Urban			
Intervention	323	134.2 (1/7)	NA
Control	230	0.0 (0/5)	Reference

MR: Mortality rate, PYRS: Person years, HR: Hazard ratio, CI: Confidence interval, NA: Not applicable

Supplementary table 2. Mortality by cause of death

	MR per 1000 PYRS (deaths/PYRS)	HR (95% CI)
Malaria		
Intervention	0.0 (0/105)	N/A
Control	9.9 (1/101)	Reference
Respiratory infection		
Intervention	9.5 (1/105)	0.53 (0.06 - 5.04)
Control	29.6 (3/101)	Reference
Sepsis		
Intervention	28.5 (3/105)	0.51 (0.16 - 1.65)
Control	98.7 (10/101)	Reference
Gastrointestinal disease		
Intervention	0.0 (0/105)	N/A
Control	9.9 (1/101)	Reference
Other		
Intervention	28.5 (3/105)	0.37 (0.11 - 1.23)
Control	128.4 (13/101)	Reference

Supplementary table 3. Effect of providing BCG and OPV vaccines at a home visit on neonatal mortality

	n	MR per 1000 PYRS (deaths/PYRS)	HR (95% CI)
Intervention	1007	110.2 (7/64)	0.50 (0.21 - 1.21)
Control	1208	357.3 (24/67)	Reference
Rural			
Intervention	684	148.3 (6/40)	0.45 (0.18 - 1.16)
Control	978	424.9 (24/56)	Reference
Urban			
Intervention	323	43.4 (1/23)	NA
Control	230	0.0 (0/11)	Reference

MR: Mortality rate, PYRS: Person years, HR: Hazard ratio, CI: Confidence interval, NA: Not applicable

Supplementary table 4. Effect of BCG and OPV vaccines provided at a home visit on non-accidental mortality, with follow-up censored at vaccination campaigns

	n	MR per 1000 PYRS (deaths/PYRS)	HR (95% CI)
Intervention	986	63.5 (6/95)	0.37 (0.15 - 0.88)
Control	1193	290.3 (27/93)	Reference
Rural			
Intervention	670	86.2 (5/58)	0.32 (0.12 - 0.83)
Control	966	341.5 (27/79)	Reference
Urban			
Intervention	316	27.4 (1/36)	NA
Control	227	0.0 (0/14)	Reference

MR: Mortality rate, PYRS: Person years, HR: Hazard ratio, CI: Confidence interval, NA: Not applicable

Supplementary table 5. Effect of BCG and OPV vaccines provided at a home visit on non-accidental mortality until day 60 of life without censoring at next vaccination/mobile team visit

	n	MR per 1000 PYRS (deaths/PYRS)	HR (95% CI)
Intervention	1007	50.2 (8/159)	0.45 (0.21 - 0.98)
Control	1218	158.1 (30/190)	Reference
Rural			
Intervention	684	64.6 (7/108)	0.41 (0.18 - 0.93)
Control	988	195.6 (30/153)	Reference
Urban			
Intervention	323	19.6 (1/51)	NA
Control	230	0.0 (0/36)	Reference

MR: Mortality rate, PYRS: Person years, HR: Hazard ratio, CI: Confidence interval, NA: Not applicable

Supplementary table 6. Effect of BCG and OPV vaccines provided at a home visit on non-accidental mortality in the intention-to-treat cohort

	n	MR per 1000 PYRS (deaths/PYRS)	HR (95% CI)
Intervention	1018	65.8 (7/106)	0.38 (0.17 - 0.84)
Control	1224	282.6 (29/103)	Reference
Rural			
Intervention	691	94.3 (6/64)	0.34 (0.15 - 0.79)
Control	992	335.9 (29/86)	Reference
Urban			
Intervention	327	23.4 (1/43)	NA
Control	232	0.0 (0/16)	Reference

MR: Mortality rate, PYRS: Person years, HR: Hazard ratio, CI: Confidence interval, NA: Not applicable

Supplementary table 7. Effect of providing BCG and OPV vaccines at a home visit on growth

	MUAC enrolment Mean (SD)	MUAC follow-up* Mean (SD)	Difference in MUAC at follow-up (95% CI)	WAZ enrolment Z-score (SD)	WAZ follow-up* Z-score (SD)	Difference in WAZ at follow-up (95% CI)
Intervention	98 (9)	118 (14)	0·82 (-0·39 - 2·03)	-0·78 (1·01)	-0·50 (1·08)	0·04 (-0·05 - 0·13)
Control	97 (10)	115 (14)	Reference	-0·91 (1·04)	-0·67 (1·10)	Reference
Rural						
Intervention	97 (9)	114 (13)	1·02 (-0·43 - 2·46)	-0·83 (1·02)	-0·59 (1·11)	0·05 (-0·06 - 0·17)
Control	97 (10)	112 (13)	Reference	-0·96 (1·03)	-0·76 (1·10)	Reference
Urban						
Intervention	98 (8)	126 (10)	-0·28 (-2·48 - 1·91)	-0·67 (0·98)	-0·33 (1·01)	-0·02 (-0·19 - 0·15)
Control	99 (11)	126 (11)	Reference	-0·70 (1·02)	-0·33 (1·06)	Reference

MUAC: Mid-upper-arm circumference, SD: Standard deviation, CI: Confidence interval, WAZ: Weight-for-age Z-score

* Measured at first follow-up visit within 6 months of age.

Supplementary table 8. Adverse events (lymphadenitis and suppurative lymphadenitis)

	n	Lymphadenitis n(%)	Suppurative lymphadenistis n(%)
Intervention	887	3 (0%)	0 (0%)
Control	1046	0 (0%)	0 (0%)
Rural			
Intervention	592	0 (0%)	0 (0%)
Control	845	0 (0%)	0 (0%)
Urban			
Intervention	295	3 (1%)	0 (0%)
Control	201	0 (0%)	0 (0%)