Statistical analysis plan:

Can Earlier BCG Vaccination Reduce Early Infant Mortality? A Randomised Trial

Protocol ID: BCG150501

Clinical Trial Registration number: NCT02504203

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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
<u>Outline stata code:</u> 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.

References

1. Benn CS, Netea MG, Selin LK, et al. A small jab - a big effect: nonspecific immunomodulation by vaccines. *Trends in immunology* 2013;34(9):431-9. doi: 10.1016/j.it.2013.04.004
2. Aaby P, Benn CS. Non-specific and sex-differential effects of routine vaccines: what evidence is needed to take these effects into consideration in low-income countries? *Human vaccines* 2011;7(1):120-4. [published Online First: 2011/02/01]
3. Stensballe LG, Ravn H, Birk NM, et al. BCG Vaccination at Birth and Rate of Hospitalization for Infection Until 15 Months of Age in Danish Children: A Randomized Clinical Multicenter Trial. *J Pediatric Infect Dis Soc* 2018;0(0):1-8. doi: 10.1093/jpids/piy029 [published Online First: 2018/04/11]
4. Martins CL, Benn CS, Andersen A, et al. A randomized trial of a standard dose of Edmonston-Zagreb measles vaccine given at 4.5 months of age: effect on total hospital admissions. *J Infect Dis* 2014;209(11):1731-8. doi: 10.1093/infdis/jit804 [published Online First: 2014/01/18]