Study protocol:

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

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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

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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.

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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.

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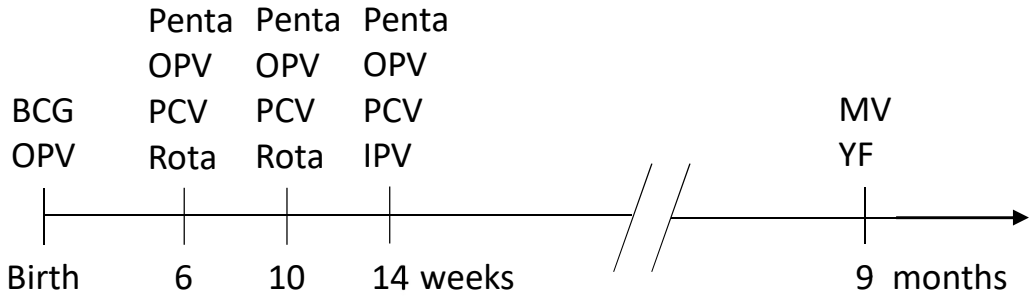
ABBREVIATIONS AND ACRONYMS

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	