Dear SDU REC and the external reviewers

We appreciate the input received from the two external reviewers and have provided our responses to them with blue font below.

Udtalelse fra ekstern ekspert 1

Expert Opinion

Regarding the research project

The Effects of Hepatitis B Vaccination at Birth on All-Cause Early-Life Morbidity and Mortality: An Investigator-Initiated Randomized Controlled Trial in Guinea-Bissau (REC762)

Background

The study is conducted in Guinea-Bissau and has been approved by the local Research Ethics Committee (National Committee for Ethics in Health Research, 036-CNES-INASA-2025). In light of the most recent revision of the Declaration of Helsinki, an assessment has been requested as to whether the study meets Danish research ethics requirements.

1. Background and Description of the Study

The prevalence of hepatitis B virus (HBV) infection in sub-Saharan Africa is estimated by the WHO to be approximately 6%. A smaller study conducted in Guinea-Bissau in 2014–2016 reported a considerably higher prevalence of 18.7% (15% among women and 24% among men).

Particularly when HBV infection is transmitted perinatally or during early childhood, there is a substantial risk of progression to chronic infection, which may subsequently lead to liver failure and hepatocellular carcinoma.

There is evidence that administration of hepatitis B vaccine immediately after birth provides better protection than later vaccination among infants born to infected mothers. There is also some evidence that vaccination immediately after birth results in a stronger antibody response. However, in the WHO position paper addressing vaccination at birth versus vaccination at 2–3 months of age, the level of evidence is graded as moderate or low.

On the other hand, there is some evidence that aluminium-containing vaccines may increase the risk of atopic dermatitis and that this risk may be reduced by delaying vaccination. However, both the absolute risk (approximately 0.9% in Denmark and presumably considerably lower in Guinea-Bissau) and the reported risk reduction (RR 0.57 with a one-month delay) are modest.

There have additionally been hypotheses concerning a possible association between aluminium-containing vaccines and neurodevelopment, particularly when vaccination is administered close to the time of birth.

The present study is a randomized, single-blind, multicentre trial comparing hepatitis B vaccination administered shortly after birth, together with BCG and oral polio vaccine—as recommended by the WHO—with the current practice in Guinea-Bissau, whereby hepatitis B vaccination is administered at approximately six weeks of age together with a number of other routine vaccinations.

Prior to enrolment, mothers are offered testing for active hepatitis B infection. Infants born to mothers with evidence of active infection are excluded from randomization and offered hepatitis B vaccination immediately after birth.

The primary outcomes are **infant mortality and infection-related hospital admissions within 42 days**. Secondary outcomes include **atopic dermatitis at two years of age** and **neurodevelopmental outcomes in a subgroup at five years of age**.

2. Scientific Quality of the Study

The study is conducted by a research group with substantial capacity and extensive experience in research in Guinea-Bissau. The randomized controlled trial design is appropriate for addressing the scientific question.

The group has previously reported unexpected findings concerning the non-specific effects of vaccines. Against this background, the research question appears scientifically valid, although the intervention contrast is relatively small, consisting essentially of a six-week delay in hepatitis B vaccination compared with the WHO recommendation.

Nevertheless, it should be noted that the statistical power of the study is expected to be relatively limited, with a considerable risk of a type II error. The sample size calculation appears to be based on the assumption of a **25% difference in the primary outcome** in order to achieve 80% statistical power. It is therefore important to consider whether such a difference is realistically expected. If the true effect is substantially smaller, the study may be unable to detect a clinically relevant difference and could consequently produce an inconclusive result.

Author response:

We appreciate the assessment of our research group and the scientific quality of the study.

The formal sample size calculation is powered to detect a 25% increase in the composite outcome of death or non-fatal admission with infection by six weeks of age, and a 22% decrease, which gives 7,184 participants in each group. For simplicity, this number was rounded to 7,250, for a trial size of at least 14,500 participants, which provides adequate power to detect a 24% increase in the risk of the composite outcome, and a 22% decrease. It should be noted that this number of participants is the anticipated minimum; we will continue the trial until HBV0 is introduced in Guinea-Bissau or when 25,000 neonates have been enrolled in the trial, whichever comes first. If 25,000 neonates are enrolled in the trial, we will be able to detect an increase in the rate of the composite outcome of 18% and a decrease of 17%.

In previous trials of non-specific effects associated with neonatal vaccines, conducted in our setting, we have seen larger effects in similar cohorts. For example, across three trials of at-birth BCG vs delayed BCG (Biering-Sørensen et al, CID 2017, PMID: 29579158) with ~6,500 participants, BCG was associated with a 38% (17% to 54%) decrease in neonatal mortality.

In an at-birth OPV vs delayed OPV trial with 7,012 participants (Lund et al, CID 2015, PMID 26219694) conducted in the same setting, by six weeks of age, the OPV vs no-OPV estimate was a 24% (-31% to 56%) reduction all-cause mortality (not significant).

Finally, in a cluster RCT from rural Guinea-Bissau (Thysen et al, BMJ GH 2024, PMID: 38350670) that enrolled 2,226 newborns, receiving BCG+OPV within 72 hours after birth was associated with a 59% (8% to 82%) decrease in mortality by six weeks of age, compared to delayed vaccines.

As can be appreciated from these trials, the provision of the neonatal vaccines BCG + OPV markedly decreased overall mortality; they have beneficial non-specific effects. The proposed trial should be interpreted considering both the previous findings from the same setting, the number of participants in the previous trials, and the major limiting factor which is that the proposed trial must be discontinued when HBV0 is implemented nationally in Guinea-Bissau. We regard a large-scale RCT that will enroll at least 14,500 and up to 25,000 participants as a major contribution to science. In our view, if a trial of this scale cannot document non-specific effects of HBV0 (positive or negative), then HBV0 is unlikely to have clinically impactful non-specific effects when administered together with BCG and OPV0; we would see this as a finding equally important to a significant effect, because both would equal an important contribution to science and both would be useful to guide vaccination policy in Guinea-Bissau.

3. Risk Assessment

I agree with the investigators that the risk associated with the two allocation groups can be regarded as **bidirectional**, provided that infants born to mothers who test positive for HBsAg, or whose test result is inconclusive, are excluded from randomization.

For the remaining participants, there is a plausible possibility of both benefit and harm associated with either allocation. The participating infants therefore do not appear to be placed at a greater overall risk than they would be in the absence of participation, given the current local vaccination practice.

However, particular attention should be paid to the **psychological burden placed on the mothers**. The decision as to whether the infant should participate, followed by the possibility of personally drawing the envelope determining the allocation, may be experienced as highly consequential or even as a matter of fate.

There may consequently be a substantial risk of maternal feelings of guilt and/or criticism from family members if the child subsequently develops an illness, even when such an event is entirely unrelated to participation in the study. This potential psychological burden should be explicitly considered and balanced against the anticipated societal and scientific value of the research.

Author response:

We appreciate the considerations regarding the impact of randomization, specifically the physical act of drawing an envelope determining the allocation.

It is noted in the participant information that it is voluntary to participate in the trial, and that BCG and OPV are the recommended vaccines in Guinea-Bissau, with HBV0 being recommended by WHO and used in many neighboring countries, but not yet implemented in Guinea-Bissau. We will ensure that our inclusion assistants also emphasize these factors in their oral explanation of the trial.

It is worth mentioning that the Bandim Health Project has had a presence at the Maternity Ward of HNSM (the main recruitment site) since 2002, since which we have conducted a series of newborn trials assessing overall health effects of BCG, OPV, and head-to-head of different strains (types) of BCG. The latest trial enrolled participants up to 2024. The trials have enrolled a combined total of ~70,000 neonates, both healthy neonates as in the present study, but also more frail low birth weight neonates and neonates admitted to the intensive care unit. All these studies have used individual randomization where the mother drew an envelope from a stack of envelopes.

During the conduct of all these trials, we have not experienced that the physical act of drawing an envelope has been a psychological burden for the mothers. Had it been the case, then we would have applied a different randomization procedure (e.g., electronic randomization through REDCap or similar, which has been used in other studies by our group).

We have likewise not received reports or questions regarding the trial or the randomization among mothers whose child fell ill. Culturally, these two aspects have not been an issue, and had they been then they would likely have been flagged by the Guinea-Bissau Ethics Committee, who are knowledgeable about how specific studies and their operational aspects may be perceived locally. As an example, remuneration for participation in a study or trial is essentially banned in Guinea-Bissau.

A reason why we chose to use randomization with an envelope was also to make sure that the mother understands that her child is being randomized, e.g., that the allocation is a lottery. In addition, other options such as the use of REDCap relies on the provision of stable electricity and internet, which is not always at hand in Bissau. The manual randomization approach therefore has the added advantage of always being operational, and the resulting paper trail faciliates supervision and data audits. Through the years, this approach has worked very well in Guinea-Bissau.

Due to our experience with this approach and our deeply-rooted knowledge of the setting and the sensitivities surrounding randomized trials, and since it has been accepted by the Guinea-Bissau Ethics Committee for the present study and many prior studies, and was accepted as well by the Danish Ethical Committee, we prefer to maintain this approach. We will make sure that our inclusion teams thoroughly emphasize the voluntary nature of the study, ensuring that we minimize the possible number of mothers that come to feel any psychological burden because of the conduct of the study.

While we strongly recommend maintaining physical randomization to ensure maternal comprehension and operational resilience, we are fully prepared to implement digital randomization should the committee deem it a strict requirement for approval. We note that this modification would require a formal protocol amendment and re-submission to the Guinea-Bissau Ethics Committee.

4. Vulnerable Populations

4.1 Research involving children

The study involves newborn infants in Guinea-Bissau, with their mothers acting as legal representatives.

Research in these newborn infants cannot appropriately be replaced by research in adults or older children, since the research question specifically concerns the effects of vaccination during the neonatal period. Moreover, the results may potentially benefit newborn infants in the future.

In Denmark, such a study would require parental consent in accordance with the applicable rules, including consent from both parents where both have parental responsibility. Children of mothers who are unable to provide valid informed consent in a sufficiently reliable manner would need to be excluded.

Author response:

In Guinea-Bissau, the mother must provide informed consent and if she is unable to due to e.g. illness, hospitalization at another ward or having passed away, a legal guardian can step in and provide valid informed consent. The study will only enroll newborns for whom a valid informed consent was provided and signed by the mother or a legal guardian of the child.

In practice, the consent will be given by the person who is responsible for taking the child for vaccination. This is virtually always the mother. Many ethnic groups with varying religion (Animist, Catholic, Muslim) are present in Bissau. Sometimes the mother will phone the father to get paternal consent (usually Muslim), but usually not. The National ethical committee has never raised the issue of paternal consent.

4.2 Research involving vulnerable populations and communities

Could the study be conducted in another population?

The randomized trial could theoretically be conducted in other populations. For example, it could be conducted in the United States, where recommendations concerning hepatitis B vaccination at birth have recently changed in favour of vaccination at a later age (6 weeks)

Such a study could potentially address the questions concerning an increased risk of atopic dermatitis and possible effects on neurodevelopment in a population with a lower baseline risk of hepatitis B infection.

However, as the investigators appropriately point out, the effects on mortality and hospital admissions are highly **context-dependent** and, importantly, also **time-dependent**.

Results obtained in high-income countries in the Global North cannot therefore be assumed to be informative with regard to mortality and morbidity outcomes in the Global South. At the same time, even results obtained in the relevant geographical setting of Guinea-Bissau may have a relatively limited period of external validity.

One would expect continued improvements in infant survival, as well as declining hepatitis B prevalence and transmission, over time. Consequently, the absolute effects of the two vaccination strategies may change substantially, potentially limiting the duration for which the study results remain directly applicable.

Against this background, it may be relevant to reconsider whether an **observational assessment of mortality and hospital admissions in the local population before and after change of vaccination scedule**, combined with a randomized study focusing on the secondary outcomes in a less vulnerable population with better opportunities for long-term follow-up, could provide a more appropriate study design.

Author response:

We appreciate these considerations regarding the epidemiological context, the potential for studies in other settings, and alternative study designs.

While a randomized trial in a high-income setting such as the United States or Denmark could theoretically address secondary endpoints like atopic dermatitis or neurodevelopment, it cannot answer our primary research question for two compelling reasons:

1. **Fundamental differences in baseline infectious disease burden**:

The primary composite outcome - infant mortality and infection-related hospital admissions within 42 days - is driven by the high baseline infectious disease burden present in sub-Saharan Africa. In

high-income settings, baseline early-life mortality and infection-related hospitalization rates are orders of magnitude lower and they have different underlying causes. A trial conducted in a high-income, low-burden setting would be underpowered for these clinical endpoints and would yield little actionable insight into the real-world morbidity and mortality impacts of neonatal vaccination in high-burden settings like Guinea-Bissau.

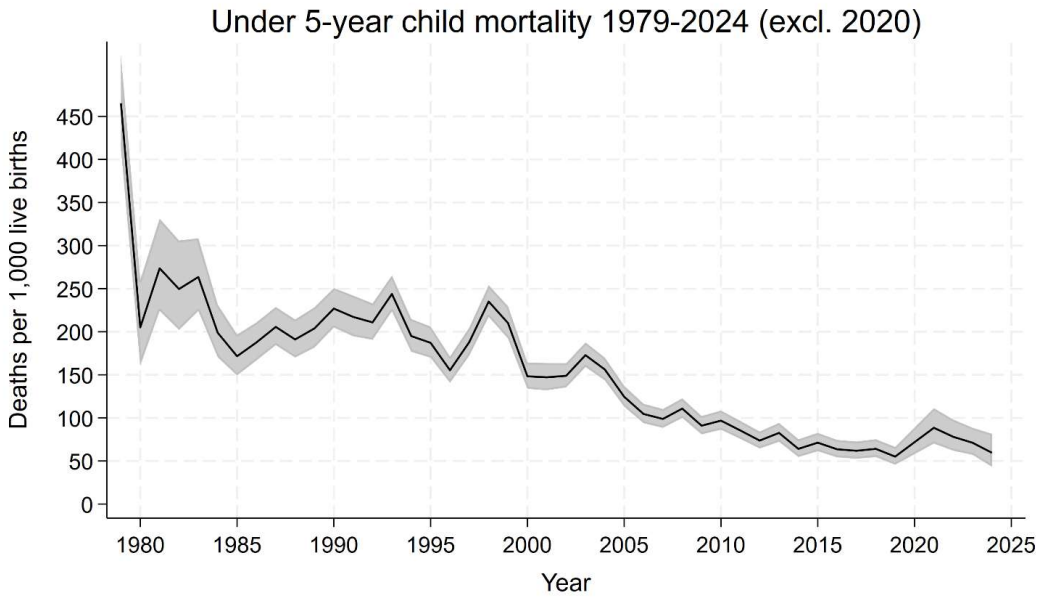
2. **Immunological context and vaccine contrast**:

Non-specific effects are highly context-dependent and influenced by co-administered vaccines. In Guinea-Bissau, we are evaluating the addition of HBV0 to the existing birth schedule of live vaccines: BCG + OPV0 vs. BCG + OPV0 + HBV0. Because birth-dose BCG and OPV are not administered in high-income countries like the US or Denmark, any non-specific effects measured in those populations would occur against a completely different immunological and epidemiological background and would have little to no comparative value for guiding vaccination policy in Sub-Saharan Africa.

Regarding the suggestion of an observational before/after study:

We agree that infant mortality and hepatitis B transmission patterns evolve over time. However, observational before/after assessments inherently suffer from residual confounding (e.g., temporal shifts in healthcare infrastructure, seasonality, and co-interventions). We have previously conducted two observational studies on hepatitis B vaccination - in Guinea-Bissau and The Gambia - both of which indicated potential negative non-specific effects among females. Despite sparking international interest, these observational findings lacked the definitive causal weight required to influence national or global policy; The Gambia was one of the first African nations to roll out universal HBV0, and Guinea-Bissau plans national implementation for 2028. To meaningfully inform vaccine policy - whether confirming benefit, reassuring safety, or identifying harm - high-quality evidence from a large-scale randomized controlled trial is therefore needed.

Finally, while national infant survival has improved historically, long-term surveillance data from the Bandim Health Project demonstrate that under-5 mortality in our setting has plateaued since approximately 2014 (see figure below). The baseline risk of mortality and severe infection in Bissau remains substantial, making this trial both urgently needed and locally relevant:



5. Will the Study Benefit the Local Population?

There is some uncertainty as to whether the results of the study will ultimately benefit families in Guinea-Bissau.

The study appears to depend on rapid access to HBsAg testing around the time of delivery. It is not clear that such testing can routinely and reliably be made available in the Guinea-Bissau setting.

If reliable HBsAg testing is not available, then—given the current epidemiological situation and the reported prevalence of HBV infection among mothers—vaccination shortly after birth may constitute the preferable strategy irrespective of the results of the present trial.

This raises an important question concerning the extent to which the infrastructure required for the study, particularly timely maternal HBsAg testing, can be maintained after the study has ended and incorporated into routine healthcare.

Author response:

We believe that the results of the study will indeed benefit families in Guinea-Bissau. As an example, our previous trials of BCG vs no-BCG were conducted among newborns with low birth weight (LBW). At the time, BCG was recommended to all newborns by the WHO but not nationally recommended to LBW newborns. The local interpretation was that such newborns were “immature” and needed to put on weight before being “healthy enough” to be vaccinated. With ethical approval from both the Guinea-Bissau national ethics committee and the Danish National Committee of Ethics, and featuring physical randomization after maternal informed consent, our trials demonstrated a substantial beneficial effect (38% reduction in neonatal mortality) for newborns in Guinea-Bissau, and the Ministry of Health changed their recommendation to universal BCG at birth for all newborns, regardless of birth weight.

The proposed study benefits the local population both while it is being conducted and afterwards.

First, all newborns approached for inclusion are entitled to free consultations and certain medications free-of-charge, independent of participation in the study. This improves the current standard of care, and benefits everyone equally.

Second, BCG and OPV0 will be provided to all newborns at the ward, regardless of the day of discharge. Normally, neonatal vaccines are only provided on weekdays, not in the weekend or on public holidays. Stockout of vaccines also occurs. We have purchased all three vaccines (BCG, OPV0, HBV0) and our vaccinators will provide the vaccines on all days of the week, increasing vaccination coverage and reducing stress, anxiety and costs/inconvenience related to procurement of vaccines for the mothers and their families.

Third, all newborns at risk of vertical transmission of hepatitis B virus will be excluded from the trial and vaccinated as soon as possible. Among these neonates (approx. 10-15% of neonates born in Guinea-Bissau), this will substantially reduce the risk of perinatal hepatitis B infection, chronic infection, ongoing transmission of hepatitis B and downstream sequelae such as cirrhosis, hepatocellular carcinoma, liver failure and extrahepatic sequelae. Avoiding these outcomes among all participants screened for inclusion (n=unknown, probably 17,000-20,000) will benefit the local population and society substantially during the next decades.

Fourth, the proposed study involves significant capacity building and training of local MDs and researchers, including two PhD positions.

The proposed study has three possible outcomes:

1) HBV0 has **beneficial** non-specific effects.
   The study provides extra incentives for broad-scale vaccination rollout to harvest the benefits of both the specific, protective effect against hepatitis B infection and the beneficial non-specific effects of HBV0. The local population and the vaccination efforts will benefit from this knowledge.

2) HBV0 has **no** non-specific effects.
   This finding will ease parental concern about hepatitis B vaccination in Guinea-Bissau and neighboring countries, documenting that the planned approach of universal hepatitis B vaccination at birth is not associated with any negative effects, and that broad vaccination regardless of risk factors (e.g., whether the mother is a carrier) is the best vaccination policy. The local population will benefit from this knowledge; anyone who had doubts about the necessity of HBV0 vaccination or its side-effects can be informed by high quality data synthesized within the local context.

3) HBV0 has **detrimental** non-specific effects.
   A universal HBV0 vaccination policy may be detrimental overall because it reduces the beneficial impact of the BCG and OPV0 vaccinations, and the policy would need to be reconsidered. HBsAg-screening with vaccination of newborns born to mothers that are carriers or vaccination based on risk factors may be the best balanced approach to reduce the mother-to-child transmission of hepatitis B virus while minimizing the harm from vaccination. The local population will benefit from this finding since otherwise detrimental effects related to vaccination are avoided, because of the study.

6. Conclusion

In a hypothetical Danish setting with comparable circumstances—i.e. where a vaccine is recommended but has not yet been implemented as standard practice—the study could, in my assessment, be ethically acceptable, provided that the following conditions were met:

- **Modification of the randomization procedure:** Following provision of informed consent, mothers should not be further confronted with the randomization process by being asked to draw the allocation envelope themselves.

Author response:

As mentioned, we have conducted a series of studies in Guinea-Bissau using this approach of randomization, without experiencing issues or having concerns expressed to us from the participants or their families. In addition, the National Ethics Committee has unanimously approved the study without reservations or comments regarding the randomization procedure.

In some of our more recent trials in Guinea-Bissau, we have utilized the digital randomization tool in REDCap, which has worked satisfactorily and would be feasible for use in inclusion centers for the proposed study. The main reason that we chose the simple randomization procedure is operational simplicity and as a continuation of the procedures applied in previous studies, but several of our teams are also trained in randomization using e.g. REDCap.

Through our work in Guinea-Bissau for 48 years, we have not experienced physical randomization as an issue, and it has not been flagged by the Ethics Committee in Guinea-Bissau or by visiting

researchers in our study DSMBs or similar. For operational simplicity and to ensure that the mother indeed understands that the allocation of her child to HBV0 or placebo is in fact a lottery and not something that the inclusion assistant chooses, we prefer to maintain the protocolized physical randomization.

While we highly recommend maintaining physical randomization to ensure maternal comprehension and operational resilience, we are fully prepared to implement digital randomization should the committee deem it a strict requirement for approval. We note that this modification would require a formal protocol amendment that is re-submitted to the Guinea-Bissau Ethics Committee for their approval.

- **Protection of the informed-consent process:** Particular attention should be paid to ensuring that the information and consent process takes place in an environment that minimizes psychological pressure and anxiety.

Author response:

We highly agree and will ensure that information, consent, disclosure of test results for HBsAg + HIV, randomization and vaccination will take place in an environment which safeguards the privacy and dignity of the participants and their families and minimizes pressure and anxiety.

- **More comprehensive participant information:** The information provided to participants should be expanded, including explicit information that refusal to participate will result in the infant being offered hepatitis B vaccination during the neonatal period, as stated in the protocol.

Author response:

This is stated in the Participant information, p. 2:

"*Does my baby have to take part? No. Participation is completely voluntary. If you decide not to take part, or if you change your mind later, your baby will not be disadvantaged. It will still be offered the standard BCG and OPV vaccines and, if less than 8 days old, the hepatitis B birth-dose vaccine. The same applies if you prefer not to have the hepatitis B screening test.*"

We will also ensure that this is explained clearly in the oral explanation of the study, which will be given individually to each mother.

- **Consent from both parents:** Where both parents hold parental responsibility, consent should be obtained from both parents.

Author response:

At the main recruitment center, the maternity ward of Hospital Nacional Simão Mendes (HNSM), the formal registration of the newborn's parents takes place after discharge.

At HNSM's maternity ward, the father is furthermore not allowed to be admitted with the mother and child. Aside from during a short designated visit time, which family members mainly utilize to bring food and other necessary items to the mother, fathers and other family members are not allowed to be present at the Ward.

There are no stipulations or regulations in Guinea-Bissau that require consent from both parents. Through a series of trials conducted by BHP, dually approved by the Guinea-Bissau and Danish board of Ethics, maternal informed consent has been the standard.

A general requirement of paternal consent for participation in the study is therefore incompatible with the conditions in the setting where the study is to be conducted.

We do rarely experience mothers that requests to telephone their husband to ask them about participation in the trial, and provide them the time and space to do so, and time to reflect. To make it clearer that this is both possible and acceptable, we have added the following to the Information Material, in the subheading "Invitation":

"*Please take your time to consider whether you wish for your child to participate in this study and consider whether you wish to consult your husband or another family member before you decide, and whether you prefer to request time to discuss and reflect regarding your child's participation in the study.*"

7. Issues Requiring Clarification Before Conducting the Study in a Vulnerable Setting

In order to determine whether it is ethically reasonable to conduct the study in a vulnerable population and healthcare setting such as Guinea-Bissau, the following issues should be clarified:

7.1 Parental responsibility and involvement of fathers

What are the applicable local rules concerning parental responsibility?

Author response:

The local rules and laws allow for the mother to make decisions regarding neonatal vaccines and participation in trials and studies. This approach has been the practice through dozens of trials conducted in Guinea-Bissau and Bandim Health Project and others; these trials and their informed consent procedures were dually approved by the National Ethics Committee in Guinea-Bissau and the Danish National Ethics Committee (VEK).

If the father has parental responsibility from the time of birth, consent should, in principle, be obtained from both parents. Is such a requirement compatible with local legislation and practice?

Author response:

The father does not have parental responsibility from the time of birth. Responsibility is determined when the newborn is registered, which does happen at an adjacent location at the hospital, but only after the mother and child has been discharged from the maternity ward, and the newborn has been vaccinated. There is no requirement of dual parental consent in the local legislation and practice.

Furthermore, is it practically feasible, under the relevant circumstances, to involve fathers in the decision-making process? This is particularly important in order to avoid placing the sole responsibility for the decision—and any subsequent adverse outcome—on the mother.

Author response:

Since fathers are not allowed to enter the Maternity Ward aside from brief visits, it is not practically feasible that they are involved in the decision-making process. It should be mentioned as well that

there is no established system for paternity leave in Guinea-Bissau, so fathers will often be at work or away from the hospital while their partner is admitted with the child. A demand of involvement of the fathers in the decision-making process would therefore make the study unfeasible, because it would infer unreasonable delays in the vaccination of the newborns.

Aside from the sentence also mentioned above ("*Please take your time to consider whether you wish for your child to participate in this study and consider whether you wish to consult your husband or another family member before you decide, and whether you prefer to request time to discuss and reflect regarding your child's participation in the study.")*, we suggest adding, to the informed consent material (immediately following the above sentence) and to the oral explanation of the study, the following:

"*You can request that your husband/partner is involved in this decision. We can either help facilitate that you can telephone your partner, where the BHP personnel can help explain the study, or in case that your husband can come to the inclusion center, our personnel is ready to explain the trial to your partner, as it was explained to you, so that he can be involved in the decision.*"

In this way, we find that the question of dual parental informed consent is handled in a reasonable way, ensuring that the potential need of the mother for assistance for the decision of participation is met, while at the same time not jeopardizing the feasibility of the study.

7.2 Availability of HBsAg testing after completion of the study

Is it realistic to expect that HBsAg testing at the time of delivery could be offered to the majority of pregnant women in Guinea-Bissau after completion of the study, both logistically and economically?

Author response:

Logistical and financial sustainability:

Yes, offering maternal point-of-care HBsAg testing is both logistically feasible and economically realistic in Guinea-Bissau.

From a health economics and logistics standpoint, targeted screening combined with selective neonatal vaccination is likely more cost-effective than universal vaccine rollout:

- **Unit and supply costs**: Through UNICEF Procurement Services, rapid HBsAg test kits cost approximately $1.00 USD per test. This cost is offset by avoiding universal purchase of hepatitis B vaccine doses and administration supplies (syringes and safety boxes) for infants who do not require immediate birth-dose protection since they are born to HBsAg-negative mothers.

- **Cold-chain logistics**: The single largest logistical barrier to universal HBV0 implementation in Guinea-Bissau is maintaining a continuous 2-8 °C cold chain during the shipment from the international manufacturer through central storage and onwards to regional delivery wards and smaller health centres. Rapid diagnostic tests do not require strict cold-chain infrastructure, occupy far less storage volume, and carry significantly lower transport costs. They are simple to perform.

- **Integration into existing antenatal care (ANC)**: Rather than relying solely on rapid testing at the maternity ward during delivery, screening can be integrated into routine ANC visits (where WHO recommends 8 antenatal contacts). The HBsAg result would be annotated directly on the mother's pregnancy card - a system that already operates with high coverage and success for antenatal HIV screening in Guinea-Bissau. Mothers presenting without an ANC card would be offered testing upon admission to the maternity ward.

Integrating targeted HBsAg screening into routine antenatal care provides a highly sustainable, low-cost model for low-resource settings compared to universal cold-chain vaccine distribution.

If this is not realistic, how do the investigators envisage that the results of the study could subsequently benefit families in Guinea-Bissau?

Author response:

In the hypothetical scenario where broad-scale testing could not be immediately maintained post-trial, generating high-quality trial data on overall health effects would still provide vital public health benefits to families in Guinea-Bissau and similar settings. The trial will yield one of three definitive findings:

1. Beneficial non-specific effects: universal HBV0 reduces overall early-life mortality and hospitalizations beyond the specific protection against HBV. This provides policy-makers with strong evidence to prioritize funding and cold-chain infrastructure for universal HBV0 rollout.

2. No non-specific effects: reassures international bodies, local clinicians, and parents that the planned universal HBV0 implementation is safe and carries no unintended off-target mortality or morbidity risks when co-administered with BCG and OPV0.

3. Detrimental non-specific effects: the study reveals that universal HBV0 impairs the non-specific protective benefits of BCG and OPV0. In this case, a targeted screening-and-vaccination strategy (or risk-based administration) would likely be superior to universal newborn vaccination, preventing widespread off-target harm while still protecting infants at high risk of vertical transmission.

In all three scenarios, the trial provides essential, locally generated evidence to guide optimal vaccination policy and improve overall child survival in West Africa.

7.3 Expected effect size and statistical power

Do the investigators consider it realistic that the difference in outcomes between the two treatment groups could be as large as 25%?

What are the investigators' considerations regarding the risk of a type II error or an inconclusive result, particularly when this is weighed against the potential psychological burden placed on mothers participating in the study?

Author response:

We first of all highlight here that our experience has not been that our studies have placed a psychological burden on the participating mothers participating. To the contrary, our work ensures

that their child receives the recommended vaccines, which are not always the case otherwise, and procuring neonatal vaccines with a newborn is both time-consuming and is associated with costs. Our teams ensure that the newborn receives a vaccination card (which is also not always the case) and is followed through sequential follow-up calls, where we also remind the mother of the next vaccines in the vaccination schedule, and we offer medical consultations and some essential medications free of charge.

The sample size calculation is based on recent data from a randomized controlled trial in Guinea-Bissau, where we observed a 6-week mortality rate of 1.4% and a non-fatal infectious disease admission rate of 2.6%, for a total composite risk of 4% in the control group. Our sample size of 14,500 newborns gives us 80% power to detect a ≥22% decrease and a ≥24% increase in the risk of the primary composite outcome, corresponding to a composite risk of 3.12% to 4.96% in the intervention arm, meaning that we have power to detect fluctuations of less than one percent in the absolute risk of the composite outcome.

Our assessment is that fluctuations of a magnitude less than one percent in the absolute risk of the composite outcome are unlikely to have meaningful clinical relevance and that a trial enrolling at least 14,500 newborns represents a substantial contribution to the knowledge-base regarding neonatal hepatitis B vaccination.

Overall Assessment

Overall, the research question is scientifically relevant, and the randomized controlled trial design is appropriate for addressing the causal effects of the timing of hepatitis B vaccination. The principal ethical concerns do not appear to relate to a substantial difference in physical risk between the two allocation groups, but rather to **the validity and robustness of the informed-consent process, the psychological burden potentially placed on mothers, the involvement of both parents, and the justification for conducting the trial in a vulnerable population and healthcare setting**.

Before the study can be considered fully ethically justified in the Guinea-Bissau context, further clarification of the issues outlined above would therefore be warranted.

Author response:

We appreciate the considerations from the reviewer, and hope that our responses above are satisfactory for the study to be considered fully ethically justified. While we highly recommend maintaining physical randomization to ensure maternal comprehension and operational resilience, we are fully prepared to implement digital randomization should the committee deem it a strict requirement for approval, noting that this modification would require a formal protocol amendment and re-submission to the Guinea-Bissau Ethics Committee.

Udtalelse fra ekstern ekspert 2

Ekspertens kommentar i mail til REC:

Der ville være en diskussion af berettigelsen af at give placebo vaccinen og så om informationen er god nok.

Jeg synes ikke det vil være uetisk at gennemføre. Se kommentarerne i dokumentet, som er få.

Men informationen ville i DK skulle være bedre. Den er mangelfuld omkring atopisk dermatit og 5 års undersøgelsen. Der står kun at de måske vil blive undersøgt men ikke hvorfor. Og hvis formålet er at kontrollere dødeligheden efter 6 uger, giver det ikke mening at kontrollere så mange gange. Så derfor skal det forklares. Og er det et enten eller?

- In some children we may later check for eczema (atopic dermatitis) or do a simple developmental assessment when the child is 5 years of age.

Så bedre information på den del. Resten er god og skrevet forståeligt. Jeg ved ikke om det giver mening at angive HBsAG som undersøgelsen eller om det ikke er ok bare at skrive hepatitis B.

Så den ville blive godkendt, mod at informations materialet blev udbedret ifht ovenstående.

Author response:

We appreciate these considerations and agree that the current information level regarding eczema and the developmental assessment may be insufficient.

In the section “What will happen if I agree to take part?”, we suggest to replace the current point 6., which was highlighted by the reviewer, to the following:

At our follow-up contacts, we will ask if your child has had an itchy rash and has scratched repeatedly and/or had sleep disturbance because of the itching. If this is the case and you reside in the greater metropolitan area of Bissau, we may offer you a voluntary visit by a physician from the study for examination of your child’s skin condition and treatment guidance.

If you live in the catchment area of the Bandim Health Project, you may be offered a voluntary neurodevelopmental assessment conducted when your child is approximately 5 years of age. Both of these assessments relate to secondary research questions that help us understand whether the birth-dose hepatitis B vaccine has any longer-term effects on skin health or child development. Participation in these later assessments is voluntary and you can decline without affecting your child’s care or continued participation in the main study.

We agree that it is more understandable for the participants to write “hepatitis B virus” rather than the technical term HBsAg. We have therefore corrected this in the information material.

We have attached the updated version of the information material, also including the corrections made due to the inputs from the first external reviewer.

In regard to the repeated follow-up contacts by telephone at one week, six weeks, six months, one year and two years of age, this is done for several reasons. First, the secondary outcomes of all-cause mortality by two years of age and incidence of self-reported atopic dermatitis by two years necessitate follow-up up to two years of age. Second, the precision of the reported data is

increased when it is reported close to its occurrence. If we only implemented e.g. one telephone call at two years of age, the specific age of death would become uncertain. By using repeated telephone contacts, we will collect data continously, and are more likely to achieve successful follow-up before a mother becomes lost to follow-up due to e.g. losing her telephone or changing her telephone number. Third, the incidence of physician-diagnosed atopic dermatitis necessitates a home visit by the study physician during the time when the child has a rash that can be examined. By having more follow-up contacts, there will be more occurrences where the child has a rash at the time of our interview.

Ekspertens specifikke kommentarer til protokollen:

Side 13:

We recognize that the immediate post-partum period may limit the depth of discussion and the mother's ability to absorb complex information and ask questions regarding the trial.

- Enig. Det er ok at gøre det.

As mentioned, all families approached for enrolment – regardless of participation - are offered free consultations during infancy at the three health centers in the BHP study area and access to essential pediatric medications free of charge. In addition, maternal HBsAg screening with immediate provision of HBV0 to every newborn of an HBsAg-positive mother (and to any newborn whose mother declines testing or declines the trial) establishes a higher level of protection against vertical hepatitis B transmission than current national practice

- Hvis det kun havde været de familier der havde sagt ja til at deltage som fik tilbudt dette, ville det stadig være acceptabelt. Der er selvfølgelig et incitament til at sige ja, men jeg mener ikke at vaccinen udsætter for en risiko ud over en reaktion på vaccinen og hvis der samtidig er adgang til en vurdering vil det være ok. Men der er ingen alvorlige risici ved det. Når de samtidig tilbyder de samme fordele selvom man sige nej, er det ingen etiske bekymringer.

- I forhold til at de udsættes for en placebo vaccine. Det kan diskuteres om det er acceptabelt at udsætte en nyfødt for et extra stik og de evt smerter der måtte være forbundet med det. Hvis det er en vanlig vaccine nål vil barnet kunne mærke det og også kunne mærke det volumen der sprøjtes ind, men der vil ikke være en reaktion i vævet og derfor ingen smerter. Jeg mener det er acceptabelt taget forsøgets formål og benefit i betragtning.

Det eneste jeg synes kan være lidt problematisk er informationen den første dag. Hvor jeg er i tvivl om forældre er i stand til at forstå sammenhængen til de sekundære mål. Men da de altid kan springe fra, mener jeg ikke at det er problematisk.

Author response:

We appreciate the assessment and agree that the information may be difficult to retain on the first day. We have expanded the section regarding the secondary aims of the study and hope that the revised information material is sufficient for the trial to be approved.

The HBV-NSE study: *The effects of hepatitis B vaccination at birth*

Information Material for Research Participants

Invitation

You are being invited to allow your newborn baby to take part in a research study. Before you decide, it is important that you understand why the study is being done and what it will involve. Please read this information carefully (or have it explained to you) and ask any questions you may have. Taking part is entirely voluntary and does not influence your relationship with the hospital / health clinic or Bandim Health Project. Please take your time to consider whether you wish for your child to participate in this study and consider whether you wish to consult your husband or another family member before you decide, and whether you prefer to request time to discuss and reflect regarding your child's participation in the study. You can request that your husband/partner is involved in this decision. We can either help facilitate that you can telephone your partner, where the BHP personnel can help explain the study, or in case that your husband can come to the inclusion center, our personnel can explain the study to your partner, as it was explained to you, so that he can be involved in the decision.

What is the purpose of the study?

In Guinea-Bissau, all newborns are recommended to receive two vaccines soon after birth: BCG (against tuberculosis) and OPV (against polio).

The World Health Organization also recommends a hepatitis B vaccine at birth (called HBV0). This vaccine is recommended to protect against hepatitis B infection that can be passed from mother to child. The vaccine that we will evaluate is not new; it is WHO-prequalified and used worldwide, including in many African countries such as Cape Verde, The Gambia, Ivory Coast, Nigeria, Mauritania and Senegal.

We already know that HBV0 protects against hepatitis B infection. What is not known is whether adding HBV0 at birth to BCG and OPV has any other effects – beneficial, harmful or none – on babies' overall health. This study will help answer that question. The results will help the Ministry of Health decide the best way to introduce the hepatitis B birth-dose vaccine in Guinea-Bissau.

Why has my baby been invited?

Your baby is eligible if he/she is less than 8 days old, is in good health, and has not yet received any vaccines.

Before the study starts, we will offer you a rapid finger-prick blood test for hepatitis B (HBsAg). If the test is positive, your baby would not enter the study and will instead be offered the hepatitis B vaccine immediately to protect him/her. We will also offer you a voluntary rapid test for HIV which is not required in order to participate in the study. All test results will be kept strictly confidential, and we will provide a private space where you will receive the test results and counselling (if necessary)

Only babies whose mothers test negative for hepatitis B will be randomized. Babies whose mothers test positive will receive HBV0 and will not take part in the randomized comparison.

What will happen if I agree to take part?

1. You will be asked to give written consent.

2. Your baby will be randomly assigned (like tossing a coin) to one of two groups:

- **Group 1**: BCG + OPV + hepatitis B vaccine (HBV0)

- **Group 2**: BCG + OPV + a sterile salt-water injection (placebo) that looks the same

3. Both groups receive the normal BCG and OPV vaccines. The only difference is the extra injection (hepatitis B vaccine or salt water).

All babies in both groups are encouraged to receive the routine childhood vaccines, including the hepatitis B-containing vaccines recommended at 6, 10 and 14 weeks of age.

4. We will collect some basic information about you and your baby and your contact telephone numbers.

5. We will contact you by telephone at about 1 week, 6 weeks, 6 months, 1 year and 2 years to ask about your baby's health. If you live in the Bandim area, a field worker may visit you at home.

6. At our follow-up contacts, we will ask if your child has had an itchy rash and has scratched repeatedly and/or had sleep disturbance because of the itching. If this is the case and you reside in the greater metropolitan area of Bissau, we will offer that a physician from the study comes to your home and examines your child's skin condition.

If you live in the catchment area of the Bandim Health Project, you may be offered to have a neurodevelopmental assessmented conducted when your child is approximately 5 years of age. Both of these assessments are secondary research questions that help us understand whether the birth-dose hepatitis B vaccine has any longer-term effects on skin health or child development. Participation in these later assessments is voluntary and you can decline without affecting your child's care or continued participation in the main study.

Does my baby have to take part?

No. Participation is completely voluntary. If you decide not to take part, or if you change your mind later, your baby will not be disadvantaged. It will still be offered the standard BCG and OPV vaccines and, if less than 8 days old, the hepatitis B birth-dose vaccine. The same applies if you prefer not to have the hepatitis B screening test. Your baby will also still be offered the same care at the hospital and health centres. Nothing will change in the care you or your baby receive.

What are the possible benefits?

- All babies in the study (and all babies whose mothers are approached, even if they do not join) will receive BCG and OPV on the day of discharge from the hospital or before leaving the health center.

- Mothers will be offered free hepatitis B and HIV testing with counselling and referral if needed.

- Families approached for the study can receive free consultations and some essential medicines for the child at the Bandim Health Project health centres, whether or not they join the study.

- The study will provide important information that may improve vaccination policy for all children in Guinea-Bissau.

- There is no guaranteed individual health benefit from taking part in the randomized study itself. The testing, consultations, medicines and timely routine vaccinations described above are offered regardless of whether you agree to participate. There is also no remuneration.

What are the possible risks or disadvantages?

- Your baby will receive one extra injection (either the hepatitis B vaccine or sterile salt water). The injection is given in the thigh and uses the same safety procedures as ordinary vaccines.

- The finger-prick blood test may cause brief discomfort.

- Learning that you have hepatitis B or HIV can cause worry. Trained staff will explain the results privately and help you get any care you need.

The hepatitis B vaccine can cause temporary soreness, redness or swelling where it is given, and occasionally fever or tiredness. Serious allergic reactions are very rare. The saline injection can cause brief pain or local discomfort. Trained staff and appropriate treatment will be available in the rare event of an acute reaction.

Will the information about me and my baby be kept confidential?

Yes. All information will be securely stored using a unique study number. Only authorized study staff will have access to personal details. Results will be published in a way that no individual can be identified. De-identified research data may be shared with other researchers or placed in a scientific data repository. Names, telephone numbers and other directly identifying information will not be included.

What will happen to the results of the study?

The results will be shared with the Ministry of Health of Guinea-Bissau, the National Immunization Technical Advisory Group, and the ethics committee. They will also be published in scientific journals. You will not be personally identified in any report.

Who is organizing and funding the study?

The study is organised by the Bandim Health Project and the University of Southern Denmark. It is funded by external non-commercial funders. The funders have no role in deciding which treatment a child receives or in the analysis and reporting of the study results. The researchers have no financial conflicts of interest.

Who has reviewed the study?

The study has been reviewed and approved by the National Ethics Committee of Guinea-Bissau (CNEPS) (approval number 06-CNEPS-INASA-2026). In addition, the Ministry of Health has authorized the conduct of the study [Pending].

Who can I contact if I have questions or concerns?

You can ask the field assistant who gave you this information at any time.

You may also contact:

Inclusion supervisor: Gabriel Marciano Gomes, Tel 95 5320060

Data entry supervisor: Ivan Monteiro, Tel 95 5319017

Trial physician: Isaquel da Silva, Tel 95 5441328, email idasilva@health.sdu.dk

If you have concerns about your rights as a research participant, you may contact the National Ethics Committee (CNEPS). Thank you for taking the time to read this information.

Please ask any questions before you decide whether your baby will take part.