

Specific and Nonspecific Effects of Measles and BCG Vaccines in Women of Childbearing Age in Guinea-Bissau

Impacts on Maternal and Child

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Specific and Non-specific Effects of Measles and BCG Vaccines in Women of Childbearing Age in Guinea-Bissau: Impacts on Maternal and Child



PhD Thesis
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PhD thesis

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01 Preface

In countries like Guinea-Bissau, every data point that signals a trend toward saving lives is not just a number; it's an opportunity that must be assessed and pursued.

I am a descendant of ancestors from Jeta Island, a small island in Guinea-Bissau rich in history, resilience, and cultural identity. My journey began in the markets of Bandim, where I sold water to support my family and pursue my dreams.

When I started working at the Bandim Health Project, I believed my role would be focused on vaccine delivery to save children's lives. But today, I understand that my work goes beyond saving lives in the present; it is also about shaping the future. It's about searching for new patterns and new formulas in science.

From those humble beginnings, I found an opportunity to transform my reality. Before even completing my medical degree, I was recruited to work at the Bandim Health Project (BHP) by three remarkable figures in public health: Peter Aaby, Dra. Amabelia Rodrigues, and Dr. Cesario Martins. Their trust in me marked the beginning of a scientific journey that allowed me to contribute to impactful international research.

This work is the result of a path filled with challenges, learning, and achievements. It represents not only a personal milestone but also a tribute to my origins, my community, and everyone who believed me.

If we know that a vaccine can save more lives than we imagined, why continue to use it as if we didn't

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My sincere appreciation goes to the academic team at the Bandim Health Project, especially Ane, Julie, Sabine, Kim for their valuable collaboration and support.

This thesis was only possible thanks to the trust and acceptance of the community, especially the women who participated with their children. I am profoundly grateful for their willingness to contribute to research that aims to improve maternal and child health.

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I also acknowledge the Graduate School of Health Sciences at the University of Southern Denmark for their academic guidance and institutional support throughout my doctoral training.

03 Abbreviations

Abbreviation Full Term

95%CI	95% Confidence Interval
AEs	Adverse Events
BCG	Bacillus Calmette–Guérin (Vacina contra tuberculose)
BHP	Bandim Health Project (Projecto de Saúde Bandim)
BMI	Mean Body Mass Index
CI	Confidence Interval (Intervalo de confiança)
CMV gB	Cytomegalovirus glycoprotein B
COVID-19	Coronavirus disease 2019 caused by coronavirus SARS-CoV-2
DTP	Vaccine against diphtheria, tetanus, and pertussis (included in Penta)
EPI	Expanded Programme on Immunization
EPIV-ULB	European Plotkin Institute of Vaccinology – Université Libre de Bruxelles
GMT	Geometric Mean Titer
Gavi	Gavi, the Vaccine Alliance
HDSS	Health and Demographic Surveillance System (Sistema de Vigilância Demográfica e de Saúde)
HepB	
Hib	
IA2030	The Immunization Agenda 2030 (a WHO strategy towards the SDGs)
IPV	Inactivated Polio Vaccine
LMIC	Low- and Middle-Income Countries
MCV	Measles-Containing Vaccine
MFI	Mean Fluorescence Intensity
MRR	Mortality Rate Ratio
MTB	Mycobacterium tuberculosis
MV	Measles Vaccine
MatAb	Maternal Antibody
NSEs	Non-Specific Effects
OPEN	Odense Patient data Explorative Network
OPV	Oral Polio Vaccine
PCV	Pneumococcal Vaccine
PCV13	Pneumococcal Conjugate Vaccine (13-valent)
PENTA	Pentavalent Vaccine (DTP-HepB-Hib)

RCT	Randomized Controlled Trial
RR	Relative Risk
Rota	Rotavirus Vaccine
SAE	Serious Adverse Event
SDG	Sustainable Development Goals
SDG3	Sustainable Development Goal number 3
SDU	University of Southern Denmark
TB	Tuberculosis
TT	Tetanus Toxoid
ULB	Université Libre de Bruxelles
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization
YFV	Yellow Fever Vaccine

04 Paper included in the thesis

Paper I: Silva I., Nielsen S., Aaby P., Marchant A., Kollmann T., Amenyogbe N., Benn CS., Scholtz-Buchholzer F. **Adverse events after measles and Bacillus Calmette-Guérin (BCG) vaccinations of women of fertile age in Guinea-Bissau. A nested study within an open-label placebo-controlled randomised trial** (Trop Med Int Health. 2025 Oct 15. doi: 10.1111/tmi.70052)

Paper II: Silva I., Argondizo Correia C., Nielsen S., Aaby P., Metcalf-Clausen M., Scholtz-Buchholzer F., Kollmann T., Amenyogbe N., Benn CS, Marchant A. **Humoral Immune Responses to Measles Vaccination in Women of Reproductive Age: A Nested Study within a Randomised Trial in Guinea-Bissau** (Manuscript)

Paper III: Silva I., Aaby P., Kollmann T., Amenyogbe N., Ca E. Scholtz-Buchholzer F., Benn CS., Nielsen S. **Impact of Pre-pregnancy BCG and Measles Vaccination on Pregnancy Outcomes: A Randomised Controlled Trial in Guinea-Bissau** (Manuscript)

05 Summaries

05.01 Summary in English

Introduction

Vaccination is one of the most effective public health interventions, significantly contributing to the reduction of maternal and infant mortality. Traditionally, effects of vaccines have been assumed to be limited to protection against target diseases. However, accumulating evidence suggests that live attenuated vaccines, such as measles vaccine (MV) and *Bacillus Calmette-Guérin* (BCG), may exert non-specific effects (NSEs), enhance general immunity and improve survival. Additionally, previous studies have indicated that maternal immunity against the antigens introduced by BCG and measles vaccine may improve their infants' non-specific benefits from the same vaccines and thereby reduce all-cause mortality.

In Guinea-Bissau, where maternal and neonatal health indicators remain at concerning high levels, the possibility that pre-pregnancy vaccination in the women of reproductive age could provide women with a beneficial effect and positively influence maternal and newborn health, represents a promising avenue for public health strategies. Limited data exist on the effects of common vaccines such as MV and BCG vaccination in adult women, particularly regarding safety, immunogenicity, and impact on pregnancy outcomes. This thesis addresses these knowledge gaps as it is based on a large randomized, placebo-controlled clinical trial that enrolled HIV-negative, non-pregnant women aged 15–35 years (at the time of enrolment) living in urban Bissau. The thesis studies are focussing on three core components: (1) safety of MV and BCG vaccines in adult women (2) immune response to vaccination, and (3) impact of pre-pregnancy MV and BCG vaccination on pregnancy outcomes and early neonatal health.

Methods

The randomised trial was conducted at the Bandim Health Project (BHP), a demographic and health surveillance system (HDSS) established in 1978 that monitors over 200,000 individuals in Guinea-Bissau. Eligible women were identified in the BHP HDSS and invited to participate. Provided informed consent as well as negative HIV and pregnancy tests, women were randomised 1:1:1 to receive MV, BCG, or placebo (saline solution). Follow-up included home visits and phone calls to monitor adverse events and pregnancy. Blood samples were

collected at baseline, four weeks post-vaccination, and during the third trimester of pregnancy. The samples were analysed by our collaborators at the European Plotkin Institute of Vaccinology (EPIVULB) in Bruxelles, Belgium using Luminex-based multiplex assays to detect IgG antibodies against Measles virus, *Mycobacterium bovis* and Cytomegalovirus (CMV - control antigen). Adverse events were recorded immediately (10–15 minutes after vaccination) and during scheduled follow-up visits on days 7, 14, 28, and 90 post-vaccination. Pregnancy incidence and outcomes were monitored over a four-year period, with additional visits during pregnancy to collect clinical and obstetric data. Statistical analyses were performed using Stata. Group comparisons employed Kruskal-Wallis tests, logistic regression, binomial regression, and Cox proportional hazards models, as appropriate.

Results

Between May 2021 and February 2026, a total of 2,860 participants were randomised: Placebo (n=953), MV (n=954), and BCG (n=953). With 63 (Placebo: 19; MV: 19; BCG: 25) participants excluded from the study since they did not receive any follow-up contact after their inclusion and 16 due to withdrawal of consent (Placebo: 3; MV: 7; BCG: 5), 2,781 participants (Placebo: 931; MV: 930; BCG: 920) were included in the final analysis. Baseline characteristics were well balanced across groups. The median age was 27 years (interquartile range: 23–30 years).

Safety: A total of 363 women (placebo: 121, MV: 121, and BCG 121) were included in the analysis for this study arm. A total of 97% (1414/1452) of planned follow-up contacts were successfully completed. Most adverse events were mild and transient. The only statistically significant difference was the high frequency of skin reactions in the BCG group (96.7%), consistent with known vaccine effects. On day 7, a higher frequency of headache episodes was reported among participants receiving MV and BCG compared to placebo (Placebo: 2.5% (3/120); MV: 8.3% (10/121) and BCG: 9.2% (11/120)), $p = 0.08$ for MV vs placebo and $p = 0.05$ for BCG vs placebo. No serious adverse events or hospitalizations were reported.

Immunogenicity: A total of 462 samples were included (placebo: 162, MV: 150, and BCG 150). MV vaccination led to a significant increase in measles-specific antibody level four weeks post-intervention, with a mean geometric mean titre (GMT) fold change of 3.84 (95% CI: 2.22 to 5.47). In contrast, the BCG group showed a modest significant increase in antibody levels against

Mycobacterium bovis antigens (fold change ~1.5), while the placebo group showed no significant changes, compared to levels in the baseline sample.

Fertility: Over the four-year follow-up, 38% (1062/2781) of participants became pregnant. Pregnancy incidence rates per 100 person years were similar across groups: placebo 16.2 (363/815938); (MV: 16.5 (363/804120) and BCG: 14.9 (336/825249).

Pregnancy outcomes: No statistically significant differences were observed in early neonatal death or failed pregnancy rates between the MV and BCG groups compared to placebo. Early neonatal death rates were similar for MV and placebo, while BCG showed a higher rate, but without statistical significance. Risk ratios for failed pregnancy were also comparable across groups.

Conclusions

This thesis provides evidence on the safety and immunogenicity of MV and BCG vaccines in women of reproductive age. Follow-up is still ongoing, MV vaccination elicited a strong humoral immune response, while BCG showed modest antibody increases consistent with its cellular immunogenic profile. Both vaccines were well tolerated, with mild adverse events.

The pre-pregnancy BCG and MV vaccination during reproductive, showed no measurable impact on fertility or pregnancy outcomes, but a signal in relation to BCG vaccine and early neonatal mortality warrants further investigation in the full dataset.

05.02 Dansk resumé [Summary in Danish]

Introduktion

Vaccination er en af de mest effektive folkesundhedsinterventioner og bidrager væsentligt til reduktion af mødre- og spædbarnsdødelighed. Traditionelt har vaccineeffekter været antaget til at være begrænset til beskyttelse mod målsygdomme. Akkumulerende evidens peger dog på, at levende svækkede vacciner, såsom mæslingevaccinen (MV) og Bacillus Calmette-Guérin (BCG), kan udøve uspecifikke effekter (NSEs), styrke den generelle immunitet og forbedre overlevelsen. Derudover har tidligere studier indikeret, at maternel immunitet over for antigener introduceret ved MV og BCG kan forstærke spædbørns uspecifikke udbytte af de samme vacciner og derved reducere total dødelighed.

I Guinea-Bissau, hvor indikatorer for mødre- og neonatal sundhed fortsat er bekymrende, er muligheden for, at vacciner givet til kvinder i den reproduktive alder både kan have direkte gavnlige effekter og samtidig forbedre sundheden for mødre og nyfødte, en lovende strategi for folkesundheden. Der findes begrænsede data om effekterne af almindelige vacciner som MV og BCG hos voksne kvinder, særligt vedrørende sikkerhed, immunogenicitet og betydning for graviditetsudfald. Denne afhandling adresserer disse ukendte effekter som del af et stort randomiseret, placebokontrolleret klinisk forsøg blandt HIV-negative, ikke-gravide kvinder i alderen 15–35 år bosiddende i det urbane Bissau. Afhandlingens studie fokuserer på tre hovedområder: (1) sikkerhed af MV og BCG hos voksne kvinder, (2) immunrespons efter vaccination samt, (3) effekten af MV og BCG på fertilitet, graviditetsudfald og tidlig neonatal sundhed.

Metoder

Det randomiserede forsøg blev gennemført ved Bandim Health Project (BHP), et Health and Demographics Surveillance System (HDSS) etableret i 1978, der følger over 200.000 individer i Guinea-Bissau. Kvinder, som opfyldte inklusionskriterierne, blev identificeret via BHP og inviteret til deltagelse. Efter informeret samtykke samt negative HIV- og graviditetstests blev kvinder randomiseret 1:1:1 til MV, BCG eller placebo (saltvandsopløsning). Opfølgning omfattede hjemmebesøg og telefoninterviews med henblik på monitorering af bivirkninger og graviditeter. Blodprøver blev taget ved baseline, fire uger efter vaccination samt i tredje trimester. Prøverne blev analyseret på European Plotkin Institute of Vaccinology (EPIVULB) ved hjælp af Luminex-baserede multiplex assays til påvisning af IgG-antistoffer mod henholdsvis mæslingevirus, *Mycobacterium bovis* og cytomegalovirus (CMV, kontrolantigen). Bivirkninger blev registreret ved

planlagte besøg dag 7, 14, 28 og 90 efter vaccination. Graviditetsincidens og -udfald blev monitoreret over fire år med supplerende graviditetsbesøg til indsamling af kliniske og obstetriske data. Statistiske analyser blev udført i Stata 18.0 med anvendelse af Kruskal-Wallis-test, logistisk regression og Cox proportional hazards-model.

Resultater

Mellem maj 2021 og februar 2026 blev i alt 2.860 deltagere randomiseret: Placebo (n=953), MV (n=954) og BCG (n=953). Med 63 deltagere (Placebo: 19; MV: 19; BCG: 25) udelukket fra studiet, da de ikke modtog nogen opfølgende kontakt efter inklusion, og 16 på grund af tilbagetrukket samtykke (Placebo: 3; MV: 7; BCG: 5), blev 2.781 deltagere (Placebo: 931; MV: 930; BCG: 920) inkluderet i den endelige analyse. Baseline-karakteristika var velafbalancerede mellem grupperne (medianalder 27 år, interkvartilrange 23–30 år).

Sikkerhed: I alt 363 kvinder (placebo: 121, MV: 121 og BCG: 121) blev inkluderet i analysen for denne studiearm. I alt 97 % (1414/1452) af planlagte opfølgningskontakter blev gennemført. De fleste bivirkninger var milde og forbigående. Den eneste statistisk signifikante forskel var den høje forekomst af hudreaktioner i BCG-gruppen (96,7 %), i overensstemmelse med kendte vaccineeffekter. På dag 7 blev en højere forekomst af hovedpineepisoder rapporteret blandt deltagere, der modtog MV og BCG sammenlignet med placebo (Placebo: 2,5 % (3/120); MV: 8,3 % (10/121) og BCG: 9,2 % (11/120)), $p=0,08$ for MV vs placebo og $p=0,05$ for BCG vs placebo. Ingen alvorlige bivirkninger eller indlæggelser blev rapporteret.

Immunogenicitet: I alt 462 prøver blev inkluderet (placebo: 162, MV: 150 og BCG: 150). MV-vaccination medførte en signifikant stigning i mæslinge-specifikke antistofniveauer fire uger efter interventionen med en geometrisk middelværdi (GMT) fold-change på 3,84 (95 % CI: 2,22 til 5,47). I modsætning hertil viste BCG-gruppen en beskeden, men signifikant stigning i antistofniveauer mod *Mycobacterium bovis*-antigener (fold-change ~1,5), mens placebogruppen ikke viste signifikante ændringer sammenlignet med baseline. Antistofniveauer for alle antigener faldt i tredje trimester af graviditeten på tværs af alle grupper, sandsynligvis som følge af fysiologiske ændringer under graviditet.

Fertilitet: Over en fireårig opfølgning blev 38% (1062/2781) af deltagerne gravide. Forekomsten af graviditet pr. 100 personår var ens på tværs af grupperne: placebo 16,2 (363/815938); MV: 16,5 (363/804120); og BCG: 14,9 (336/825249).

Graviditetsudfald: Der blev ikke observeret statistisk signifikante forskelle i tidlig neonatal død eller mislykkede graviditeter mellem MV- og BCG-grupperne sammenlignet med placebo. Tidlig neonatal dødsrate var ens for MV og placebo, mens BCG viste en højere rate, men uden statistisk signifikans. Risikofaktorer for mislykket graviditet var også sammenlignelige på tværs af grupper.

Konklusioner

Dette forsøg giver robust evidens for sikkerhed og immunogenicitet af MV og BCG hos kvinder i den reproduktive alder. MV-vaccination fremkaldte en stærk humoral immunrespons, mens BCG viste beskedne antistofstigninger i overensstemmelse med dens cellulære immunprofil. Begge vacciner blev godt tolereret med kun milde bivirkninger.

BCG- og MV-vaccination før graviditet hos kvinder i den reproduktive alder viste ingen målbar effekt på fertilitet eller graviditetsudfald. Dog indikerer et signal relateret til BCG-vaccination og tidlig neonatal dødelighed, at der er behov for yderligere undersøgelser i det fulde datasæt

05.03 **Resumo em português português [Summary in Portuguese]**

Introdução

Vacinação é uma das intervenções de saúde pública mais eficazes, contribuindo significativamente para a redução da mortalidade materna e infantil. Tradicionalmente, os efeitos das vacinas têm sido considerados limitados à proteção contra doenças-alvo. No entanto, evidências crescentes sugerem que vacinas vivas atenuadas, como a vacina contra o sarampo (MV) e a *Bacillus Calmette-Guérin* (BCG), podem exercer efeitos não específicos (NSEs), fortalecer a imunidade geral e melhorar a sobrevivência. Além disso, estudos anteriores indicaram que a imunidade materna contra os antígenos introduzidos pela BCG e pela vacina contra o sarampo pode potencializar os benefícios não específicos dessas vacinas nos lactentes e, assim, reduzir a mortalidade por todas as causas.

Na Guiné-Bissau, onde os indicadores de saúde materna e neonatal permanecem em níveis preocupantes, a possibilidade de que vacinas administradas a mulheres em idade reprodutiva possam proporcionar um efeito benéfico às mulheres e influenciar positivamente a saúde materna e neonatal representa uma via promissora para estratégias de saúde pública. Existem dados limitados sobre os efeitos de vacinas comuns, como MV e BCG, em mulheres adultas, particularmente no que diz respeito à segurança, imunogenicidade e impacto nos desfechos gestacionais. Esta tese aborda essas lacunas de conhecimento, baseando-se em um grande ensaio clínico randomizado, controlado por placebo, que incluiu mulheres HIV-negativas, não grávidas, com idades entre 15 e 35 anos (no momento da inclusão), residentes na área urbana de Bissau. Os estudos da tese concentram-se em três componentes principais: (1) segurança das vacinas MV e BCG em mulheres adultas; (2) resposta imunológica à vacinação; e (3) impacto da vacinação com MV e BCG nos desfechos gestacionais e na saúde neonatal precoce.

Métodos

O ensaio randomizado foi conduzido no Bandim Health Project (BHP), um sistema de vigilância demográfica e de saúde (HDSS) estabelecido em 1978 que monitora mais de 200.000 indivíduos na Guiné-Bissau. Mulheres elegíveis foram identificadas no HDSS do BHP e convidadas a participar. Após consentimento informado e testes negativos para HIV e gravidez, as participantes foram randomizadas 1:1:1 para receber MV, BCG ou placebo (solução salina). O acompanhamento incluiu visitas domiciliares e chamadas telefônicas para

monitorar eventos adversos e gravidez. Amostras de sangue foram coletadas no início, quatro semanas após a vacinação e durante o terceiro trimestre da gestação. As amostras foram analisadas por nossos colaboradores no European Plotkin Institute of Vaccinology (EPIVULB), em Bruxelas, Bélgica, utilizando ensaios multiplex baseados em Luminex para detectar anticorpos IgG contra vírus do sarampo, *Mycobacterium bovis* e citomegalovírus (CMV – antígeno controle). Eventos adversos foram registrados imediatamente (10–15 minutos após a vacinação) e durante visitas programadas nos dias 7, 14, 28 e 90 pós-vacinação. A incidência e os desfechos gestacionais foram monitorados por quatro anos, com visitas adicionais durante a gestação para coleta de dados clínicos e obstétricos. As análises estatísticas foram realizadas utilizando Stata. As comparações entre grupos empregaram testes de Kruskal-Wallis, regressão logística, regressão binomial e modelos de riscos proporcionais de Cox, conforme apropriado.

Resultados

Entre maio de 2021 e fevereiro de 2026, um total de 2.860 participantes foi randomizado: Placebo (n=953), MV (n=954) e BCG (n=953). Com 63 participantes (Placebo: 19; MV: 19; BCG: 25) excluídos do estudo por não terem recebido qualquer contacto de seguimento após a inclusão e 16 devido à retirada de consentimento (Placebo: 3; MV: 7; BCG: 5), 2.781 participantes (Placebo: 931; MV: 930; BCG: 920) foram incluídos na análise final. As características basais estavam bem equilibradas entre os grupos. As características basais estavam bem equilibradas entre os grupos. A idade mediana foi de 27 anos (intervalo interquartil: 23–30 anos).

Segurança: Um total de 363 mulheres (Placebo: 121, MV: 121 e BCG: 121) foram incluídas na análise deste braço do estudo. Um total de 97% (1414/1452) dos contatos de acompanhamento planejados foram concluídos com sucesso. A maioria dos eventos adversos foi leve e transitória. A única diferença estatisticamente significativa foi a alta frequência de reações cutâneas no grupo BCG (96,7%), consistente com os efeitos conhecidos da vacina. No dia 7, uma maior frequência de episódios de dor de cabeça foi relatada entre participantes que receberam MV e BCG em comparação ao placebo (Placebo: 2,5% (3/120); MV: 8,3% (10/121) e BCG: 9,2% (11/120)), $p=0.08$ para MV vs placebo e $p=0.05$ para BCG vs placebo. Não foram relatados eventos adversos graves ou hospitalizações.

Imunogenicidade: Um total de 462 amostras foram incluídas (Placebo: 162, MV: 150 e BCG: 150). A vacinação com MV levou a um aumento significativo

no nível de anticorpos específicos contra sarampo quatro semanas após a intervenção, com uma média geométrica (GMT) de mudança de 3,84 (IC 95%: 2,22 a 5,47). Em contraste, o grupo BCG apresentou um aumento modesto, porém significativo, nos níveis de anticorpos contra antígenos de *Mycobacterium bovis* (mudança ~1,5), enquanto o grupo placebo não apresentou alterações significativas em relação aos níveis da amostra basal.

Fertilidade: Ao longo de quatro anos de seguimento, 38% (1062/2781) das participantes engravidaram. As taxas de incidência de gravidez por 100 pessoas-ano foram semelhantes entre os grupos: placebo 16,2 (363/815938); MV: 16,5 (363/804120); e BCG: 14,9 (336/825249).

Desfechos gestacionais: Não foram observadas diferenças estatisticamente significativas na morte neonatal precoce ou nas taxas de gravidez malsucedida entre os grupos MV e BCG em comparação ao placebo. As taxas de morte neonatal precoce foram semelhantes para MV e placebo, enquanto BCG apresentou uma taxa mais alta, mas sem significância estatística. As razões de risco para gravidez malsucedida também foram comparáveis entre os grupos.

Conclusões

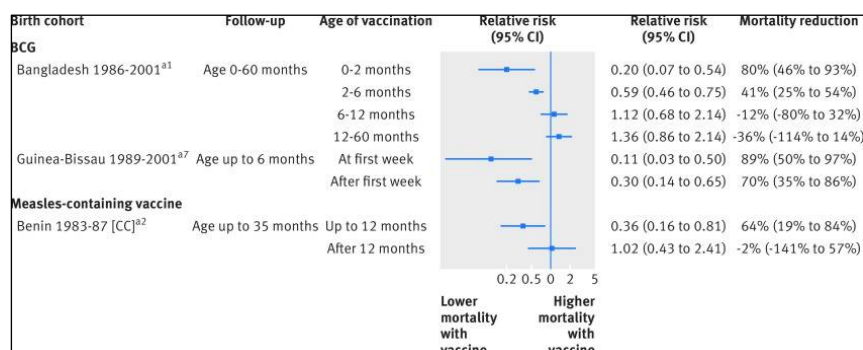
Este ensaio fornece evidências robustas sobre a segurança e imunogenicidade das vacinas MV e BCG em mulheres em idade reprodutiva. A vacinação com MV induziu uma forte resposta humoral, enquanto a BCG apresentou aumentos modestos nos anticorpos, consistentes com seu perfil imunogênico celular. Ambas as vacinas foram bem toleradas, com apenas eventos adversos leves. A vacinação com BCG e MV antes da gravidez, em mulheres em idade reprodutiva, não mostrou impacto mensurável sobre a fertilidade ou os desfechos da gravidez. Contudo, um sinal relacionado à vacina BCG e à mortalidade neonatal precoce merece investigação adicional no conjunto completo de dados.

06 Introduction

Vaccination is one of the most powerful public health interventions, contributing significantly to reducing infant and maternal mortality (1,2).

Traditionally, the effects of vaccines have been evaluated based on protection against the target disease (2).

While the specific effects of vaccines are widely documented, accumulating evidence suggests that live attenuated vaccines, such as BCG, oral polio vaccine (OPV), and measles vaccine (MV), may exert nonspecific effects (NSEs), positively influencing the immune system and child survival (3,4). These beneficial effects appear to be stronger when the vaccines are administered at the optimal age, as illustrated in **Figure 1**, which shows significantly greater mortality reduction when BCG and MV are given early in life.



Source: Higgins et al, BMJ 2016

Figure 1: Differential vaccine effects by age at vaccination. CC=case-control study; RR=relative risk. Reference numbers correspond to those in appendix 3.

In Guinea-Bissau, where maternal and neonatal health indicators are still worrying, with high rates of neonatal mortality and stillbirths (5,6), the possibility that vaccines administered to women may influence newborn survival represents a promising opportunity for public health interventions (7).

06.01 Importance of maternal priming

Recent studies have shown that vaccination of children in the presence of maternal immunity may enhance the non-specific benefits when the same vaccines are administered to their offspring, leading to improved survival outcomes (8–12).

For MV, it has been shown that early measles vaccination in the presence of maternal measles antibodies is associated with significantly improved child survival, possibly due to non-specific immune benefits or enhanced priming of the infant immune system (8,11).

Maternal antibodies against measles are stronger following natural infection than vaccination, so children of vaccinated mothers may become susceptible to measles at an earlier age (13). Today, many mothers have weaker or no antibodies due to lack of exposure or waning immunity, yet the vaccination schedule remains unchanged, potentially leaving infants vulnerable in the time period before MV vaccination (14).

For BCG vaccine, it has been shown that maternal BCG vaccination indicated by the presence of a BCG scar is linked to better child survival, especially in male infants, possibly due to non-specific immune benefits passed from mother to child (15).

Thus, pre-pregnancy vaccination with BCG and MV, combined with earlier childhood vaccination of their offspring, may be an effective strategy. It enhances maternal immunity, improves the quality of immune components transferred to the child, and potentially extends the duration and benefits of the child's specific and non-specific immune profile.

Little is known about the effects of MV and BCG vaccination in adult women, however, especially regarding safety, immune response, and impact on pregnancy and newborn health outcomes. To fill this gap, we conducted a randomised, placebo-controlled clinical trial enrolling non-pregnant, HIV-negative women aged 15 to 35 years living in urban Bissau.

This thesis reports a subset of outcomes from the larger ongoing randomized trial. The primary and secondary outcomes of the main trial are summarised below.

Primary Outcomes

- Measles antibody levels in infants at 20 weeks, 9 months, and 13.5 months of age.
- Yellow fever viral load following administration of the yellow fever vaccine at 9 months of age.

Secondary Outcomes

- Maternal immune response to measles vaccination, assessed prior to pregnancy and during the third trimester, including both antibody concentration and functional quality.
- Transplacental transfer of maternal measles antibodies, evaluated by comparing maternal antibody levels in the third trimester with infant pre-vaccination levels at 20 weeks and 9 months of age, considering both quantitative and qualitative antibody measures.
- Maternal morbidity following allocation to BCG, MV, or placebo, assessed through monthly home visits conducted for at least 12 months.
- Child morbidity among infants born to mothers receiving BCG, MV, or placebo.

Analyses related to longer-term maternal and child morbidity, as well as evaluation of the broader trial hypotheses, will be conducted once full cohort follow-up is finalised.

This thesis is thus structured around three main areas:

- A. Safety of MV and BCG vaccines in adult women, assessing the occurrence of short- and medium-term adverse events after vaccination.
- B. Immune response to vaccination, focusing on measles and BCG antibody levels before and after vaccination of adult fertile women, including levels during pregnancy for those that became pregnant during the trial.
- C. Impact of pre-pregnancy vaccination on pregnancy incidence, pregnancy outcomes, and neonatal health, including rates of miscarriage, stillbirth, and early neonatal mortality.

By integrating these three components, this thesis seeks to contribute to the understanding of the specific and non-specific effects of pre-pregnancy MV and BCG vaccines given in the women of childbearing age, with potential implications for vaccination policies and maternal and child health strategies in low-income contexts. **The Figure 2** summarises the general overview of the thesis structure.

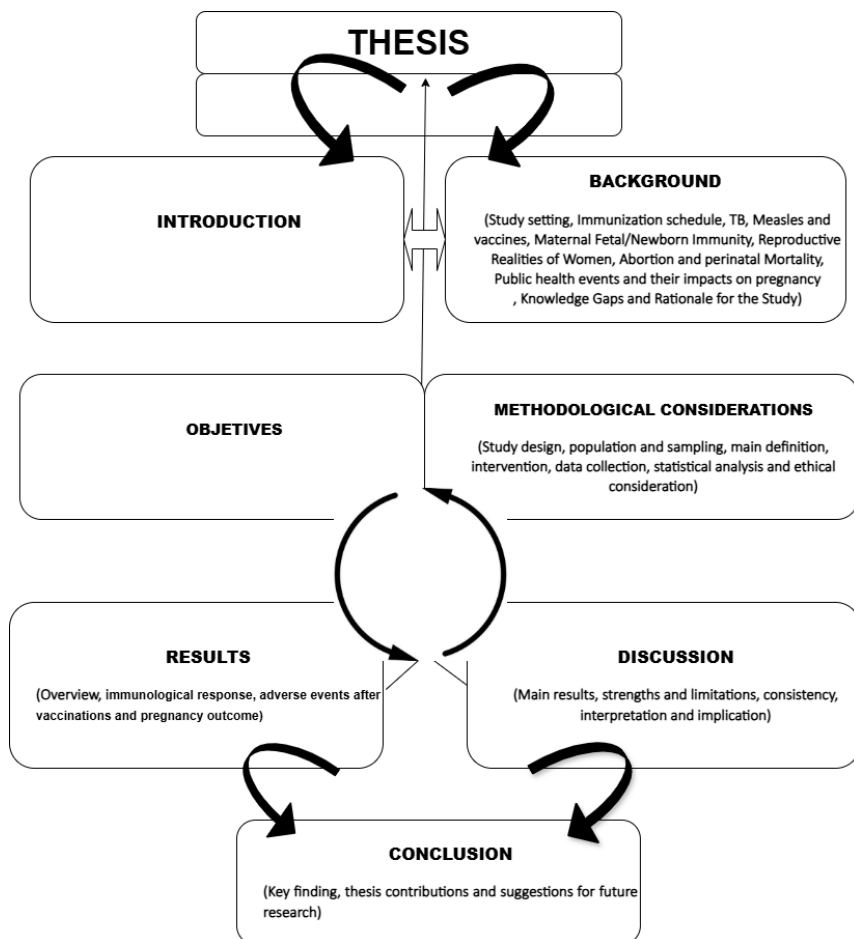


Figure 2: Thesis structure

07 Background

07.01 Study setting

Guinea-Bissau is a West African country with an estimated population of around 2.20 million inhabitants and a territorial area of 36,125 km² (16). Bordered to the north by Senegal, to the south and east by Guinea, and to the west by the Atlantic Ocean, Guinea-Bissau has a tropical climate with two distinct seasons: the rainy season (May to November) and the dry season (December to April). The capital, Bissau, is the country's political, economic, and administrative centre (17).

Historically, Guinea-Bissau was part of the Kingdom of Gabu and the Empire of Mali and was later colonized by Portugal. It became independent in 1973, becoming the first Portuguese colony in Africa to have its independence recognized by Portugal (18). Guinean society is marked by strong cultural diversity and significant socioeconomic challenges, including high poverty rates; around 70% of the population lives on less than USD 1.90 per day (19).

Administratively, the country is divided into eight regions and one autonomous sector (Bissau). For public health purposes, the country is organized into 11 health regions and 114 health areas. Despite these health structures, more than 40% of the population lives more than 5 km from a basic health unit, often in areas with poor infrastructure which poses a significant challenge to accessing primary care (20).

07.02 Bandim Health Project

Bandim Health Project (BHP) www.bandim.org (Figure 3), is a demographic and health surveillance system (HDSS) established in 1978. The BHP HDSS monitors more than 200,000 individuals in urban and rural areas of Guinea-Bissau, with regular visits to record births, deaths, vaccinations, and other health indicators. The urban study area includes six neighbourhoods in the capital, Bissau, while the rural area is monitored by mobile teams that regularly visit villages to collect data on maternal and child health.

BHP offers a robust platform for conducting epidemiological studies and clinical trials, including research on measles, tuberculosis, HIV, nutrition, vaccination,

and mortality. Its infrastructure allows for the longitudinal monitoring of women of childbearing age and children under five, making it an ideal setting for investigating the effects of BCG and MV vaccination on women of reproductive age.

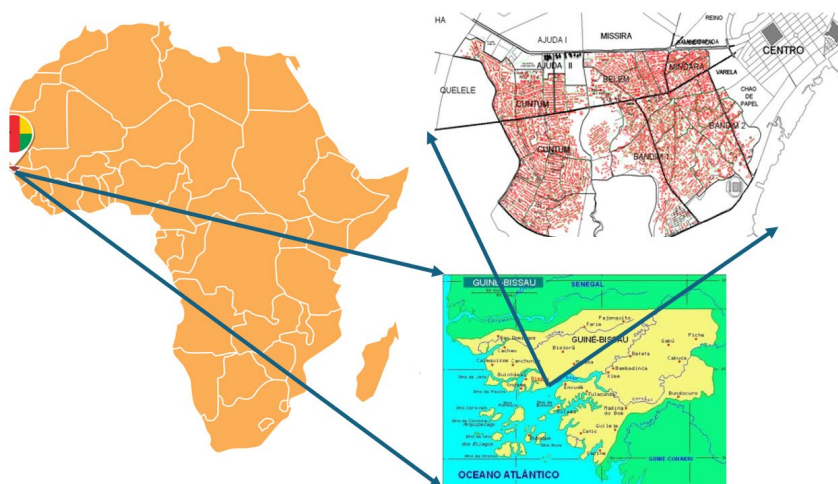


Figure 3: Geospatial location of the Bandim Health Project, Guinea-Bissau

07.03 Immunisation schedule in Guinea-Bissau

The Expanded Programme of Immunisation (EPI) was launched in 1974 and currently covers 11 diseases to be prevented by 9 vaccines. The schedules for children and women are described in the following **Tables 1 and 2**:

Table 1: Routine immunisation schedule for children 0-24 months

Contact	Age	Antigens
1	Birth	BCG, VPO-0
2	6 weeks	PENTA1, PCV13-1, OPV1, Rota 1
3	10 weeks	PENTA2, PCV13-2, OPV2, Rota 2
4	14 weeks	PENTA3, PCV13-3, OPV-3, IPV
5	9 months	Measles vaccine-1, Yellow fever vaccine

Source: Immunization and Epidemiological Surveillance Service of Guinea-Bissau

BCG: Bacillus Calmette–Guérin – tuberculosis vaccine; OPV: Oral Polio Vaccine (doses 0, 1, 2, 3); PENTA: Pentavalent vaccine (diphtheria, tetanus, pertussis, hepatitis B, Haemophilus influenzae type b); PCV13: 13-valent pneumococcal conjugate vaccine; Rota: Rotavirus vaccine; IPV: Inactivated Polio Vaccine.

Table 2: Routine immunisation schedule for women

Pregnant women or women of childbearing age		
Contact	Age	Antigens
1	1st contact (from the age of 14 or during pregnancy)	TT1
2	4 weeks after TT1	TT2
3	6 weeks after TT2	TT3
4	1 year after TT 3	TT4
5	1 year after TT 4	TT5

Source: Immunization and Epidemiological Surveillance Service of Guinea-Bissau
BCG: Bacillus Calmette–Guérin: tuberculosis vaccine; OPV: Oral Polio Vaccine (doses 0, 1, 2, 3); PENTA: Pentavalent vaccine (diphtheria, tetanus, pertussis, hepatitis B, Haemophilus influenzae type b); PCV13: 13-valent pneumococcal conjugate vaccine; Rota: Rotavirus vaccine; IPV: Inactivated Polio Vaccine.

In response to measles outbreaks in 2021 and 2022, Guinea-Bissau launched a national measles-rubella vaccination campaign in February 2025, targeting approximately 900,000 children aged 6 months to 14 years. The campaign also included vitamin A supplementation and albendazole administration (21).

In 2025, Guinea-Bissau carried out Tetanus Toxoid (TT) vaccination campaigns targeting women of reproductive age. The campaign aimed to prevent maternal and neonatal tetanus.

07.04 Measles and measles vaccines

07.04.01 Measles virus

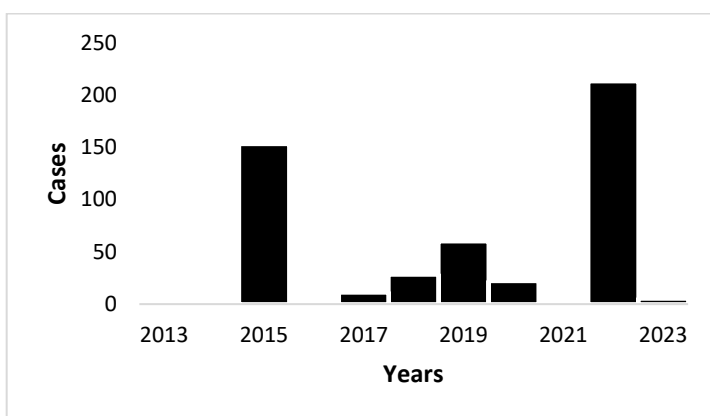
Measles virus (MeV), a member of the Paramyxoviridae family, is a non-segmented, negative-sense RNA virus known for its high contagiousness. Despite causing millions of infections worldwide each year, there are currently no licensed antiviral therapies available to treat it (22,23).

In low-income settings, measles can often be a fatal infection. Before the introduction of measles vaccine there was a popular saying in Guinea-Bissau **“a child during a measles epidemic is not a child”**, referring to the risk of becoming childless if one only has 1 child during an epidemic, reflecting the collective perception of the high child mortality during measles outbreaks.

In 2023, the African region experienced a significant resurgence of measles, accounting for nearly half of all major or disruptive outbreaks reported globally.

Low vaccination coverage, exacerbated by the disruption of immunisation campaigns during the COVID-19 pandemic, contributed to thousands of preventable cases and deaths, particularly among children under five years of age (24).

Between 2013 and 2023, Guinea-Bissau recorded an annual average of approximately 36 measles cases. 2022 stood out as the year with the highest number of notifications, totalling approximately 200 cases, representing a significant increase compared to the average observed over the decade (**Figure 4**) (25).



Source: WHO/UNICEF Joint Reporting Form on Immunization (JRF). Country data

Figure 4: Measles Cases in Guinea-Bissau, year 2024

07.04.02 Measles vaccines

Measles immunity can be acquired through two main pathways: active immunization, which occurs after infection with either the wild-type or attenuated virus, and passive immunisation, which is conferred by maternal antibodies transferred across the placenta (26).

The currently used live attenuated MV is recommended in two doses in childhood. It is both safe and highly effective, having prevented millions of deaths worldwide (27,28). It elicits strong and long-lasting antibody and T-cell responses (28). In 2024, global coverage for the first dose of MV was 83%, while the second dose reached only 74% both falling significantly short of the 95% threshold required to prevent large outbreaks (29).

The current measles vaccination policy was originally developed based on children born to mothers, who had acquired natural immunity through infection (30). In contrast, most mothers today have obtained immunity through vaccination rather than natural infection, and there is evidence that their infants are born with lower levels of maternal measles antibodies (14). This shift has important implications, as the original policy aimed to balance the need for early protection in infancy with concerns that maternal antibodies might interfere with the immune response to the vaccine (26). Since MV also has marked beneficial NSEs, especially when provided in the presence of vertically transferred antibody to the offspring, the strategy to wait until maternal antibody have waned may be misguided.

Between 2013 and 2024, measles vaccination coverage in Guinea-Bissau ranged from 63% to 90% (**table 3**), but consistently remained below the elimination target set by WHO. The fluctuations reflect both logistical and programmatic limitations as well as the impact of the COVID-19 pandemic. In 2024, coverage dropped to around 65% (31).

Table 3: Measles vaccination coverage

Ano	Administrative (%)	Official (%)	WHO/UNICEF (WUENIC) (%)
2013	88.9	89	81
2014	74.1	81	81
2015	75.0	90	80
2016	71.5	71	80
2017	66.0	66	66
2018	79.0	79	79
2019	79.0	79	79
2020	72.3	90	72
2021	63.1	93	63
2022	74.8	63	75
2023	71.8	63	72
2024	65.1	65	65

Source: WHO Immunization Data portal

World Health Organization, WHO, 2026, All rights reserved

07.04.03 Adverse reactions of measles vaccine

Measles vaccines are safe, with adverse reactions that are mild and transient. Local reactions such as pain and tenderness at the injection site may occur within 24 hours and typically resolve within 2–3 days. Systemic reactions include fever ($\geq 39.4^{\circ}\text{C}$) which occurs in 5–15% of recipients between 7–12 days post-vaccination, sometimes accompanied by a rash (2–5%) or rarely by febrile seizures (1 in 3,000 doses). Severe adverse events are rare. Adverse events are less frequent after the second dose, as most individuals are already immune (27,32).

07.04.04 Non-specific effects of MV

When the MV was introduced in low-income countries during the 1970s and 1980s, a striking reduction in all-cause mortality, often exceeding 50%, was observed among vaccinated children, despite the fact that only around 10% of deaths were directly attributable to measles (33).

The reduction in all-cause mortality suggested that MV conferred broader health benefits beyond its specific protection against the measles virus.

Subsequent evidence from randomised controlled trials and observational studies has consistently shown that MV is associated with substantial reductions in all-cause childhood mortality in low-income settings (34–36). In particular, measles vaccination was associated with a reduction in mortality that could not be explained solely by the prevention of measles infection (37).

These findings challenged the traditional view that the sole benefit of vaccination was pathogen-specific protection (33).

In parallel with these findings, evidence suggests that the non-specific beneficial effects of the measles vaccine are stronger when administered in the presence of maternal antibodies (MatAb).

Children vaccinated in the presence versus absence of MatAb had a 71% lower mortality (95% CI: 9–91%) (11). Furthermore, having MatAb at the time of measles vaccination was associated with a 55% reduction (95% CI: 35–78%) in overall mortality compared with measles vaccinated in the absence of MatAb in five studies (8).

07.05 Tuberculosis (TB) and Bacille Calmette-Guérin (BCG) vaccine

07.05.05 Tuberculosis disease

Tuberculosis (TB) is a preventable and treatable disease caused by *Mycobacterium tuberculosis* (MTB). Despite the availability of highly effective therapies, it remains the leading cause of death worldwide from a single infectious agent (38).

In 2022, approximately 10.6 million people developed tuberculosis (TB) worldwide, resulting in about 1.3 million deaths, according to estimates from the World Health Organization (WHO) (39). In Guinea-Bissau, the TB incidence rate was 361 cases per 100,000 population, as recorded in the WHO Global Report on Tuberculosis (40).

07.05.06 Bacillus Calmette-Guérin vaccine

The attenuated strain of *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) is widely used as a vaccine against tuberculosis and has attracted interest as a vector for delivering antigens targeting infectious diseases (41). Although widely used, the exact mechanisms by which the BCG vaccine provides protection against tuberculosis is not precisely known, but CD4⁺ T cells, particularly TH1 and TH17 subsets, have been identified as key players in mediating immune protection against TB (42).

The BCG vaccination coverage in Guinea-Bissau ranged from 34% to 95%. Despite high levels in earlier years, the COVID-19 pandemic and logistical barriers led to sharp declines, followed by partial recovery in subsequent years (31) (**table 4**).

Table 4: BCG vaccination coverage

Ano	Administrative (%)	Official (%)	WHO/UNICEF (WUENIC) (%)	#sdu.dk
2013	95	95	93	
2014	90.4	94	93	
2015	89	97	92	
2016	82.9	83	91	
2017	87	87	87	
2018	89	89	89	
2019	84	84	84	
2020	66.9	90	67	
2021	34.2	93	34	
2022	79.8	34	80	
2023	81.4	34	81	
2024	71.2	71	71	

Source: WHO Immunization Data portal

World Health Organization, WHO, 2026, All rights reserved

07.05.07 Adverse events of BCG

The BCG vaccine is widely used globally, with approximately 100 million new-borns vaccinated annually (43). Despite its extensive use, serious adverse events in children are very rare. Mild adverse events include local reactions at the injection site, such as erythematous and indurated papules that ulcerate and heal within a couple of months, and ipsilateral regional lymphadenopathy. Serious adverse events can be local or systemic. Local adverse events include subcutaneous abscesses, keloids, and suppurative lymphadenitis, the latter being more common when the vaccine is not administered correctly (44).

07.05.08 Non-specific effects of BCG

It has been shown that BCG vaccination induces beneficial non-specific effects beyond tuberculosis prevention.

Historical and clinical data show significant reductions in infant mortality following BCG introduction in Europe and Guinea-Bissau (33,45,46). Randomised trials in Guinea-Bissau revealed a reduction in neonatal mortality among low-birth-weight infants vaccinated at birth (47). A WHO-commissioned meta-

analysis confirmed a 30–53% reduction in all-cause mortality in children under five (4).

The recent BLOW2 trial in India further demonstrated that early BCG vaccination combined with OPV significantly reduced all-cause and infection-related mortality among low-birth-weight neonates (48)

In adults, the Activate trial in Greece reported BCG vs placebo associated with a 45% reduction in infections and 79% in respiratory infections among elderly patients post-discharge (49).

Although several studies have suggested that the BCG vaccine may offer non-specific protection against disease in children and adults, not all studies have confirmed this effect, indicating that protective effects may vary depending on the population, epidemiological context, age of vaccination and coadministration of other vaccines (50).

Immunological studies indicate that BCG stimulates trained innate immunity, promoting a more robust immune response against unrelated pathogens (42,51).

Studies suggest that newborns whose mothers were vaccinated with BCG, especially those who develop a vaccination scar, may have a better prognosis and a lower risk of mortality from various infectious causes.

In fact, BCG vaccination was associated with a 35% reduction in hospitalisation risk (95% CI: 6–55%) among children of BCG-vaccinated mothers (9). This effect was even stronger (66% reduction, 95% CI: 33–83%) when the mother had a visible BCG scar, no reduction in mortality was observed if the mother had no scar ($P = 0.04$) (52).

07.06 Maternal Immunity and Foetal/Newborn Immunity

Maternal immunity plays a crucial role in protecting the fetus and newborn from infections during the first months of life, a period when the infant's immune system is still developing (53). There are two distinct mechanisms through which maternal vaccination may influence the child's health:

Transfer of maternal passive protection:

During pregnancy, maternal IgG antibodies are transferred through the placenta, providing temporary passive immunity to the infant (54). This transfer is most effective in the third trimester and depends on maternal antibody levels

and placental health (53,54). It offers specific protection against the same pathogens targeted by maternal vaccination. Several studies, as shown in **Table 5**, support the positive impact of maternal immunity on child health outcomes (53,55,56).

Maternal priming for enhanced non-specific effects in the child:

Beyond passive protection, maternal immunity may also prime the infant's immune system, potentially enhancing the non-specific effects of the same vaccine administered to the child. This hypothesis suggests that maternal immune activation during pregnancy could modulate the infant's response to vaccines, leading to broader immunological benefits(11,57).

Table 5: Previous studies on the influence of maternal immunity on the fetus or newborn

Author, years and reference	Study title	Conclusions
Maternal immunity and transfer of passive protection		
Boonyaratana Kornkit et al, 2019 (53)	Why Should We Advocate Maternal Immunization	Pregnant women and neonates are at risk for severe complications from infectious diseases. Routine vaccination in pregnancy protects the mother, the fetus as well as the infant by boosting transplacental and breast milk antibody.
Fernandes et al, 2019 (55)	The effectiveness of maternal pertussis vaccination in protecting newborn infants in Brazil: A case-control study	Vaccination during pregnancy is highly effective in protecting infants from pertussis and that it may have substantial impact on morbidity and mortality in infants too young to have been vaccinated.
Keller-Stanislawski et al, 2014 (56)	Safety of immunization during pregnancy: A review of the evidence of selected inactivated and live attenuated vaccines	The benefits of vaccinating pregnant women generally outweigh the potential risks, if the woman is at risk of being exposed to a particular infection and the disease would pose a risk for her or her unborn child.
Maternal immunity and non-specific priming of the newborn		
Nielsen et al., 2022 (8)	Effect of early two-dose measles	The studies of maternal priming with measles and BCG open a new field of

	vaccination on childhood mortality and modification by maternal measles antibody in Guinea-Bissau, West Africa	interventions. Earlier vaccination with live vaccines, when maternal antibody is still present, is likely to have beneficial effects on overall child survival.
Schaltz-Buchholzer, F. et al, 2022 (57)	Maternal BCG primes for enhanced health benefits in the newborn	There was a trend of reduced risk of in-hospital death associated with maternal BCG priming, a protection that was strongest against neonatal sepsis, and for males. Children of mothers with a scar are not more likely to develop a scar after vaccination.
Aaby et al, 2014 (58)	Measles Vaccination in the Presence or Absence of Maternal Measles Antibody: Impact on Child Survival	Childhood survival was better for children who received MV in presence of maternal antibody than for those who had no detectable maternal antibody, and better than for those who received only a single MV at 9 months of age
Berendsen et al, 2019 (52)	Maternal Priming: Bacillus Calmette-Guérin (BCG) Vaccine Scarring in Mothers Enhances the Survival of Their Child with a BCG Vaccine Scar	Maternal BCG priming might be important for the effect of BCG vaccination on child survival. Ensuring better BCG vaccine scarring among mothers and children could have a considerable effect on child mortality levels

07.07 Reproductive Realities of Women in Guinea-Bissau

In Guinea-Bissau, early marriage and pregnancy are common (59). Motherhood is not only seen as a personal choice but as a deeply rooted social and cultural obligation (59–61). In many communities, women who have not had children by the age of 30 face significant family and societal pressure, often being questioned or stigmatized.

Women's reproductive health in Guinea-Bissau is shaped by a complex interplay of poverty, illiteracy, cultural norms, and limited access to quality

healthcare. Women often lack autonomy in health-related decisions, relying on their husbands due to entrenched gender roles. The burden of domestic responsibilities remains heavy, even during pregnancy (**Figure 5**). These factors contribute to poor pregnancy outcomes (59).



A - Women selling at the central market — this represents the main economic activity for many women in urban areas (In the markets of Bissau, Photo: DW/L. Danso).

B - Pregnant woman preparing to fish in the river — a common activity among women living in rural and peri-urban areas.

C - Women simultaneously caring for the household and children — reflecting the multiple roles women often assume in daily life.

Figure 5: Women engaged in work, including those who are already pregnant, Guinea-Bissau

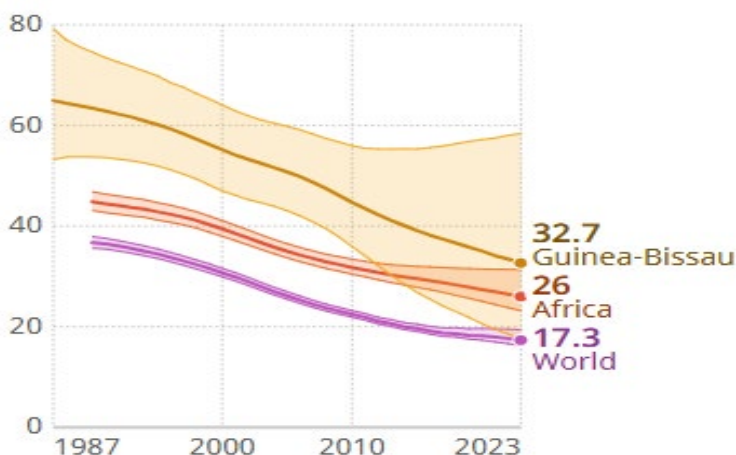
07.08 Abortion and Perinatal Mortality

In Guinea-Bissau, high rates of pregnancy loss and perinatal mortality have shaped cultural practices around pregnancy. *“Many women avoid disclosing early pregnancies or preparing for childbirth due to fear of loss. These norms such as not naming the fetus or buying baby items before birth, reflect a collective awareness of pregnancy fragility and serve as emotional protection against repeated losses.”*

Miscarriage is the most common complication of pregnancy, affecting approximately one in four pregnant women. The rate of clinically recognized miscarriages ranges from 12% to 15% in the general population, although this number represents only a fraction of total reproductive losses (62).

Perinatal mortality and abortion cases continue to be sensitive and often silenced topics (63). Perinatal mortality, which includes late foetal deaths (from the 22nd week of gestation) and early neonatal deaths (up to the seventh day of life), is a key indicator of the quality of care provided during prenatal, delivery, and immediate neonatal periods (62,63).

The limited quality of health data in Guinea-Bissau poses a significant challenge to accurately understanding perinatal mortality and stillbirths. Even so, available estimates paint an alarming picture (stillbirth: 30/1,000 births; neonatal mortality: 34/1,000 live births) (**Figure 6**) (5,64).



Source: World Health Organization Data. Global report Published 21 May 2024

Figure 6: Neonatal mortality, Guinea-Bissau

07.09 Public health events and their impacts on pregnancy in Guinea-Bissau

Pregnancy in low-income settings, such as Guinea-Bissau, is often marked by additional challenges related to public health pathologies (65). These conditions not only affect maternal health but also compromise fetal development and neonatal outcomes.

07.09.09 Obstetric haemorrhage

West Africa ranks first in the proportion of obstetric haemorrhage, with a prevalence of 31.3%. Obstetric haemorrhage is the leading category of maternal complications and a major contributor to maternal and neonatal mortality across Africa (65). In Guinea-Bissau, limited resources hinder the capacity to mitigate

these complications, with national availability of blood transfusion services averaging only 9% (66)

07.09.10 Hypertension and Pre-eclampsia

Hypertensive disorders of pregnancy, particularly pre-eclampsia and eclampsia, are among the leading causes of maternal and perinatal mortality worldwide (67).

Pre-eclampsia affects up to 8% of pregnancies worldwide and is strongly linked to complications such as sustained postpartum hypertension, cardiovascular disease, preterm birth, and neonatal death (68). Hypertension and pre-eclampsia are major contributors to maternal and neonatal morbidity and mortality in Africa, including Guinea-Bissau (65,69).

07.09.11 Malaria

According to the World Malaria Report 2025, the prevalence of malaria exposure during pregnancy in 2024 was highest in West Africa, where approximately 6 million women and adolescent girls representing 38% of an estimated 15.8 million pregnancies were affected by malaria infections (70).

In 2024, a total of 52,263 long-lasting insecticidal nets (LLINs) were distributed to pregnant women during their first antenatal care visit, achieving a coverage rate of 98%.

Regarding intermittent preventive treatment, 31,602 pregnant women received the third dose of sulfadoxine-pyrimethamine (IPTp3), corresponding to a coverage of 59%, which remained below the established target of 64%.

In terms of case management and diagnosis, a total of 98,392 malaria cases were confirmed, of which 96,869 (98.5%) were treated in accordance with the National Program guidelines. Malaria accounted for 12% of all cases attended nationwide, with an incidence rate of 40 per 1,000 inhabitants. The case fatality rate was 1.2%, while the mortality rate reached 5.4 per 100,000 inhabitants (71,72).

07.09.12 Anemia and Malnutrition

Malnutrition is one of the main determinants of anemia, a condition that significantly affects maternal and fetal health. In contexts of high nutritional

vulnerability, such as in many African countries and particularly in Guinea-Bissau, anemia during pregnancy represents a serious public health problem (73). Maternal anemia has been consistently linked to adverse obstetric outcomes, such as heightened risk of intrapartum hemorrhage, preterm birth, low birth weight, and increased neonatal mortality (74).

Anemia is acknowledged as a major contributor to maternal mortality across Africa. In Guinea-Bissau, the prevalence of anemia in women of reproductive age is estimated to be 51% (75,76). Although Guinea-Bissau has implemented a national program offering free ferrous sulfate and folic acid supplementation during antenatal care, both coverage and adherence remain suboptimal. Late initiation of prenatal visits and inadequate supplementation significantly diminish the effectiveness of this intervention (77).

07.09.13 HIV/AIDS

Maternal deaths among women living with HIV may arise from the aggravating effects of pregnancy on the progression of HIV infection. In these circumstances, the interaction between pregnancy and HIV is considered the underlying cause of death, complicating pregnancy, childbirth and the puerperium (67)

Guinea-Bissau has one of the highest HIV prevalence rates in West Africa, with approximately 5.3% of the reproductive-age population living with HIV, making it the second highest in the region. Women account for more than 70% of all HIV cases, highlighting the disproportionate burden on maternal health (78).

07.09.14 Challenges in Prenatal Care and Health Infrastructure

Maternal and neonatal health in Guinea-Bissau remains one of the country's most pressing public health challenges. It is characterized by fragile hospital infrastructure, a shortage of qualified professionals, and limited coverage of prenatal care services (66). Evidence indicates that less than half of pregnant women attend the minimum number of antenatal consultations recommended by the World Health Organization (79).

07.10 Knowledge Gaps and Rationale for the Study

Despite promising evidence, significant gaps remain in our understanding of the non-specific effects (NSEs) of vaccines in adult women and their impact on maternal and child health.

It is well known that vaccination in women of reproductive age may boost the transfer of passive immunity to their children. New data suggests a promising potential for enhancing beneficial non-specific effects of live vaccines BCG and MV in children by priming their mother with the same vaccines.

BCG and MV are well-known childhood vaccines and have mainly been studied as such. This study aims to address these gaps by generating data on the safety, immunogenicity, and impact of BCG and MV in women of reproductive age in Guinea-Bissau.

08 Objectives

To investigate the specific and non-specific effects of pre-pregnancy MV and BCG administered to women of reproductive age in Guinea-Bissau, with a focus on adverse events, immunological outcomes and impacts on maternal and neonatal health.

Article I: To describe and compare the occurrence of adverse events after MV or BCG vaccination in women of childbearing age.

Article II: To assess measles antibody levels in women of childbearing age before and after vaccination with MV and BCG.

Article III: To analyse the effects of MV and BCG vaccination on pregnancy outcomes, such as miscarriage, stillbirth, and early neonatal mortality.

09 Methodological considerations

09.01 Study Design

The three studies in the present PhD thesis were conducted within an ongoing randomised clinical trial, with the goal of allocating at least 2,400 women 1:1:1 to MV, BCG or placebo. The inclusion criteria were HIV-negative, non-pregnant women of childbearing age (15 to 35 years), who had already completed one to three pregnancies, with the last pregnancy ending at least 12 months ago (or less if the last child had died). These criteria were determined to avoid vaccinating pregnant or HIV positive women as there are contraindications for MV/BCG vaccinations in these populations.

09.02 Population and Sampling

Eligible women were identified through the Health and Demographic Surveillance System (HDSS) of the Bandim Health Project (BHP). They received a home visit in the morning from a trial assistant, who explained the study. If interested, women were invited to a nearby health centre, where they received detailed information about the study's purpose, potential risks, and benefits, including free medical consultations and basic medications. They were informed about the HIV and pregnancy tests required prior to inclusion, and assured that participation was voluntary, would not affect their relationship with the health staff, and that they could withdraw consent at any time. After oral and written informed consent procedures, the women were then tested for HIV and pregnancy and screened for any illness or contraindications to receiving a livevaccine. Those eligible were subsequently randomised using REDCap in blocks of four or six and vaccinated according to their allocation.

The study was conducted within covering six urban districts and approximately 100,000 inhabitants. All women of reproductive age living in this area are

monitored through monthly home visits to identify new pregnancies, and each pregnancy receives a unique identification number for longitudinal follow-up. Based on current HDSS census data, the annual incidence of new deliveries among these women is around 15%. Therefore, from a cohort of 2,400 enrolled women, more than 300 births are expected to occur between the 5th and 10th trimesters after enrollment.

For the maternal–child components of the study, the first pregnant women were consecutively invited, to ensure the enrolment of 100 children from each group in the child-part of the trial for a total of 300 children.

09.01 Main definitions

09.01.01 Article I

An adverse event after vaccination is defined as any unwanted medical occurrence after vaccination that does not necessarily have a causal relationship with the use of a vaccine (80).

Adverse Event (AEs) are classified into two categories:

Serious Adverse Event (AEs): includes cases requiring hospitalisation (or its extension), causing permanent disability, congenital anomalies, life-threatening conditions, or resulting in death.

Non-serious Adverse Event: any event that does not meet the criteria for seriousness (81).

Local adverse events: include reactions at the site of vaccination such as burning sensation, erythema, induration, and, in some cases, nodules or ulcers. For BCG, this may also involve abscesses and regional lymphadenopathy (32,44).

Systemic adverse events: refer to broader reactions such as fever, headache, conjunctivitis, rash, and hypersensitivity. In the case of BCG, systemic effects may include persistent fever, hepatosplenomegaly, respiratory symptoms, and poor weight gain (32,44).

09.01.02 Study II

To better understand the immunological basis underlying involved in study II, this section provides a concise overview of the immune system and its key components.

An antigen is any substance foreign to the body that triggers the production of **antibodies** - proteins produced by B lymphocytes that specifically recognize and neutralize antigens (82).

Types of immunity

Immunity is broadly categorized into:

Innate Immunity: The body's first line of defence, acting rapidly and non-specifically and **adaptive Immunity:** A more specific response that develops after antigen exposure (82–84).

Immune system cells

Specialized cells that work together to defend the body against infections, foreign substances, and abnormal cells. They are primarily divided into two categories: **B Lymphocytes** - responsible for humoral immunity through antibody production. **T Lymphocytes** - include helper T cells (CD4⁺), which coordinate immune responses, and cytotoxic T cells (CD8⁺) (82–84).

Vaccines and Antibody Seroprevalence

Vaccines are biological preparations that mimic infection to activate the immune system. They contain antigens that stimulate antibody production and immune memory, offering protection without causing disease. (85). **Antibody seroprevalence** refers to the proportion of individuals in a population with detectable antibodies against a specific pathogen in their blood serum (86).

Immune response refers to the set of coordinated reactions carried out by the immune system in response to the presence of an antigen (83).

Immunological interference/immune blunting refers to the alteration of an immune response to a vaccine or infection due to the presence of another pre-existing or concurrent immune response (87).

09.01.03 Study III

The incidence of pregnancy

Refers to the number of new pregnancies occurring in a specific population during a defined time period, typically expressed per 1,000 women of reproductive age. It includes all pregnancy outcomes: live births, abortions, and stillbirth

Stillbirth (or intrauterine fetal death)

Babies born with no signs of life after 24 completed weeks of gestation. In contrast, **miscarriage** is defined as the termination of pregnancy before 24 weeks (62,88,89).

Neonatal death

The death of a live-born infant within the first 28 days of life. It is further categorized into **early neonatal death** (occurring within the first 7 days) and **late neonatal death** (occurring from day 8 to day 27) (38).

09.02 Interventions

The interventions consisted of administering measles vaccine (MV), BCG vaccine, or placebo (saline) to randomised women of reproductive age. All vaccines were administered according to international guidelines and manufacturer instructions. Apart from these interventions, no other medication

was provided or administered by our study team, either at enrolment or during the different phases of follow-up, including throughout pregnancy.

MV:

The MV used was a live-attenuated virus vaccine. It was administered as a 0.5 mL dose via subcutaneous injection (90).

BCG Vaccine:

The BCG vaccine used, a live attenuated preparation of *Mycobacterium bovis*. The vaccine was administered intradermally, in the right upper arm (deltoid region). It was administered as a 0.1 mL dose (91).

Placebo:

Participants in the control group received 0.5 mL of sterile saline solution via the same route of administration as the MV to ensure blinding.

Vaccinations were registered, and participants were advised not to receive any other vaccines within the following four weeks. Additionally, women who had received a COVID-19 vaccine within the past 14 days were not eligible for enrolment.

09.03 Data Collection

The Bandim Health Project (BHP) carries out continuous monitoring of women of childbearing age in the study area, with the aim of identifying new pregnancies and births. For this study, baseline data including reproductive history and participant identification were extracted from the Bandim Health Project (BHP) database.

Prior to inclusion, each participant underwent a detailed evaluation. General and update information was collected on clinical history (assessed by a physician), recent vaccination status, obstetric history, and social and demographic characteristics. This information was obtained through interviews conducted by trained field assistants. After inclusion in the study, participants were followed up through regular telephone contacts. Initially, these calls were made every two months, but the frequency was later adjusted to every four months to optimize follow-up procedures. During follow-up, information on morbidity and

mortality, medication use, and pregnancy-related events occurring after inclusion were updated (**Table 6**).

Table 6: Data sources and structure

	Data origin	Data collection
Baseline data	BHP data and interview during inclusion	Existing HDSS data and in-person information from time of enrolment at the health centres
Study I (AEs)	Data from the interviews in a series of 4 scheduled visits	Face-to-face at home
Study II (Immunity)	Sample collected during inclusion, at 4 weeks and in third trimester of pregnancy	Plasma separated from whole blood sample
Study III (Pregnancy outcome)	Data from the woman	Telephone and face-to-face information in homes

09.03.04 Study I Adverse event

After vaccination, participants were observed for 15 minutes to identify possible immediate adverse reactions. Subsequent follow-up was conducted through home visits by field assistants on days 7, 14, 28, and 90 after randomisation, to record symptoms and adverse events (**Figure 7**). If the first visit was unsuccessful, a second attempt was made. If both visits failed, follow-up was conducted by telephone.

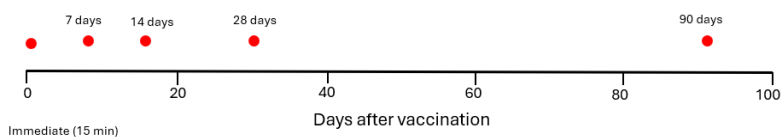


Figure 7: Follow-up timeline for adverse events monitoring

In accordance with the requirements of the ethics committee, the substudy initially included the first 100 women enrolled in the main trial in each group. However, this number was slightly exceeded to compensate for potential loss to

follow-up. A total of 122 women per randomisation group were ultimately included in the analysis.

09.03.05 Study II Immune response

Blood samples (3 mL) were collected from all participants at baseline, prior to study enrolment. A subgroup of women was consented to have an additional blood sample collected 4 weeks after enrolment. Among those who became pregnant, we attempted to collect samples in the third trimester of pregnancy (Figure 8).

The immunological study arm was composed of the first women enrolled who had available samples. Many of the women who were enrolled in the adverse-event substudy, were also included in the immunological analyses because they were among the earliest participants with samples available. The first 30 women per group were designated for analysis of the vaccine response and had a blood sample obtained at 4 weeks post-randomisation. Thus, the immunological study population was defined by the earliest participants with accessible samples, following the same enrolment sequence.

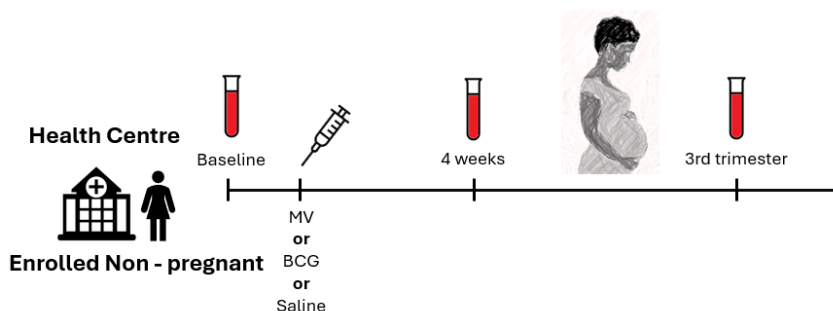


Figure 8: Enrolment of non-pregnant women with blood sampling to assess vaccine-induced immune responses.

The blood was centrifuged at 700 g for 10 minutes at room temperature to separate the plasma, which was stored in cryovials at -20°C. Two 1,000 µL aliquots of plasma and one of plasma-free whole blood were obtained for epigenetic analyses. The plasma samples were transported under controlled temperature

to the European Plotkin Institute of Vaccinology (EPIV-ULB) in Brussels and stored at -80°C.

Antigen-specific IgG detection was performed at EPIV-ULB using a Luminex-based multiplex assay. Measles-related antigens, BCG, and CMV (control) were coupled to MagPlex® microspheres (**Table 7**).

Serum samples were diluted and incubated with the microspheres for 2 hours, followed by incubation with PE-labelled anti-human IgG antibody.

Readings were performed on a Bio-Plex 200 reader, with results expressed as mean fluorescence intensity (MFI). Standard curves and controls were included to ensure assay reproducibility and sensitivity.

Table 7: Antigens used in serological evaluation

Antigen	Pathogen	Purpose
Measles virus nucleoprotein	Measles virus	Associated with viral encapsidation
Measles virus lysate	Measles virus	Measles bulk antigens
<i>M. bovis</i> lysate	<i>M. bovis</i>	<i>M. bovis</i> (BCG) bulk antigens
CMV gB	Cytomegalovirus	Non-vaccine antigen (control antigen)

09.03.06 Article III Pregnancy outcomes

In Bissau, all women have access to a telephone, either because they themselves have a phone or through a close relative or neighbour. This facilitated communication and follow-up.

When a pregnancy was reported during the telephone follow-up or come through the HDSS data collection, a field assistant conducted a home visit to confirm the pregnancy and record relevant information. After confirmation, follow-up visits were scheduled to monitor the pregnancy's progress and update the participant's clinical status.

Most women received two or three visits during the pregnancy, depending on the gestational age at the time of registration. Gestational age was estimated based on multiple data sources, prioritizing those with the greatest reliability and availability, such as the date of the last menstrual period, uterine fundal height,

clinical records, and, when available, ultrasound scans. In some cases, however, pregnancy was only identified retrospectively, after the follow-up period had ended. Women were followed in the following schemes (**Figure 9**):

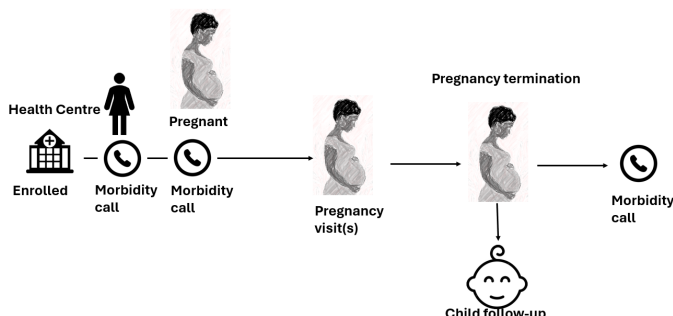


Figure 9: Pregnancy and child follow-up process

09.04 Statistical analysis

09.04.07 Baseline characteristics

Data analysis was conducted using Stata software. Baseline characteristics (**Table 8**) were analysed separately for all enrolled women, for those with confirmed pregnancies, and for women reporting adverse events (AEs).

Table 8: Variable of baseline characteristics

Category	Variable
Sociodemographic characteristics	Mean age at enrolment
	Woman lives together with husband
	Enrolled during rainy season (June–November)
	Mean Body Mass Index (BMI)
Pregnancy history	Number of previous births
	– One birth
	– Two births
	– Three or more births
	Last child born had died/stillbirth
	– Last child born had died
Vaccination history	– Last child was stillborn
	Mean time since last pregnancy (months)
	Has at least one BCG scar
Health-related risk factors	Recent vaccination (last 4 weeks)
	Chronic disease
	Medical consultation in the last three month

09.04.08 Study I Adverse event

Symptoms were analysed based on the number and proportion of women who reported them, stratified by randomisation group. Comparisons between the groups (MV vs. placebo and BCG vs. placebo) were performed for all follow-up and by follow-up time (15 minutes after vaccination and 7, 14, 28, and 90 days after enrolment). In the analysis of all follow-up interviews, each participant contributed only with one observation. No corrections were applied for multiple testing.

09.04.09 Study II Immune response

Individual baseline variables associated with antibody changes were identified in univariable analyses, and variables with $p \leq 0.05$ were included in multiple regression models to identify independent predictors of increased or decreased in immune response.

Logarithmic mean antibody titres were compared between the randomisation groups (BCG, MV, and placebo) at baseline, four weeks, and third trimester, using the Kruskal-Wallis rank sum test. Changes in titres over time were analysed by linear regression, adjusted for age and baseline values, for each antigen. Seroconversion was defined as a two-fold or more increase in titres and the seroconversion was compared between randomisation group in logistic regression models. A two-fold conversion was used mainly because very few women reached the standard four-fold conversion in antibody levels (92).

09.04.10 Study III Pregnancy outcomes

To ensure a clear temporal relationship between exposure and outcome, we focused exclusively on the first pregnancy occurring after enrolment in the present study.

Pregnancy incidence was calculated per 100 person-years and compared between groups using Cox regression (HR, 95% CI), with results shown overall and by follow-up year. Kaplan-Meier curve mainly used to illustrate the differences in pregnancy between randomisation groups over time.

Rates of early neonatal death and failed pregnancy (stillbirth + miscarriage) were calculated per 1,000 pregnancies using RR from binomial regression (95% confidence interval) for comparisons between groups. Since risk of adverse

pregnancy outcomes are significantly higher among twins, analyses were also stratified by twinning status.

09.05 Ethical Considerations

The trial was approved by the National Health Research Ethics Committee of Guinea-Bissau, reference number [043/CNES/INASA/2019](#), and the trial was pre-registered in the ClinicalTrials.gov with the ID [NCT04899765](#).

All participants were duly informed about the objectives, methods, risks and benefits of the study. Informed consent was obtained in writing, in accessible language and in the local language, ensuring that participants fully understand their voluntary participation.

All data collected was treated with strict confidentiality. Personal information was stored securely, ensuring the anonymity of the participants. Data entry and management were conducted using REDCap, a secure, web-based platform. Field assistants used password-protected tablets and computers to collect and store data, further ensuring data protection and restricted access.

10 Results

10.01 Overview

Figure 10, presents a consolidated overview of the key findings from the different studies included in this thesis.

Study	Exposure	Outocome	N	Point of comparison	Main change		P value / HR 95%(Cis)
I	Adverse events	Adverse events after vaccination reported following vaccination	363	Overall any symptoms 90 day follow-up. %(n), chi-squared	Proportion (%) of (events/total women)		
					any symptoms	Placebo 27.3 (33/121)	ref.
						MV 35.5 (43/121)	0.17
						BCG 33.9 (41/121)	0.26
II	Immunological response	Baseline seroprevalence and post-vaccination dynamics of Measles and BCG antibodies	462	Fold chang	Mean change in Log10 titer (95% CI); Mean fold change in GMT (95% CI)		
					Measles lysate	change from Baseline to four week MV: 0.30 (0.18 to 0.42); 3.84 (2.22 to 5.47)	(0.001)
					M. bovis lysate	change from Baseline to four week BCG: 0.08 (0.04 to 0.12); 1.48 (1.27 to 1.69)	(0.001)
III	Pregnants	Pregnancy Incidence and outcomes among randomized groups	2781	Incidence - hazard ratio (HR) estimated by the Cox model	Incidence rate per 100 years (events / total days) [total women]		
					Incidence of pregnancy	Placebo: 16.2 (363/815938) [931]	ref.
						MV: 16.5 (363/804120) [930]	1.01 (0.87-1.17)
						BCG: 14.9 (336/825249) [920]	0.91 (0.79-1.06)
			997	RR from Binomial regression	Incidence rate per 1000 pregnancies with event (events / total women (pregnancy end))		
					Failed pregnancy	Placebo: 74 (25/338)	ref.
						MV: 83 (28/338)	1.12 (0.67-1.88)
						BCG: 78 (25/321)	1.05 (0.62-1.79)
			940	Incidence of children dead (within the first 7 days)	Incidence rate per 1000 children (events / total days) [total children]		
					Neonatal death	Placebo: 25.0 (8/2192) [320]	ref.
						MV: 31.3 (10/2165) [319]	1.26 (0.50-3.19)
						BCG: 53.2 (16/2025) [301]	2.15 (0.92-5.00)

Figure 10: Main results in the included studies

10.01 Baseline characteristics

The baseline characteristics among participants in the overarching randomised trial were well balanced between the randomisation arms. The mean age was 27 years (interquartile range: 23–30 years). Most women lived with their husbands: Placebo (63.9%), MV (63.4%), and BCG (61.6%). Regarding reproductive history, approximately half of the participants had one previous birth, while a third reported two births.

Most participants had at least one BCG scar (74%) (Table 1 of the study 3).

10.01 Article I (Adverse events after vaccinations)

After 90 days of follow-up, the only adverse manifestation that stood out significantly was the skin reaction in the BCG group, which was entirely anticipated as BCG is known to give a skin reaction and a scar following a correctly applied vaccination. While the Placebo and MV groups presented low frequencies of skin reactions 7.4% (n=9) and 6.6% (n=8), respectively, the BCG group registered a markedly higher incidence, with 96.7% (n=117) of participants reporting skin reactions. This difference was statistically significant ($p<0.01$) (**Table 9**).

Headache was more frequently reported within the first 7 days after vaccination among women who received either BCG or MV, compared to those in the placebo group. The prevalence was 2.5% (3/120) in the placebo group, 8.3% (10/121) in the MV group, and 9.2% (11/120) in the BCG group, with p-values of 0.08 and 0.05, respectively, when compared with placebo (Table 3 of the study 1).

During the first 14 days of follow-up, the proportion of women reporting any symptom tended to be higher in the groups that received BCG or MV. This was particularly due to the BCG skin reaction. Among those vaccinated with BCG, the development of skin reactions was particularly notable, with a progressive increase in prevalence over time: 38% at day 7 (46/120), 59% at day 14 (71/121), 79% at day 28 (92/118), and reaching 97% by day 90 (113/117), as shown in Figure 2 of Study 1.

Table 9: Occurrence of adverse reactions reported during follow-up after inclusion by follow-up by randomisation group.

	Placebo	MV	BCG	MV vs placebo	BCG vs placebo
All follow-up	(N=470)	(N=468)	(N=476)	p-value	p-value
Local Manifestations (Variable % (n))					
Local or regional pain	0.8 (1)	2.5 (3)	2.5 (3)	0.62	0.62
Skin reactions	7.4 (9)	6.6 (8)	96.7 (117)	0.80	0.00
Lymph node inflammation	3.3 (4)	5.0 (6)	2.5 (3)	0.75	1.00
Systemic Manifestations (Variable % (n))					
Fever	6.6 (8)	11.6 (14)	6.6 (8)	0.18	1.00
Body pain	2.5 (3)	2.5 (3)	4.1 (5)	1.00	0.72
Acute gastrointestinal disease	2.5 (3)	4.1 (5)	2.5 (3)	0.72	1.00
Headache	8.3 (10)	15.7 (19)	14.0 (17)	0.07	0.15
Eye irritation	3.3 (4)	5.0 (6)	2.5 (3)	0.75	1.00
Skin irritation or inflammation	0.0 (0)	1.7 (2)	0.8 (1)	0.50	1.00
Respiratory illness	9.1 (11)	5.8 (7)	9.1 (11)	0.33	1.00
Medical consultation	3.3 (4)	4.1 (5)	1.7 (2)	1.00	0.68

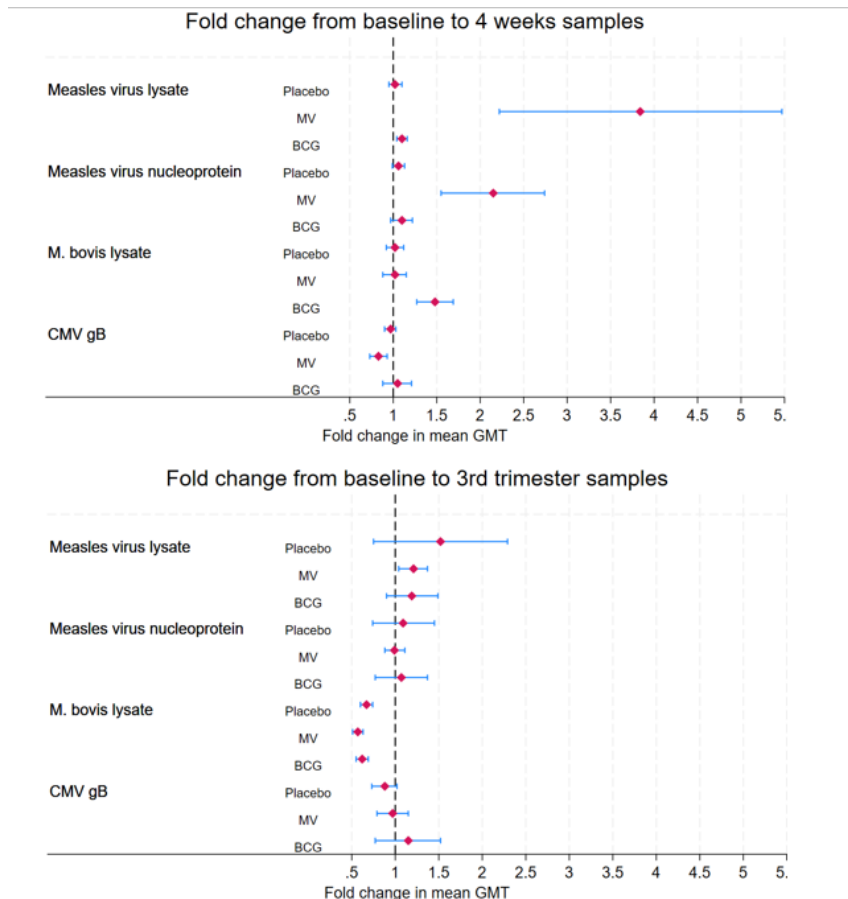
10.02 Article II (Humoral immune responses to measles and Bacillus Calmette-Guérin)

The Baseline log₁₀ antibody titres were similar across all three study groups for each antigen evaluated (Table 2 of study 2). There were no strong determinants of baseline antibody levels against measles lysate and *M. bovis* lysate (Supplementary tables 1 and 2 of Study 2).

In Figure 11, among the antigens evaluated, the most notable immune response was observed for the measles lysate antigen. Participants in the MV group showed an increase in antibody titres from baseline to 4 weeks post-intervention, with a fold change of 3.84 (2.22–5.47) in geometric mean titres (GMT). In contrast, the Placebo group showed a fold change of 1.02 (0.95–1.10), and the BCG group 1.10 (0.97–1.22), indicating natural variation (Figure 11).

For the *Mycobacterium bovis* lysate antigen, immune responses were low in the Placebo 1.02, (0.92–1.12) and MV 1.02, 0.88–1.15) groups. However, the BCG group showed an increase, with a fold change of 1.48 (1.27–1.69) (Figure 11). These responses, however, did not persist into the third trimester of pregnancy, particularly for the *M. bovis* lysate antigen, where titres returned closer to

baseline in all groups. By contrast, for measles-related antigens, a modest but non-significant increase was observed in both the MV and the non-MV groups (Figure 11). It reached statistical significance for the measles virus lysate.



Abbreviations: BCG, Bacille Calmette-Guérin; CI, Confidence Interval; CMV gB, Cytomegalovirus; MV, Measles vaccine

Figure 11: Antigen analysis panel by randomisation Groups: Fold change from baseline to 4 Weeks and third trimester of pregnancy.

Participants with high baseline antibody levels showed reduced booster immune responses four weeks after vaccination, significant for MV (measles lysate, RR 0.44, (0.28–0.69)) and a non-significant trend for BCG (*M. bovis* lysate, RR 0.68, (0.45–1.02; $p=0.065$) (table 3 and 4 of study 2).

10.03 Article III Pregnancy Outcome

Over the four-year follow-up period, 38.1% (1062/2,781) of women became pregnant. **Figure 12** presents the cumulative estimate of pregnancy incidence based on Kaplan-Meier analysis, revealing nearly overlapping trajectories among the three randomisation groups.

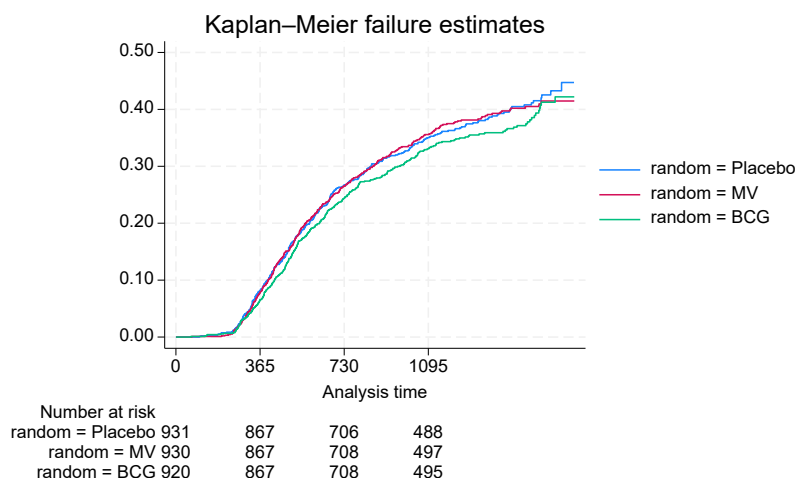


Figure 12: Time to pregnancy after enrolment in trial by randomisation groups

Tables 3 and 4 of study 3 summarises the main pregnancy outcomes across the randomised groups, focusing in pregnancy incidence, on early neonatal death and pregnancy failure (including miscarriage and stillbirth). Early neonatal death rates were comparable between the Placebo (25.0 per 1,000 live births) and MV (31.3 per 1,000) groups, while the BCG group showed a higher rate (53.2 per 1,000). However, these differences were not statistically significant, with risk ratios of 1.26 (0.50-3.19) for MV versus placebo and 2.15 (0.92-5.00) for BCG versus Placebo.

Regarding pregnancy failure, slightly higher proportions were observed in the placebo (74 per 1,000 pregnancies), MV (83 per 1,000) and BCG (78 per 1000). No statistically significant differences were found: RR for MV vs Placebo = 1.12 (0.67-1.88); RR for BCG vs Placebo = 1.05 (0.62-1.79).

Pregnancy incidence, failed pregnancies, and early neonatal deaths fluctuated across follow-up years, no consistent trend was observed (Table 5 of study 3).

11 Discussion

11.01 Main results

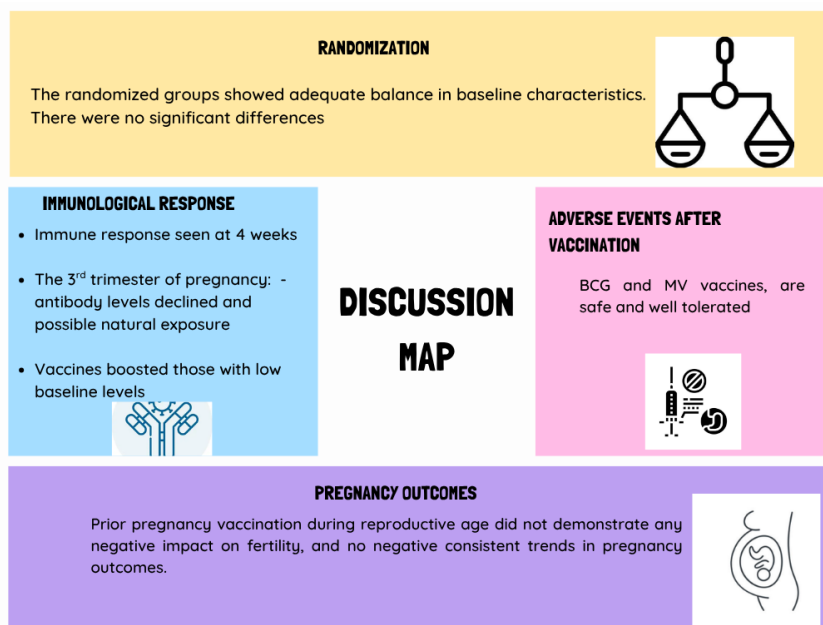


Figure 13: Overview: evaluation of safety, immunological response, and pregnancy outcomes.

There were no significant differences in sociodemographic characteristics, obstetric history, previous vaccination status, or initial immunological levels for the antigens tested, among women included in the randomised groups. These results indicate that randomisation was effective, ensuring comparability between groups and strengthening the internal validity of the study.

The results of this study indicate that the BCG and MV vaccines, when administered in adults, are safe and well tolerated, with a very low incidence of adverse events within three months.

In both MV and BCG vaccination groups, a measurable immune response was observed, evidenced by increased antibody levels against measles lysate and *M. bovis* lysate 4 weeks post-vaccination, respectively, confirming the immunogenic capacity of the evaluated vaccines. The vaccines primarily boosted immune responses in those with low antibody levels at baseline. The effects were not maintained in pregnancy, at an average of 16 months post-vaccination.

The third trimester of pregnancy was characterised by a natural decline in antibody levels across groups compared with the response four weeks post-vaccination. However, there was still a significant increase in the response to measles lysate in the MV group. Pregnancy seemed to affect the three antibody types differently, with larger declines in the antibody response to BCG than to MV and control antigens.

Pre-pregnancy vaccination in women of reproductive age did not demonstrate influence on fertility or pregnancy outcomes, reinforcing the feasibility of these vaccine in adult, but a signal of elevated risk in early neonatal mortality in the BCG group merits additional follow-up.

11.01 Strengths and limitations

Strengths

One of the main strengths of the study is its rigorous design: all trials used randomised, placebo-controlled methods with well-balanced group allocation. This approach helped reduce selection bias and strengthened the internal validity of the findings.

The studies achieved high retention rates of participants included in the analysis after randomisation, with minimal and balanced losses to follow-up, further supporting the robustness of the findings.

The use of a multiplex Luminex assay provided high analytical sensitivity and reproducibility, supported by international quality control standards.

The inclusion of control antigens such as cytomegalovirus glycoprotein B (CMV gB) enabled assessment of baseline immune comparability and vaccine-specific responses.

The study populations consisted of parous women, which may have enhanced the reliability of self-reported pregnancy related outcomes due to prior experience with pregnancy and childbirth.

The monitoring system in this clinical trial allowed for early identification of pregnancies and their outcomes, contributing to a more complete dataset on pregnancy failures than is commonly captured in routine reporting.

Weaknesses

Despite these strengths, several limitations must be acknowledged. The generalisability of the findings is constrained by the study setting a single urban area in Guinea-Bissau and the specific population studied (young, healthy, parous women). These factors limit extrapolation to broader populations, including nulliparous women and men.

Furthermore, pregnancy outcomes and time-to-pregnancy data were primarily self-reported, often without clinical confirmation (e.g., ultrasound), introducing the possibility of recall bias (article III). Also, results are preliminary as the data collection is still ongoing.

Additionally, the absence of a formal sample size calculation and power estimation specifically for the outcomes of this thesis constitutes a methodological limitation.

11.02 Consistency with previous studies

The findings from this research align with the literature on the immunological and clinical effects of measles (MV) and Bacillus Calmette-Guérin (BCG) vaccination.

Regarding adverse events, the safety profile observed in this study corroborates findings from children. BCG and MV have been part of childhood routine immunisation programs in low-income countries for decades and are generally well known among local communities (33). As in previous studies, the most common complaints reported following BCG vaccination is skin reactions at the injection site (93–95), which did not interfere with daily activities, and no serious adverse events were reported in this adult female population.

Similarly, MV was associated with very few adverse events, with headache being the only symptom that showed a slight increase consistent with prior reports from children (96–98).

In the immunogenicity study, the observed increase in antibody levels four weeks after MV and BCG administration is consistent with previous reports demonstrating an increase in humoral responses following vaccination (99–103), followed by possibility of a gradual decline (101,99), particularly during late pregnancy (56,104,105). The reduced response in individuals with high baseline antibody levels aligns with prior findings indicating that pre-existing immunity may attenuate booster-induced activation in adults (106–108).

The pregnancy outcomes study also aligns with existing literature on maternal and perinatal health in West Africa (5,109). Pregnancy loss remains a significant public health concern in the region, with intrauterine deaths often underreported compared to neonatal deaths (63).

11.03 Interpretation and implications

We observed consistency in relation to the main known adverse events (AEs) of BCG and MV, the reported complaints being moderate, tolerable and transient. The only non-transient observation was the skin reactions for the BCG group in all stages of follow-up, which was expected and had been explained to the trial participants at enrolment. Therefore, this study indicates that the vaccines can be used among adults.

The absence of statistically significant differences in baseline antibody levels between groups, together with the lack of variation in the control antigen cytomegalovirus glycoprotein B (CMV gB), indicates that participants were immunologically balanced at the start of the study. Therefore, post-intervention outcomes can be interpreted as a direct result of the vaccine-induced immune responses.

The immunological dynamics during the third trimester of pregnancy appear to be influenced by several factors: (i) the natural decline in antibody levels due to the interval between study enrolment and the third trimester; (ii) biological modulation inherent to pregnancy, including haemodilution (110), immune system adjustments (111), and elevated levels of progesterone and estrogen (105,112); and (iii) natural infection exposure. The latter may explain the modest, non-significant increases in measles-related antibodies observed in non-MV groups.

Notably, after study initiation, small measles outbreaks occurred in the region, which were not fully documented by governmental authorities, reinforcing the likelihood of natural exposure (25).

Individuals with high baseline measles antibodies showed reduced booster responses, consistent with immune blunting from pre-existing immunity, common in settings like Guinea-Bissau, where vaccination rates are high and natural exposure also occurs (25).

This increase in antibody levels opens the possibility of vaccinating with MV and BCG before pregnancy. At the same time, it raises a question that may not be answered in this PhD thesis but will be studied in the full cohort: to which extent might these antibody levels be beneficial for the newborn, considering they are possibly influenced by the pregnancy itself and natural exposure.

Guinea-Bissau, like many countries in Sub-Saharan Africa, faces significant maternal health challenges, including malaria during pregnancy, pre-eclampsia and eclampsia (65), limited infrastructure and personnel for adequate antenatal care (79) among other conditions that contribute to one of the highest maternal and infant mortality rates worldwide (5,64). Within this context, our study shows that pre-pregnancy vaccination, during reproductive age, did not demonstrate any influence on fertility or pregnancy outcomes, though a signal in relation to BCG and early neonatal mortality will be followed up in the full cohort. It did, however, not show any consistent pattern with time since randomization, speaking against a biological effect.

This study was initiated during the COVID-19 pandemic, a period marked by widespread disinformation surrounding vaccines. Much of this misinformation focused on alleged negative effects on health, fertility, and pregnancy outcomes, particularly among women (113). In this context, the findings of this study contribute meaningfully to clarifying doubts within the community and reinforcing trust in some vaccination programs in adult.

The data presented here, obtained through close monitoring, probably reflects the reality more accurately, highlighting the importance of closer surveillance as a tool to improve the quality and reliability of estimates.

12 Conclusion

12.01 Key Findings

The MV and BCG vaccination in women were well tolerated, with few mild and transient adverse events. No serious adverse events or hospitalisations were observed study subset.

Both vaccines induced detectable antigen-specific antibody responses four weeks after vaccination, but effects waned by the third trimester, except from a modest effect of MV on measles lysate antibody. Antibody levels appear to have been influenced by natural waning over time, natural exposure to measles virus, and pregnancy-related immunological modulation.

It was not possible to observe any influence of pre-pregnancy BCG or MV vaccination during reproductive age, on fertility or pregnancy outcomes. The trends observed were based on small numbers and were inconsistent, limiting the strength of the conclusions

12.02 Thesis Contributions

The findings largely reinforce the short-term safety and efficacy of MV and BCG vaccines administered in adults. Vaccination in adulthood may be influenced by pre-existing antibodies, reflecting the high endemicity of these diseases. Importantly, these results help dispel growing misinformation in Africa regarding fertility and pregnancy outcomes.

12.03 Suggestions for future research

Conduct longitudinal studies to monitor the long-term effects of pre-pregnancy vaccination in women of reproductive age on the health of mothers and children and across multiple pregnancies to assess long-term fertility and maternal and child outcomes.

Explore the feasibility of combining pre-pregnancy vaccination in women of reproductive age with other public health interventions, to maximize maternal and newborn health benefits.

Investigate antibody levels in umbilical cord blood at birth to better understand the final maternal antibody transfer and its potential protective effects for the newborn.

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14 Appendices

14.01 Paper I:

Silva I., Nielsen S., Aaby P., Marchant A., Kollmann T., Amenyogbe N., Benn CS., Scholtz-Buchholzer F. **Adverse events after measles and Bacillus Calmette-Guérin (BCG) vaccinations of women of fertile age in Guinea-Bissau. A nested study within an open-label placebo-controlled randomised trial** (Trop Med Int Health. 2025 Oct 15. doi: 10.1111/tmi.70052)

14.02 Paper II:

Silva I., Argondizo Correia C., Nielsen S., Aaby P., Metcalf-Clausen M., Scholtz-Buchholzer F., Kollmann T., Amenyogbe N., Benn CS., Marchant A. **Humoral Immune Responses to Measles Vaccination in Women of Reproductive Age: A Nested Study within a Randomised Trial in Guinea-Bissau** (Manuscript)

14.03 Paper III:

Silva I., Aaby P., Kollmann T., Amenyogbe N., Ca E. Scholtz-Buchholzer F., Benn CS., Nielsen S. **Impact of Pre-pregnancy BCG and Measles Vaccination on Pregnancy Outcomes: A Randomised Controlled Trial in Guinea-Bissau** (Manuscript)



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RESEARCH ARTICLE

Adverse Events After Measles and Bacillus Calmette-Guérin Vaccinations of Women of Fertile Age in Guinea-Bissau. A Nested Study Within an Open-Label Placebo-Controlled Randomised Trial

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ABSTRACT

Background: Within a randomised controlled trial providing Bacillus Calmette-Guérin, measles vaccine, or placebo to women of fertile age, we aimed to study the risk of adverse events following vaccination. This study addresses the lack of data on adverse reactions in adults, especially women of childbearing age, to inform broader vaccination policies.

Methods: The trial included HIV-negative, non-pregnant women aged 15 to 35 years, who had given birth to at least one child, and residing in the Bandim Health Project study area in the capital Bissau of Guinea-Bissau. Excluded were individuals who were frail, had serious illnesses like active tuberculosis, or had no prior births. Participants were randomised 1:1:1 to bacillus calmette-guérin, measles vaccine, or placebo. A subset of participants was enrolled into the adverse events study. Adverse events were registered immediately 15 min post-vaccination and at home visits 7, 14, 28 and 90 days after enrolment to register symptoms that could be related to the trial interventions. We compared symptom distributions by randomisation using chi-squared tests.

Results: Of the total 366 women included in the study, 99% (363) were eligible to follow up for adverse events (Placebo, 121; measles vaccine, 121; Bacillus Calmette-Guérin, 121) and 97% (1414/1452) of the planned study contacts were successful, 1.3% (18/1414) of which were mobile phone interviews. The baseline characteristics did not differ between the randomisation groups. There were no immediate reactions within the first 15 min post-vaccination. Seven days after vaccination, there was a tendency for more episodes of headache reported among both the Bacillus Calmette-Guérin and measles vaccine vaccinated participants compared to placebo. We registered 11 medical consultations (placebo: 4, measles vaccine: 5, Bacillus Calmette-Guérin: 2) and no deaths or hospitalisations during 90 days of post-enrolment follow-up.

Conclusion: Bacillus Calmette-Guérin and measles vaccine were safe and tolerable for adult women of childbearing age, with few short- and medium-term adverse events.

Sustainable Development Goal: Good Health and Wellbeing

1 | Introduction

The prevention of infectious diseases through vaccination is considered one of the greatest successes of public health. Vaccinations have prevented millions of deaths and disabilities throughout history, eradicating and controlling various diseases in the world [1, 2].

The idea that vaccines can have broader effects on the immune system and on overall health is becoming increasingly consolidated. Effects on the risk of other diseases that are not explained by preventing the disease targeted by the vaccine have been termed non-specific effects (NSEs) [3–5]. The live *Bacillus Calmette-Guerin* (BCG) and measles vaccine (MV) have been associated with beneficial NSEs, improving overall child survival in low-income settings [6–9].

The recommended timing of infant measles vaccination, at 9 months of age, was decided five decades ago, when practically all mothers would have had exposure to natural measles infection. The 9-month policy represents a trade-off between protecting infants as early as possible and avoiding interference with maternally transferred measles antibody with the infant's post-vaccination antibody response [8]. It is now clear that infants of vaccinated mothers are born with lower levels of maternal measles antibody, compared with infants of mothers who were exposed to measles infection; most infants now have no detectable maternal antibody by 20 weeks of age according to studies carried out in Guinea-Bissau [10, 11].

Intriguingly, recent studies indicate that for both MV and BCG, the beneficial NSEs are particularly strong if the vaccines are given to children of mothers who had been primed with the same antigen [11–14], or if the vaccine was a revaccination [15], suggesting that vaccination in the presence of pre-existing immunity, be it from the mother or previous vaccination, amplifies the beneficial NSEs.

If these hypotheses are confirmed, this information can be used to optimise the health of young children by making sure their mothers are vaccinated prior to pregnancy or by providing measles vaccination at an earlier age when the infant still has circulating maternal antibody. However, there is a lack of knowledge regarding the possible adverse reactions or discomfort that these vaccines can cause in adults since most of the known side effects and adverse reactions were assessed only for routine child vaccinations.

In Guinea-Bissau, healthcare resources are limited, and child mortality remains significantly above the global average [16, 17]. Between 2013 and 2023, the country reported an average of 36 measles cases annually. However, in 2022, this number rose sharply to approximately 200 cases, representing a substantial increase compared to the previous decade [18]. Additionally, the tuberculosis (TB) incidence rate was 361 cases per 100,000 population, as reported in the WHO Global Tuberculosis Report [19]. In this context, adult vaccination, particularly among women of childbearing age, may offer considerable public health benefits both for adults and to improve offspring outcomes [20].

Although the positive impacts of vaccines for humanity are irrefutable, various barriers lead to insufficient coverage. One of the barriers can be fear and misinformation related to adverse events (AEs) [21]. While some level of AEs is anticipated for all vaccines, it is important that they are monitored, investigated, and documented. There is limited data available regarding vaccine-related adverse effects in adult women, despite growing interest in maternal vaccination strategies [22]. Monitoring these events is essential to ensure safety, community acceptance, and providing inclusive public health policies [23] especially in settings where vaccination efforts focus primarily on children. This study helps fill that gap by evaluating the safety of BCG and MV in women of childbearing age. We therefore assessed AEs within the first 3 months post-vaccination in a substudy nested within an RCT testing the effects of BCG vs. MV vs. placebo provided to women of fertile age in Guinea-Bissau.

2 | Methods

2.1 | Setting

The trial was conducted at the Bandim Health Project (BHP, www.bandim.org), in Bissau, Guinea-Bissau. The BHP's Health and Demographic Surveillance System (HDSS) in urban Bissau includes six districts in the capital, covering approximately 100,000 individuals, including approximately 8000 women of childbearing age who have had at least one child. Within this study area, the BHP follows all women of childbearing age with bi-monthly visits to register new pregnancies and migration in or out of the study area.

2.2 | Trial Design, Participants and Interventions

The present substudy of adverse events took place within an ongoing randomised trial, aiming to allocate at least 2400 women 1:1:1 to MV, BCG, or placebo. Inclusion criteria were being HIV-negative, non-pregnant, female of fertile age (15 to 35 years old), who had given birth to at least one child at least 12 months ago (or less if the last child had died). Participants who refused to provide informed consent, were in a clinically fragile state of health, had a history of active tuberculosis or other serious illnesses, or were unable to understand the objectives of the study were excluded.

We aimed to enrol 300 women included early in the trial for the present safety sub-study in order to conduct in-depth monitoring of short-term adverse events and assess whether there were any major side effects that could influence the trial; the main trial will follow all women for at least 12 months, providing data on long-term serious adverse reactions as part of its overall aims.

2.3 | Enrolment and Randomisation of Women

In the main clinical trial, the eligible women were identified through the HDSS. They received a home visit from a project assistant, who explained the study. Provided they were interested

TABLE 1 | The main known adverse events after MV and BCG [18, 26].

Classification	BCG [26]	MV [18]
Local	Mild: Injection site papule (onset 2–4 weeks); Mild ulceration (1–2 months); Scar (2–5 months) Severe: Injection site reactions; Skin lesions distinct from the vaccination site; Local abscess; Keloid; Lymphadenitis Suppuration (onset 2–6 months)	Pain and tenderness at injection site within 24 h of vaccination (resolved within 2–3 days)
Systemic	Persistent fever; Osteitis and Osteomyelitis, Disseminated BCG disease; Immune reconstitution inflammatory syndrome (IRIS)	Mild: Fever > 39.4 degrees for 5%–15% of vaccinees between 7th–12th day after vaccination (duration 1–2 days) Rash in 2%–5% of vaccinees, 7–10 days after vaccinations, duration about 2 days. Severe: Anaphylaxis, Seizures, Thrombocytopenia; Encephalopathy/ Encephalitis;

Abbreviations: BCG, Bacillus Calmette-Guérin; MV, measles vaccine.

in participating, women were invited to go to a nearby health centre in the afternoon for a more detailed explanation. Following oral and written informed consent procedures, those who tested negative for HIV and pregnancy were interviewed, randomised, and vaccinated according to the randomisation allocation.

2.4 | Follow-Up for Possible Adverse Reactions Linked to Vaccines

Participants were monitored for 15 min after vaccination for possible immediate reactions to the vaccination. Subsequently, home visits were performed by a project assistant at intervals of 7, 14, 28, and 90 days after randomisation to register symptoms. The team conducted a 2nd visit if the 1st visit was unsuccessful, and telephone follow-up was attempted if both home visits were unsuccessful.

2.5 | Outcomes

AEs after vaccination were defined as any symptoms reported following vaccination [23–25].

Serious adverse events (SAEs) were defined as an event that requires hospitalization for at least 24 h; causes significant dysfunction and/or persistent disability (sequelae); causes risk of death, or causes death [23, 25].

Adverse events, depending on the manifestation, could be local or systemic. Table 1 presents the main known adverse events of BCG and MV [26, 27].

In the present study, we grouped some of the symptoms experienced by participants. Any symptom of pain aside from abdominal pain was grouped in one category “Body pain”, irrespective

of location. “Acute gastrointestinal disease” was any symptom of abdominal pain, diarrhoea, or vomiting. “Respiratory illness” was any of the following symptoms: cough, difficulties breathing, or runny nose. For each visit time point and across all visits, we assessed whether the participant had reported one or more symptoms (any type, yes/no).

2.6 | Statistical Analyses

Background characteristics were presented as percentages for categorical variables, while continuous variables were presented as mean ± standard deviation by randomisation group. Assessment of differences in proportion among the randomisation groups was carried out using chi-square or Fisher's exact test, as appropriate. Distributions of continuous risk factors were compared using the Kruskal-Wallis rank-sum test.

For the analysis of outcomes, we present the number and proportion of women reporting outcomes by randomisation group. The analyses were conducted overall and by follow-up visit time point (7, 14, 28, and 90 days after randomisation), comparing MV vs. placebo and BCG vs. placebo. For the overall analysis, women could only contribute one observation. We did not perform any correction for multiple testing.

All statistical analyses were conducted using Stata 18.0.

2.7 | Ethical Considerations

The trial and the substudy were approved by the National Committee for Ethics in Health Research of Guinea-Bissau (CNES), reference number **043/CNES/INASA/2019**, and the trial was pre-registered with [ClinicalTrials.gov](https://www.clinicaltrials.gov) trial ID NCT04899765. Oral and written informed consent for voluntary

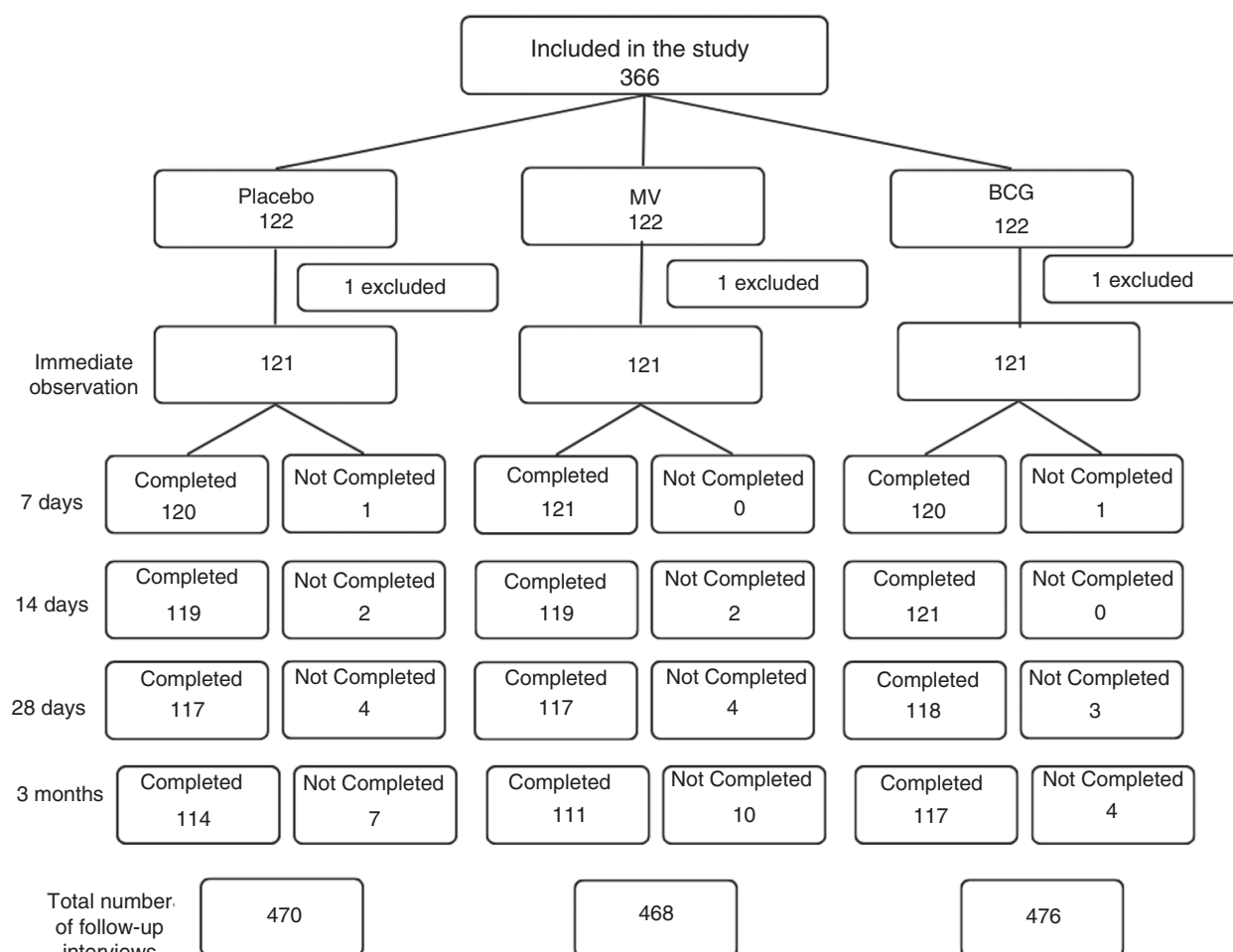


FIGURE 1 | Flow chart of inclusion. BCG, Bacillus Calmette-Guérin; MV, measles vaccine.

participation was obtained, with written consent to use the collected data obtained from all participants. Confidentiality and anonymity of participants were ensured during both data collection and analysis.

3 | Results

3.1 | Flow Chart of Inclusions

During May to December 2021, a total of 366 women were enrolled and received follow-up for adverse reactions after vaccination (placebo: 122, MV: 122, and BCG: 122). Three participants were excluded due to errors in vaccine administration (Placebo 1, MV 1, and BCG 1), where those randomised were mistakenly given the wrong intervention. Overall, 97% (1414/1452) of the scheduled contacts were successful, 1.3% (18/1414) of which were done by mobile phone, and there were no discrepancies between the randomisation groups in terms of follow-up adherence (Figure 1).

3.2 | Baseline Characteristics

The mean age of the participants was 26 years. The baseline characteristics such as sociodemographic characteristics,

vaccination history, and health risk factors at enrolment did not differ between randomisation groups (Table 2).

3.3 | Adverse Events Reported

There were no immediate adverse events detected within the first 15 min post-vaccination and no SAEs within 90 days post-randomisation. A total of 11 cases of medical consultations were reported, four in the placebo group, five in the MV group, and two in the BCG group, with a p of 1.00 for MV vs. placebo and 0.68 for BCG vs. placebo.

The main symptoms reported by the women in the trial were injection-site skin reactions, fever, headache, and respiratory symptoms. The self-reported symptoms, overall and by contact timepoint, are summarized in Table 3.

The proportion of women with any self-reported symptom tended to be higher in the groups randomised to BCG and MV vaccine during the first 14 days of follow-up (Table 3). For participants randomised to BCG, the development of skin reactions during follow-up was noteworthy, the skin reaction prevalence gradually increasing from 38% (46/120) at 7 days, 59% (71/121) at 14 days, 79% (92/118) at 28 days, to 97% (113/117) by 90 days (Figure 2).

TABLE 2 | Baseline characteristics of trial participants.

Variable	Randomisation group			Test	
				MV vs placebo	BCG vs placebo
	Placebo [N=121]	MV [N=121]	BCG [N=121]	<i>p</i> ^a	<i>p</i> ^b
Sociodemographic characteristics					
Mean (interquartile range) age at enrolment	26 (22–29)	26 (23–29)	26 (23–30)	0.96	0.84
Mean (interquartile range) body mass index	23 (20–26)	24 (20–27)	24 (20–26)	0.59	1.00
Mean (interquartile range) mid-upper arm circumference (MUAC) in mm	274 (246–298)	278 (252–304)	274 (244–300)	0.65	1.00
Woman lives together with husband % (<i>n</i>)	57.9 (70)	56.2 (68)	60.3 (73)	0.80	0.69
Mean (interquartile range) time since last ended pregnancy (months)	20 (17–22)	19 (16–22)	18 (15–21)	0.73	0.09
Last child born had died % (<i>n</i>)	6.6 (8)	5.0 (6)	9.1 (11)	0.58	0.47
Enrolled during the rainy season (June–November)	93.4 (113)	92.6 (112)	93.4 (113)	0.80	1.00
Mean (interquartile range) years of schooling [N]	8 (6–12) [88]	8 (5–12) [89]	8 (4–12) [87]	1.00	0.91
Ethnic group					
Balanta % (<i>n</i>)	9.1 (11)	4.1 (5)	7.4 (9)	0.50	0.10
Fula % (<i>n</i>)	19.0 (23)	23.1 (28)	26.4 (32)		
Mancanha % (<i>n</i>)	6.6 (8)	6.6 (8)	7.4 (9)		
Mandinga % (<i>n</i>)	8.3 (10)	5.8 (7)	4.1 (5)		
Manjaco % (<i>n</i>)	16.5 (20)	14.0 (17)	5.8 (7)		
Pepel % (<i>n</i>)	29.8 (36)	28.9 (35)	35.5 (43)		
Other % (<i>n</i>)	10.7 (13)	17.4 (21)	13.2 (16)		
Vaccination history					
Received vaccine within the last 4 weeks % (<i>n</i>)	0.8 (1)	0.0 (0)	0.0 (0)	1.00	1.00
Has at least one BCG scar % (<i>n</i>)	81.0 (98)	84.3 (102)	80.2 (97)	0.50	0.87
History of illness prior to inclusion % (<i>n</i>)					
Chronic disease ^b % (<i>n</i>)	0.0 (0)	0.8 (1)	0.8 (1)	1.00	1.00
Medication received in the last month before inclusion % (<i>n</i>)	5.8 (7)	7.4 (9)	5.8 (7)	0.60	1.00
Medical consultation during the last 3 months % (<i>n</i>)	0.8 (1)	2.5 (3)	0.8 (1)	0.62	1.00
Hospitalisation during the last 3 months % (<i>n</i>)	0.0 (0)	0.0 (0)	0.0 (0)	—	—

Note: Values are percentages (numbers) unless stated otherwise.

Abbreviations: BCG, Bacillus Calmette-Guérin; MV, measles vaccine.

^a*p* calculated with chi² test or Fisher's exact test (for factors with cells with less than six observations, approx. 5% of observations within each randomisation group) for categorical factors. *p* calculated with Kruskal-Wallis rank-sum test for continuous factors.

^bChronic disease defined as a long-term health condition requiring continuous medical care.

TABLE 3 | Occurrence of adverse reactions reported during follow-up after each inclusion by follow-up interval and by randomisation group.

Variable	Randomisation group			Test ^a	
	Placebo (N=121)	MV (N=121)	BCG (N=121)	MV vs placebo	BCG vs placebo
All follow-up	(N=470)	(N=468)	(N=476)	<i>p</i>	<i>p^a</i>
Local Manifestations % (<i>n</i>)					
Local or regional pain% (<i>n</i>)	0.8 (1)	2.5 (3)	2.5 (3)	0.62	0.62
Skin reactions % (<i>n</i>)	7.4 (9)	6.6 (8)	96.7 (117)	0.80	0.00
Lymph node inflammation % (<i>n</i>)	3.3 (4)	5.0 (6)	2.5 (3)	0.75	1.00
Systemic manifestations					
Fever % (<i>n</i>)	6.6 (8)	11.6 (14)	6.6 (8)	0.18	1.00
Body pain % (<i>n</i>)	2.5 (3)	2.5 (3)	4.1 (5)	1.00	0.72
Acute gastrointestinal disease % (<i>n</i>)	2.5 (3)	4.1 (5)	2.5 (3)	0.72	1.00
Headache % (<i>n</i>)	8.3 (10)	15.7 (19)	14.0 (17)	0.07	0.15
Eye irritation % (<i>n</i>)	3.3 (4)	5.0 (6)	2.5 (3)	0.75	1.00
Skin irritation or inflammation % (<i>n</i>)	0.0 (0)	1.7 (2)	0.8 (1)	0.50	1.00
Respiratory illness % (<i>n</i>)	9.1 (11)	5.8 (7)	9.1 (11)	0.33	1.00
Medical consultation % (<i>n</i>)	3.3 (4)	4.1 (5)	1.7 (2)	1.00	0.68
7 days follow-up	(N=120)	(N=121)	(N=120)		
One or more self-reported symptoms (any) % (<i>n</i>) ^b	11.7 (14)	17.4 (21)	16.7 (20)	0.21	0.27
Local manifestations % (<i>n</i>)					
Local or regional pain % (<i>n</i>)	2.5 (3)	2.5 (3)	0.8 (1)	1.00	0.62
Skin reactions % (<i>n</i>)	1.7 (2)	1.7 (2)	38.3 (46)	1.00	0.00
Lymph node inflammation % (<i>n</i>)	2.5 (3)	2.5 (3)	0.8 (1)	0.99	1.00
Systemic manifestations					
Fever % (<i>n</i>)	0.8 (1)	2.5 (3)	1.7 (2)	0.62	1.00
Body pain % (<i>n</i>)	0.0 (0)	0.8 (1)	0.8 (1)	1.00	1.00
Acute gastrointestinal disease % (<i>n</i>) ^b	0.8 (1)	1.7 (2)	1.7 (2)	1.00	1.00
Headache % (<i>n</i>)	2.5 (3)	8.3 (10)	9.2 (11)	0.08	0.05
Eye irritation % (<i>n</i>)	0.8 (1)	3.3 (4)	0.0 (0)	0.37	1.00
Skin irritation or inflammation % (<i>n</i>)	0.0 (0)	0.8 (1)	0.8 (1)	1.00	1.00
Respiratory illness % (<i>n</i>)	4.2 (5)	2.5 (3)	5.0 (6)	0.50	1.00
Medical consultation % (<i>n</i>)	0.0 (0)	0.8 (1)	0.0 (0)	1.00	.

(Continues)

TABLE 3 | (Continued)

Variable	Randomisation group			Test ^a	
	Placebo (N=121)	MV (N=121)	BCG (N=121)	MV vs placebo	BCG vs placebo
14 days follow-up	(N=119)	(N=119)	(N=121)		
One or more self-reported symptoms (any) % (n) ^b	5.0 (6)	10.9 (13)	9.9 (12)	0.09	0.15
Local manifestations % (n)					
Local or regional pain % (n)	0.8 (1)	0.0 (0)	0.0 (0)	1.00	0.50
Skin reactions % (n)	1.7 (2)	5.0 (6)	58.7 (71)	0.28	0.00
Lymph node inflammation % (n)	0.0 (0)	1.7 (2)	1.7 (2)	0.50	0.50
Systemic manifestations					
Fever % (n)	0.8 (1)	4.2 (5)	2.5 (3)	0.21	0.62
Body pain % (n)	0.0 (0)	0.0 (0)	1.7 (2)	—	0.50
Acute gastrointestinal disease % (n)	0.8 (1)	0.8 (1)	0.8 (1)	1.00	1.00
Headache % (n)	0.8 (1)	1.7 (2)	2.5 (3)	1.00	0.62
Eye irritation % (n)	0.8 (1)	0.8 (1)	0.0 (0)	1.00	0.50
Skin irritation or inflammation % (n)	0.0 (0)	0.0 (0)	0.0 (0)	—	—
Respiratory illness % (n)	0.8 (1)	2.5 (3)	2.5 (3)	0.62	0.62
Medical consultation % (n)	0.8 (1)	0.0 (0)	0.0 (0)	1.00	0.50
28 days follow-up	(N=117)	(N=117)	(N=118)		
One or more self-reported symptoms (any) % (n) ^b	6.8 (8)	8.5 (10)	8.5 (10)	0.62	0.64
Local manifestations % (n)					
Local or regional pain % (n)	0.0 (0)	0.0 (0)	1.7 (2)	—	0.50
Skin reactions % (n)	3.4 (4)	0.9 (1)	78.6 (92)	0.37	0.00
Lymph node inflammation % (n)	0.0 (0)	0.0 (0)	0.0 (0)	—	—
Systemic manifestations					
Fever % (n)	2.6 (3)	4.3 (5)	1.7 (2)	0.72	0.68
Body pain % (n)	0.9 (1)	1.7 (2)	0.8 (1)	1.00	1.00
Acute gastrointestinal disease % (n)	0.0 (0)	0.0 (0)	0.8 (1)	—	1.00
Headache % (n)	1.7 (2)	3.4 (4)	0.8 (1)	0.68	0.62
Eye irritation % (n)	0.9 (1)	0.0 (0)	1.7 (2)	1.00	1.00
Skin irritation or inflammation % (n)	0.0 (0)	0.9 (1)	0.0 (0)	1.00	.
Respiratory illness % (n)	1.7 (2)	0.0 (0)	0.0 (0)	0.50	0.25
Medical consultation % (n)	0.9 (1)	1.7 (2)	0.8 (1)	1.00	1.00

(Continues)

TABLE 3 | (Continued)

Variable	Randomisation group			Test ^a	
	Placebo (N=121)	MV (N=121)	BCG (N=121)	MV vs placebo	BCG vs placebo
90 days follow-up	(N=114)	(N=111)	(N=117)		
One or more self-reported symptoms (any) % (n) ^b	9.6 (11)	12.6 (14)	6.0 (7)	0.48	0.30
Local manifestations % (n)					
Local or regional pain % (n)	0.0 (0)	1.8 (2)	0.9 (1)	0.24	1.00
Skin reactions % (n)	0.9 (1)	0.0 (0)	96.6 (113)	1.00	0.00
Lymph node inflammation % (n)	0.9 (1)	1.8 (2)	0.0 (0)	0.62	0.49
Systemic manifestations					
Fever % (n)	2.6 (3)	3.6 (4)	0.9 (1)	0.72	0.37
Body pain % (n)	1.8 (2)	0.0 (0)	0.9 (1)	0.50	0.62
Acute gastrointestinal disease % (n)	1.8 (2)	2.7 (3)	0.0 (0)	0.68	0.24
Headache % (n)	3.5 (4)	7.2 (8)	1.7 (2)	0.25	0.44
Eye irritation % (n)	0.9 (1)	0.9 (1)	0.9 (1)	1.00	1.00
Skin irritation or inflammation % (n)	0.0 (0)	0.0 (0)	0.0 (0)	—	—
Respiratory illness % (n)	3.5 (4)	1.8 (2)	1.7 (2)	0.68	0.44
Medical consultation % (n)	1.8 (2)	1.8 (2)	0.9 (1)	1.00	0.62

Note: Acute gastrointestinal disease: was any symptom of abdominal pain, diarrhoea, or vomiting, and "Respiratory illness" was any of the following symptoms: cough, difficulties breathing, or runny nose.

Abbreviations: BCG, Bacillus Calmette-Guérin; MV, measles vaccine.

^ap calculated with chi² test or Fisher's exact test (for factors with cells with less than six observations, approx).

^bSince participants could report more than one symptom at each visit, the number of participants with one or more symptoms is lower than the sum of different symptoms reported.

There tended to be a higher prevalence of headache, most notably by 7 days post-vaccination, in the groups randomised to either vaccine, the prevalence being 2.5% (3/120) for placebo; 8.3% (10/121) for MV, and 9.2% (11/120) for BCG, with corresponding *p* being 0.08 and 0.05, respectively, for the comparison with placebo.

4 | Discussion

4.1 | Main Results

Although there is emerging evidence that vaccines such as BCG and MV provided in the presence of maternal antibody are more beneficial for children [11, 14], there is little information regarding the safety and tolerability of these vaccines in adults, not least in women of reproductive age.

Using a 5-timepoint follow-up and monitoring schedule for the women included in the study (15 min immediately after vaccination, 7-day, 14-day, 28-day, and 90-day visits after), we found no evidence that these vaccines have reactogenic reactions that were not well tolerated by the women included in the trial. In

particular, there were no severe adverse events recorded in this safety cohort of the trial. The short-term AEs recorded, including a trend for higher prevalence of headache by 7 days post-randomisation for both vaccines, were limited in scope and time, and within the range expected for BCG and MV. Skin reactions at the vaccination site for the group randomised to BCG were likewise expected [28] and it was not observed that the vaccines interfered with their daily life routines.

4.2 | Strengths and Limitations

For this study, we used data from closely spaced in-person active follow-up contacts, which allowed us to obtain direct and accurate information, thus limiting the possibility of serious AEs not being reported. The frequent visits limited the possibility of recall bias. We obtained a very high response rate, which reinforces the validity of the results in relation to the population studied.

On the other hand, the sample size of the adverse events sub-study was relatively small, which limits the statistical power to detect rare or subtle adverse effects. Although randomisation

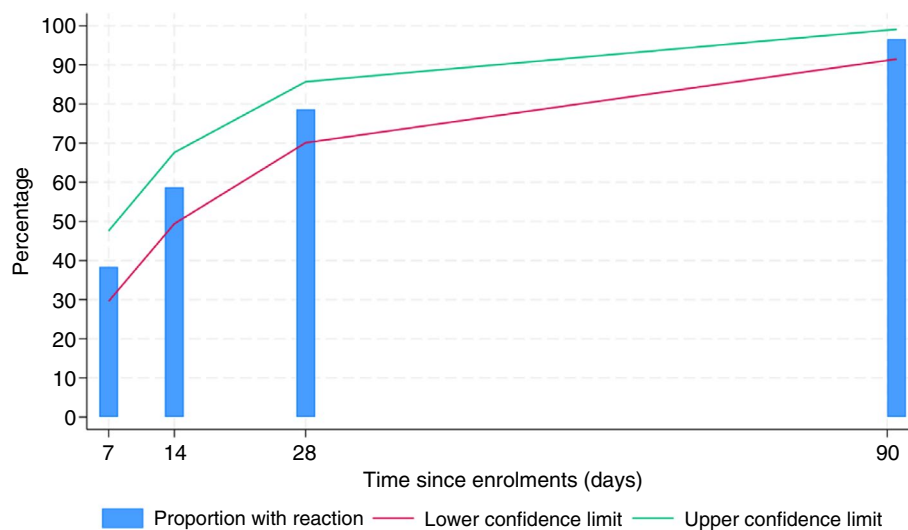


FIGURE 2 | Prevalence of BCG skin reactions (self-reported) in BCG group. BCG, Bacillus Calmette-Guérin.

was used to balance groups, there was no adjustment for potential confounding variables beyond baseline comparability. The statistical analysis was limited to basic comparisons without multivariable modelling or correction for multiple testing, which may affect the robustness of the findings.

This study was conducted during the COVID-19 pandemic period (inclusion May–September 2021; follow-up May–December 2021). There were many strikes in the health care system in Guinea-Bissau during the study period. Therefore, under normal conditions, the number of medical consultations might have been higher than those presented in this study.

The population studied here is young healthy women with documented reproductive capacity (mothers that had given birth to at least one child). Our results can therefore not be extrapolated broadly to the adult population. Noteworthy, women generally experience more adverse reactions to vaccinations than men [29], so it is likely that men would have fewer adverse events than those registered in the present study. In this study, we did not evaluate AEs occurring more than 3 months after inclusion.

4.3 | Consistency With Previous Studies

BCG and MV are vaccines that have been included in the vaccination schedules for children in low-income countries for more than 50 years and are well accepted in the community by children.

Regarding BCG, the main known AEs are presented in Table 1. In this study, as in other studies that evaluated BCG AEs, the main finding in all stages of follow-up was skin reactions at the BCG vaccination site [30–32]. We found that 97% developed a skin reaction, which is very similar to the scar rates seen in children who are vaccinated with a potent BCG strain and with a correct injection technique [32]. For those who had these reactions, none of the participants complained that the vaccines prevented them from carrying out their daily life

routines. Furthermore, among BCG-vaccinated children, it has been shown that developing a scar is associated with improved overall survival [33, 34].

MV is rarely associated with AEs; the main known MV AEs are presented in Table 1. Our study was not powered to detect rare side effects, and the only symptom that tended to be increased in the MV group was headache, which for Turkish school children has been reported as an MV-induced AE occurring in 3.5% of children in the first month after vaccination [35]. While our sample size was quite limited, the low proportion of AEs found in this study corroborates the findings of other studies that evaluated possible AEs of MV or MMR [35–37].

4.4 | Interpretation and Implications

We observed consistency in relation to the main known AEs of BCG and MV, the reported complaints being moderate, tolerable, and transient. The only non-transient AE was the skin reactions for the BCG group in all stages of follow-up, which was expected and had been explained to the trial participants at enrolment. Therefore, this adverse event study indicates that the vaccines can be used among adults, although neither the rare nor long-term AEs were addressed here.

We have advocated for revaccination or adult vaccination to increase protection of children in the first years of life [11–13], but there could be speculation regarding the AEs of these vaccines, despite their widespread use in children. This is the first study to assess the adverse events of both MV and BCG used in an adult population, demonstrating that it is possible to use these vaccines for women of childbearing age.

These findings support the safe use of BCG and MV in adult women and highlight the potential for expanding vaccination policies beyond childhood. In low-income countries such as Guinea-Bissau, integrating adult vaccination, especially among women of childbearing age, could strengthen public health measures and improve maternal and child health outcomes [22, 38].

5 | Conclusion

Our findings indicate that BCG and MV vaccines are safe and well-tolerated among adult women of childbearing age, with few short- and medium-term adverse events. These results support the feasibility of expanding vaccination strategies to include adults, particularly in low-income countries where child mortality remains high and healthcare infrastructure is limited. Further research with larger cohorts and more robust methods is essential to assess long-term safety and inform public health strategies.

Conflicts of Interest

The authors declare no conflicts of interest.

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Humoral immune responses to measles and Bacillus Calmette-Guérin vaccination in women of reproductive age: a nested study within a randomised trial in Guinea-Bissau

Short running title

Antibody responses to measles and BCG vaccination in reproductive-age women

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25 **Abstract**

26 **Background:** This study investigated the humoral immune response to measles vaccination (MV)
27 and Bacillus Calmette-Guérin Vaccine (BCG) in women of reproductive age, assessing strategies to
28 enhance maternal antibody levels prior to pregnancy.

29 **Methods:** In a randomised, placebo-controlled trial conducted in Guinea-Bissau, HIV-negative,
30 non-pregnant women aged 15–35 years were allocated 1:1:1 to receive MV, BCG, or placebo. As a
31 secondary outcome in the trial, a subgroup of participants had measles- and BCG-specific antibody
32 levels measured at baseline, four weeks post-vaccination and for women who became pregnant
33 during the third trimester of pregnancy, using a multiplex Luminex-based assay. cytomegalovirus
34 (CMV))-specific antibody was measured as a control antigen. Seroconversion was defined as a ≥ 2 -
35 fold increase in antibody levels.

36 **Results:** 459 women were included in the study. Four weeks post-vaccination, MV and BCG groups
37 showed an increase in specific antibodies. This increment was particularly seen for women whose
38 baseline level of specific antibody was below the median. Among women who got pregnant (on
39 average 16 months post-randomisation), in the third trimester of pregnancy, the overall levels of
40 measles antibody and antibodies to the control antigen were quite similar to those seen at
41 baseline, but in the MV group the levels of antibody to measles lysate remained elevated. The
42 levels of BCG antibodies were significantly lower in all randomisation groups.

43 **Conclusion:** MV and BCG vaccines elicited strong antibody responses in women of reproductive
44 age after four weeks, particularly among those with lower baseline levels.

45 This effect was not sustained into the third trimester except from a weak positive effect on
46 measles lysate antibody. Pregnancy appeared to differentially influence antibody persistence:
47 measles antibodies and antibodies to the control antigen remained stable, while BCG antibodies
48 declined.

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54 INTRODUCTION

55 Infant protection against primary measles infection can be acquired from two sources: from active
56 immunisation with measles vaccine (MV) and from passive transfer of maternal antibodies.

57 Measles immunisation contributes to herd immunity and long-term prevention of infection.

58 Specifically, two doses of measles vaccine provide long-lasting protection, which is generally
59 considered to be lifelong for most individuals (1,2). In contrast, while passive immunity plays a key
60 role in protecting infants during their most vulnerable early months, its duration is limited (1).

61 Before the introduction of MV, nearly every individual contracted the disease during childhood,
62 and maternal immunity acquired through natural infection provided robust and long-lasting
63 protection to infants during their early months of life (1). This defined the current vaccination
64 policy of providing the first dose of MV between 9 and 15 months of age, when maternal antibody
65 has waned (1,3).

66 With the introduction of MV, the natural exposure to the measles virus has drastically declined (1).
67 Mothers, who acquired immunity through vaccination, tend to have lower levels of measles-specific
68 antibodies compared to those who experienced natural infection during childhood (4). A recent
69 meta-analysis revealed that approximately 70% of infants lose maternal measles antibodies by 4
70 months of age (5). This has led to an epidemiological shift in measles cases and complications, with
71 an increasing number of infections occurring in children under 9 months of age (6,7), before
72 receiving MV.

73 In low-income countries such as Guinea-Bissau, the challenges of immunization are particularly
74 evident. Despite ongoing efforts, Guinea-Bissau has struggled to achieve MV coverage above 80%
75 (8). The country is not measles free, and cases are still reported almost yearly (9).

76 There is evidence that the MV not only protects against measles itself, but also contributes to
77 improved overall child survival (10–12). This phenomenon, known as non-specific beneficial effects,
78 suggests that measles vaccination may enhance the immune system's ability to respond to other
79 infections, thereby reducing overall mortality among children (13).

80 Parallel to these findings, evidence emerged suggesting that the non-specific beneficial effects of
81 MV may be stronger when the vaccine is administered in the presence of maternal antibodies (14–
82 16), indicating that maternal immunity not only influences the infant's direct protection against
83 measles infection but may also play a role in enhancing the broader, non-specific benefits of MV.

84 A similar association has been observed for another live vaccine, the Bacillus Calmette–Guérin (BCG)
85 vaccine. There are indications that children born to mothers previously vaccinated with BCG may
86 benefit more from its beneficial non-specific effects (17–20).

87 However, maternal antibodies are also known to interfere with the infant’s immune response to the
88 same vaccines, particularly affecting the humoral response. This interference leads to a diminished
89 antibody response following vaccination in the presence of maternal antibodies (21–23).

90 The evidence raises an important question: could pre-pregnancy vaccinating with MV or BCG
91 enhance the transfer of immunity to their children, thereby extending the duration of passive
92 immunity and additionally, potentially amplifying the non-specific benefits of these vaccines in the
93 children?

94 To address this question, we conducted a randomised placebo-controlled trial to evaluate both the
95 specific and non-specific effects of MV and BCG given during reproductive years, on women and
96 their children. Since live attenuated vaccines are contraindicated in pregnancy, we enrolled non-
97 pregnant women.

98 **METHODS**

99 **Study setting**

100 The pre-pregnancy maternal vaccination trial (MatVac) was conducted at the Bandim Health Project
101 (BHP), Bissau, Guinea-Bissau. The BHP maintains a Health and Demographic Surveillance System
102 (HDSS) that routinely follows all children in the Bandim urban study area, which includes six districts
103 in the capital of Guinea-Bissau, home to approximately 100,000 individuals. Within this area, the
104 BHP follows all women of childbearing age with bi-monthly visits to register new pregnancies. When
105 a woman is registered as pregnant, a unique identification number is assigned to the unborn foetus.
106 All children are followed with home visits every 4 months, during which information is recorded on
107 infections, hospital admissions, breastfeeding patterns, mid-upper-arm-circumference,
108 vaccinations, and vital status. Guinea-Bissau recommends MV in two doses at 9 and 15 months of
109 age. No measles vaccination campaigns were carried out during the trial period.

110 **Trial design and participants**

111 Women were eligible to participate in the study if they were HIV-negative, non-pregnant, aged
112 between 15 and 35 years, and had already given birth to a child that had reached 12 months of age
113 (or less if the last child had died).

114 **Enrolment and randomisation of women**

115 Eligible women were identified through the BHP HDSS databases. They received a home visit from
116 a project assistant who explained the study. If they were interested in participating, they were asked
117 to go to one of the three nearby health centres. At the health centre, after obtaining oral and written
118 informed consent, capillary blood samples were collected via finger prick and tested for HIV using
119 rapid diagnostic tests (RDTs), following national procedure. Subsequently, venous blood samples
120 were collected for further laboratory processing and immunological analyses. If the HIV test was
121 positive, immediate counselling by a trained psychologist was given and the woman was referred
122 for further testing and information and treatment at an HIV clinic, according to the current standard
123 of care. A urine pregnancy test was also performed. Those who tested negative for HIV and were
124 not pregnant were randomised 1:1:1 to one of three treatment arms: BCG, MV, or placebo (saline).

125 **Blood samples**

126 A 3.0 mL blood sample was collected from all women recruited prior to vaccination into a sodium
127 heparin vacutainer (BD 367869). After collection, blood samples were centrifuged at 700 x g for 10

128 minutes at room temperature to separate the plasma from cellular fraction. Plasma was stored in
129 1.0 mL cryovials and kept at -20°C , until transported under monitored and controlled
130 temperature conditions to the European Plotkin Institute of Vaccinology, Université Libre de
131 Bruxelles (EPIV-ULB), in Brussels, Belgium, where they were kept at -80°C until use.

132 The present study focused on assessing the immunological response in women enrolled. In
133 addition to the baseline sample, which was obtained from all enrolled women (which was planned
134 to 2400 women), a subgroup of 90 participants, 30 participants per group were to provide blood
135 samples at four weeks post-enrolment to assess early vaccine response. Furthermore, a subgroup
136 of 300 participants, 100 per group, who became pregnant during follow-up, would provide blood
137 samples in third trimester, to assess antibody levels during pregnancy (In addition, their children
138 would be sampled at 4.5 months or 9 months of age). The present study focusses on the samples
139 taken from women and is based on the first shipment of samples to the laboratory in Belgium. This
140 includes 462 baseline samples, 101 4-week samples and 126 third trimester samples. Thus, only
141 the sample collection for the 4-week analysis was completed.

142 **Total IgG detection assay**

143 Antigen-specific IgG responses were detected at EPIV-ULB using a multiplexed Luminex-based
144 binding antibody assay, adapted from Brown and colleagues (24). This multiplex binding antibody
145 assay was selected to enable simultaneous detection of antibody responses against multiple viral
146 and mycobacterial antigens using limited sample volumes. The antigen panel included measles virus
147 antigens (The Native Antigen Company, UK), *Mycobacterium bovis* lysate (NR-31211, BEI Resources).
148 Cytomegalovirus glycoprotein B (CMV gB) (Sino Biological, China), included as a non-related control
149 antigen (Table1).

150 Briefly, MagPlex[®] microspheres (Diasorin, Italy) were covalently coupled with antigens via carboxyl
151 chemistry using sulfo-N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)
152 carbodiimide (EDC) (Thermo Fisher Scientific, USA), at a concentration of 6,25 μg of antigen per 100
153 μL of microspheres. Plasma samples were diluted according to the detection range of each antigen
154 (1:200 for measles and *M. bovis* antigens, and 1:2,000 for the others) and incubated with antigen-
155 coated beads in 96 well plates for two hours in a plate shaker at room temperature (RT). After
156 incubation, plates were washed with a washing buffer (WB, PBS, 0,1% BSA and 0.05% Tween20) and
157 re-incubated with a goat anti-human IgG-PE (reference 2040-09, Southern Biotech, USA) at a
158 concentration of 0,65 $\mu\text{g}/\text{mL}$ for one hour. Samples and beads were washed again, resuspended in
159 WB and kept at 4°C until detection. In each plate, polyvalent intravenous immunoglobulin

160 (Sandoglobulin, CSL Behring, Switzerland) and anti-measles serum (third international standard
161 97/648, NIBSC, UK) were serially diluted and used as standard curves to determine the dynamic
162 range of the assay and to control for batch effects and assay reproducibility. Blank wells with buffer
163 solution were included to evaluate the assay background. A Bio-Plex 200 array reader (Bio-Rad) was
164 used to detect at least 50 beads from each barcoded microsphere. Results were given as mean
165 fluorescence intensity (MFI).

166 **Main study outcomes**

167 We assessed the following outcomes:

- 168 • Baseline seroprevalence of measles antibodies in women of reproductive age (15–35 years)
169 before vaccination
- 170 • Specific antibody levels four weeks after vaccination
- 171 • Specific antibody levels in the third trimester of pregnancy for trial participants who
172 became pregnant during the trial and had the sample collected

173

174

175 **Statistical analyses**

176 Data were analysed using Stata (version 18.0) software.

177 ***Baseline participant characteristics***

178 Categorical variables were described using absolute frequencies and percentages, while
179 continuous variables were presented as mean and interquartile range. To compare categorical
180 variables between groups, the chi-square test (χ^2) or Fisher's exact test were used. The Kruskal-
181 Wallis rank-sum test was used for continuous variables.

182 Chi-squared and Fischer's exact tests (for cells with less than 5 events) and Kruskal-Wallis ranksum
183 tests were used to compare the distribution of baseline characteristics in women with above vs.
184 below median antibody levels.

185 ***Antibody response analyses***

186 Antibody measurements were conducted at three distinct time points: at enrolment (before
187 vaccination n: 462), four weeks after vaccination n: 101, and during the third trimester of
188 pregnancy n: 126. MFI values were log transformed. Seroconversion was defined as a two-fold
189 increase in antibody levels from the baseline level. This outcome was chosen as very few fulfilled

190 the standard criterion of a 4-fold increase. The probability of seroconversion was compared
191 between time points in logistic regression models adjusted for the woman's age at enrolment
192 (continuous) and the baseline antibody level (continuous), comparing MV or BCG with the placebo
193 group for each antigen.

194 ***Factors associated with antibody response after vaccination***

195 A univariable binomial regression analysis was conducted to explore associations between
196 baseline characteristics and antibody levels four weeks post-vaccination (a similar analysis was not
197 conducted in third trimester, as there was no effect of vaccination at that time point). Variables
198 with a p-value < 0.05 in the univariable analysis were subsequently included in a multivariable
199 analysis to identify independent factors associated with antibody responses.

200 **Ethics**

201 The data presented is a secondary outcome of a clinical trial approved by the Guinea-Bissau ethics
202 committee (043/CNES/INASA/2019). The trial is registered at ClinicalTrials.gov (registration number:
203 [NCT04899765](https://clinicaltrials.gov/ct2/show/study/NCT04899765)). As part of the study protocol, participating women received a range of health
204 services, including free consultations, pregnancy follow-up, basic medications, and counselling, in
205 line with national health and ethical standards.

206 RESULTS

207 A total of 462 baseline samples were included (Figure 1). Baseline characteristics were balanced
208 between study arms (Table 2). The mean age of participants was 26 years (23–30 interquartile
209 range) across all three randomisation groups. More than 60% in each group were women living with
210 their husbands. The mean body mass index was approximately 24 (IQR 20–28). Three women were
211 excluded from the analysis due to randomisation errors (one from each group), resulting in 459
212 women who were included in the baseline antibody analysis (Figure 1). A total of 101 women had a
213 sample taken four weeks after vaccination (placebo: 41, MV: 29, and BCG: 31) and a total of 126
214 women provided a sample during the third trimester of pregnancy (placebo: 46, MV: 42, and BCG:
215 38).

216

217 Determinants of baseline antibody levels

218 The analysis of baseline antibody levels revealed similar values across all three groups for each
219 evaluated antigen (Table 2). We investigated the association between baseline characteristics and
220 baseline antibody levels to measles and BCG. For measles virus lysate, having just one child at the
221 time of enrolment was associated with a lower probability of having antibody levels above the
222 median, compared with having more than one child (Supplementary table 1). For *M. bovis* lysate,
223 those with longer time since last pregnancy had a higher probability of having baseline antibody
224 levels above the median (Supplementary table 2).

225 Antibody response after four weeks

226 For the measles virus lysate antigens, the MV group demonstrated the highest antibody response
227 with a mean fold change of 3.84 (2.22–5.47), while the placebo (1.02 (0.95–1.10)) and BCG (1.10
228 (0.97–1.22)) groups showed minimal changes. Similarly, for the measles virus nucleoprotein antigen,
229 a significant increase was observed in the MV group (2.15 (1.55–2.74)), whereas the placebo (1.06
230 (0.99–1.13)) and BCG (1.10 (0.97–1.22)) groups exhibited mean fold changes near 1 (Figure 2 and
231 2b).

232 Antibody responses to *M. bovis* lysate antigen remained stable in the placebo (1.02 (0.92–1.12)) and
233 MV (1.02 (0.88–1.15)) groups, while the BCG group showed an increase with a mean fold-increase
234 of 1.48 (1.27–1.69).

235 For the control antigens CMV gB, all groups exhibited fold changes close to 1 (placebo: 0.97 (0.90-
236 1.03); MV: 0.83 (0.73–0.93); BCG: 1.05 (0.88–1.21)) (Figure 2).

237 **Factors associated with antibody response after four weeks**

238 An important determinant of a specific antibody response above the median, particularly for MV,
239 was the baseline antibody level. MV-vaccinated individuals with baseline measles antibody levels
240 above the median were less likely to mount an immune response to measles lysate four weeks post-
241 vaccination compared with MV-vaccinated women with baseline levels below the median (RR 0.44,
242 95% CI: 0.28–0.69; Table 3). Among BCG-vaccinated participants, a similar trend was observed for
243 *M. bovis* lysate antibody levels, though it did not reach statistical significance (RR 0.68, 95% CI: 0.45–
244 1.02; p=0.07; Table 4).

245 These associations remained statistically significant in the multivariable analysis. The MV group
246 show a positive association with having a measles lysate antibody response (2.05 (1.26–3.33)) when
247 compared with the BCG and placebo groups. Additionally, having baseline measles lysate antibody
248 levels above the median remained associated (0.53 (0.34–0.81)) (Table 3).

249 **Vaccine response: third trimester of pregnancy**

250 Among the 126 women who became pregnant during the study, the third trimester sample was
251 collected at an average time of 16 months (IQR 10-22) after randomisation.

252 In samples collected during the third trimester, a small increase in measles lysate antibody
253 responses was observed across all groups. This reached statistical significance for the MV group:
254 placebo (mean fold change 1.52 [0.75–2.29]), MV (1.21 [1.04–1.37]), and BCG (1.19 [0.90 –1.49])
255 (Figure 3 and 3b). For measles nucleoprotein antigen, all groups demonstrated fold changes close
256 to 1, with placebo at 1.09 (0.74–1.45), measles vaccine (MV) at 0.99 (0.88–1.11), and BCG at 1.07
257 (0.77–1.37).

258 In contrast, antibody responses to *M. bovis* lysate decreased from baseline to third trimester across
259 all groups, with fold changes below 1: placebo at 0.67 (0.60–0.70), MV at 0.57 (0.51–0.63), and BCG
260 at 0.62 (0.55–0.59). For the control CMV antigen, the fold changes were close to 1 (placebo at 0.88
261 (0.73–1.02), measles vaccine (MV) at 0.97 (0.79–1.15), and BCG at 1.15 (0.77–1.52)).

262

263 **DISCUSSION**

264 This study evaluated binding antibody responses to two common childhood vaccines, the pre-
265 pregnancy MV and BCG vaccination in women of reproductive age, at four weeks post-vaccination
266 and, among those who became pregnant, during the third trimester of pregnancy.

267 As expected, MV and BCG vaccination induced detectable and specific humoral immune responses
268 at four weeks post-vaccination. Higher baseline antibody levels were negatively associated with the
269 vaccine-induced response at four weeks post vaccination, suggesting limited boosting in individuals
270 with stronger pre-existing immunity.

271 In none of the groups this effect was sustained in the third trimester, which was, on average, 16
272 months post-randomisation. A small increase in measles lysate antibody responses was observed in
273 all randomised groups and reached statistical significance in the MV group. In contrast, the BCG
274 antibodies declined in all groups, while antibodies to the control antigen, CMV; remained stable.

275 **Strengths and weaknesses**

276 This study represents a secondary outcome of a randomised, placebo-controlled clinical trial. The
277 baseline characteristics were well balanced across the randomised groups, ensuring comparability
278 between arms.

279 The immune responses were assessed at multiple timepoints and the final number of participants
280 exceeded initial projections. Antibody assessment at baseline, four weeks post-vaccination, and
281 during the third trimester of pregnancy allowed a dynamic evaluation of antibody levels over time.
282 An additional methodological strength of this study is the inclusion of CMV gB as control antigen.
283 CMV gB is immunologically unrelated to the vaccines under investigation making it a suitable control
284 for the vaccine-induced specific response. The observed similarity in CMV gB antibody levels among
285 the groups supports the effectiveness of randomisation and indicates the absence of baseline
286 immunological bias.

287 One limitation of this study is the absence of neutralizing antibody quantification, which is
288 considered the gold standard correlate of protection for measles. Even so, binding antibody levels
289 have been shown to correlate with humoral immune responses (25). Given the objective of
290 comparing the magnitude of antigen-specific antibody responses across study groups and vaccine
291 antigens, a multiplex binding assay was considered appropriate, while it ensured high analytical
292 sensitivity and reproducibility.

293 We defined seroconversion as a two-fold increase in antibody levels post-vaccination. Although the
294 standard definition is typically a four-fold increase, we adopted the two-fold threshold for practical
295 reasons, as very few participants in this cohort of women, who had likely had measles vaccine
296 previously, showed a four-fold rise using our methods (26,27).

297 Other limitations include the fact that the present study was based on the first samples shipped to
298 the laboratory and thus the sample size was limited for all analyses except for the analysis of
299 responses 4 weeks post-vaccination. The analysed samples, therefore, correspond to those women
300 with available specimens, without a predefined statistical power for comparisons. Only women of
301 fertile age were included, and the study was conducted in urban Bissau, Guinea-Bissau, potentially
302 limiting the generalisability of the findings to settings with different infectious disease epidemiology.
303 Additionally, the analysis focused exclusively on IgG antibody responses, without assessing cellular
304 immunity, which is particularly relevant for vaccines such as BCG.

305 **Consistency with previous studies**

306 This study resonates with previous literature showing an increase in antibody levels following
307 measles vaccination (6,28–30). It also contributes to the limited evidence suggesting that BCG may
308 elicit a humoral response (31), even though it is primarily known to stimulate cellular immunity
309 (32,33). However, a reduction in BCG antibody levels was observed in third trimester of pregnancy
310 (34).

311 The lower antibody response observed in individuals with higher baseline measles antibody levels
312 is consistent with prior evidence suggesting that pre-existing immunity can dampen the immune
313 activation elicited by booster vaccination (35,36,21). This effect, commonly referred to as ‘immune
314 blunting’, has been particularly noted in the context of the first dose of live attenuated vaccines in
315 the presence of maternal antibody, including the measles vaccine (21).

316 Having only one child at enrolment tended to be associated with a baseline lower probability of
317 antibody levels above the median compared with having more than one child in the univariate
318 analysis. To our knowledge there is no similar observations, and there was no strong effect of
319 maternal age, suggesting that this finding may be a chance finding.

320 **Interpretation and implications**

321 The findings of this study demonstrate that pre-pregnancy measles and BCG vaccination induced an
322 increase in antibody levels four weeks after administration, confirming the initial effectiveness of
323 the immune response.

324 However, this effect was not sustained into the third trimester of pregnancy, when measles
325 antibody levels returned to values close to baseline and for BCG declined further. A limited effect of
326 MV might still have been present for measles lysate antibody but showed some degree of increase

327 in the BCG and placebo groups. A reason for the increased levels of measles antibody in the BCG
328 and placebo may be that during the follow-up period, the country did report some small measles
329 outbreaks, in the range of 22-213/year, in between year 2019 -2022 (9). Reinforces the probability
330 that some natural boosting may have occurred.

331 The decline in the BCG antibody levels may be explained by the natural decay of antibodies over
332 time and lack of natural boosting, but also by the adaptations of pregnancy. Physiological
333 hemodilution during pregnancy further circulating antibody concentrations (37). Some degree of
334 immune system modulation also plays a role in shaping the overall response (38). In addition,
335 elevated levels of progesterone and estrogen, the shift from Th1 to Th2 responses, reduce memory
336 B-cell activity, and active transplacental transfer of IgG all contribute to modulation of humoral
337 immunity (39–42).

338 This decline does not negate the overall effectiveness of the vaccine, as other unmeasured
339 components of the immune system, such as cellular and innate immunity, are also likely to be
340 affected and contribute to protection (28).

341 Taken together, the data could indicate that the influence of pregnancy differs for different
342 antibody, as the level of BCG antibody declined and the levels of MV and CMV antibody remained
343 stable in all three groups.

344 This study could not directly address the level of measles- or BCG-specific antibodies transferred
345 from mothers vaccinated prior to pregnancy to their infants, nor how long these antibodies persist
346 in early life. However, this will be studied in a follow-up study of the children.

347 At four weeks post-vaccination, MV and to some extent BCG had a more pronounced effect in
348 women with lower baseline levels. It could be that pre-existing antibody levels had already reached
349 a saturation point, which may have limited the potential for further antibody increase following
350 vaccination.

351 **CONCLUSION**

352 The pre-pregnancy measles (MV) and BCG vaccination in women of reproductive age led to clear
353 increases in antibody levels four weeks post-vaccination, particularly among those with lower
354 baseline levels. However, these vaccine-induced responses were not sustained into the third
355 trimester of pregnancy, on average 16 months after randomisation.

356 Levels of antibodies appear to have been modulated by progressive waning, immunological
357 adjustments during pregnancy and natural measles virus exposure

358

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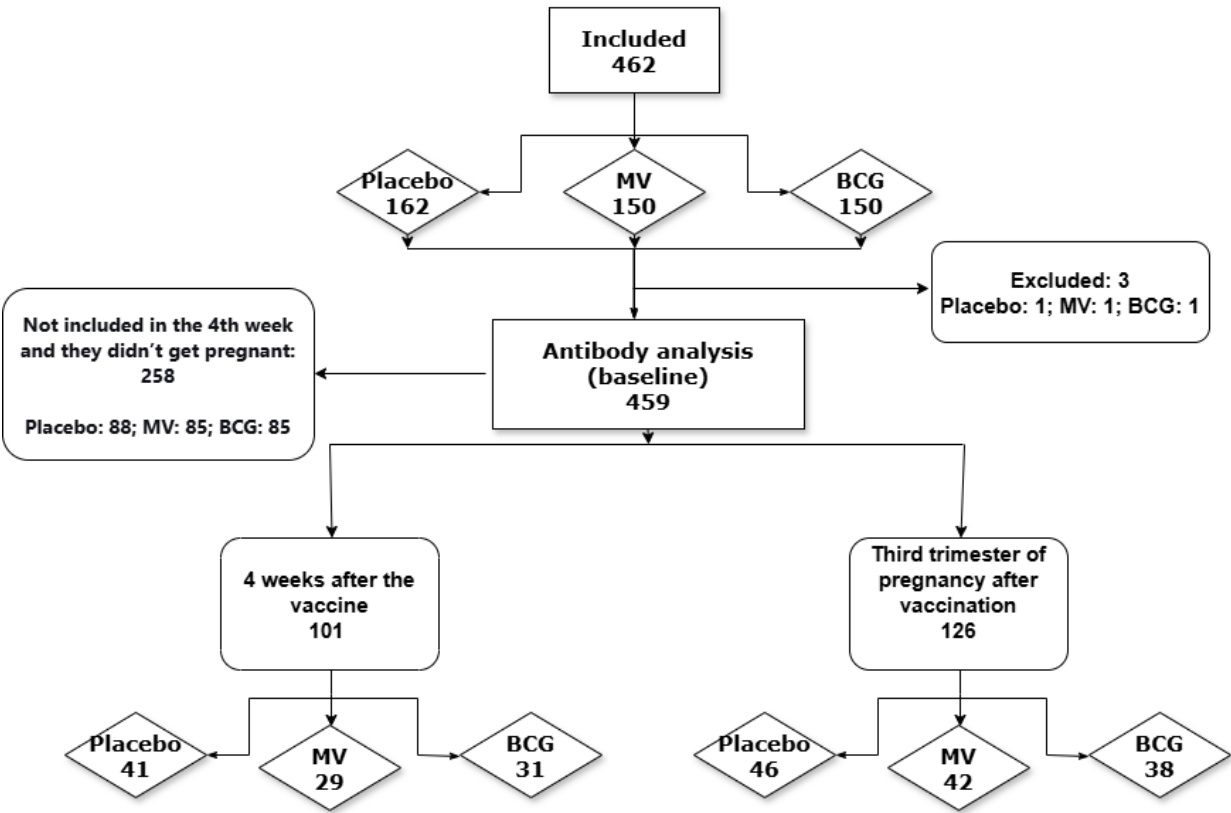
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487 **Figure 1: Study flowchart**



488

489 **Note: Among the women with samples collected at four weeks, 26 subsequently became**
490 **pregnant and are therefore also counted within the third trimester sample group.**

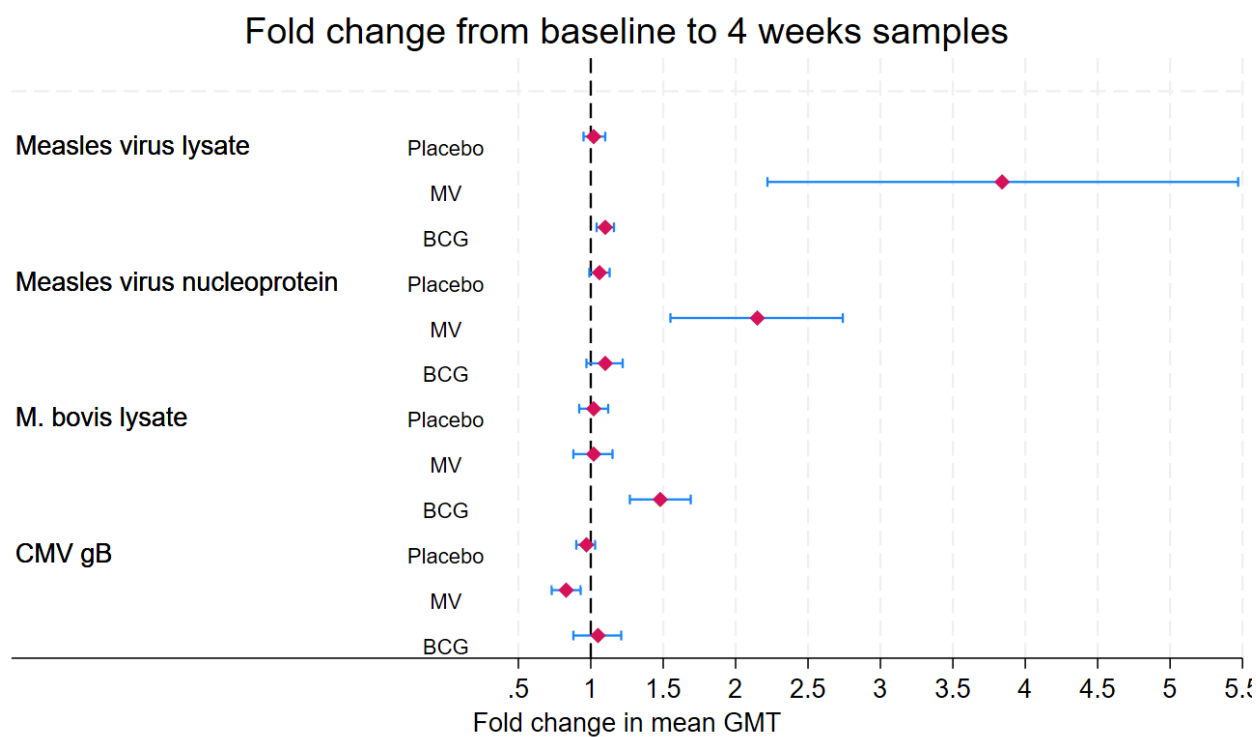
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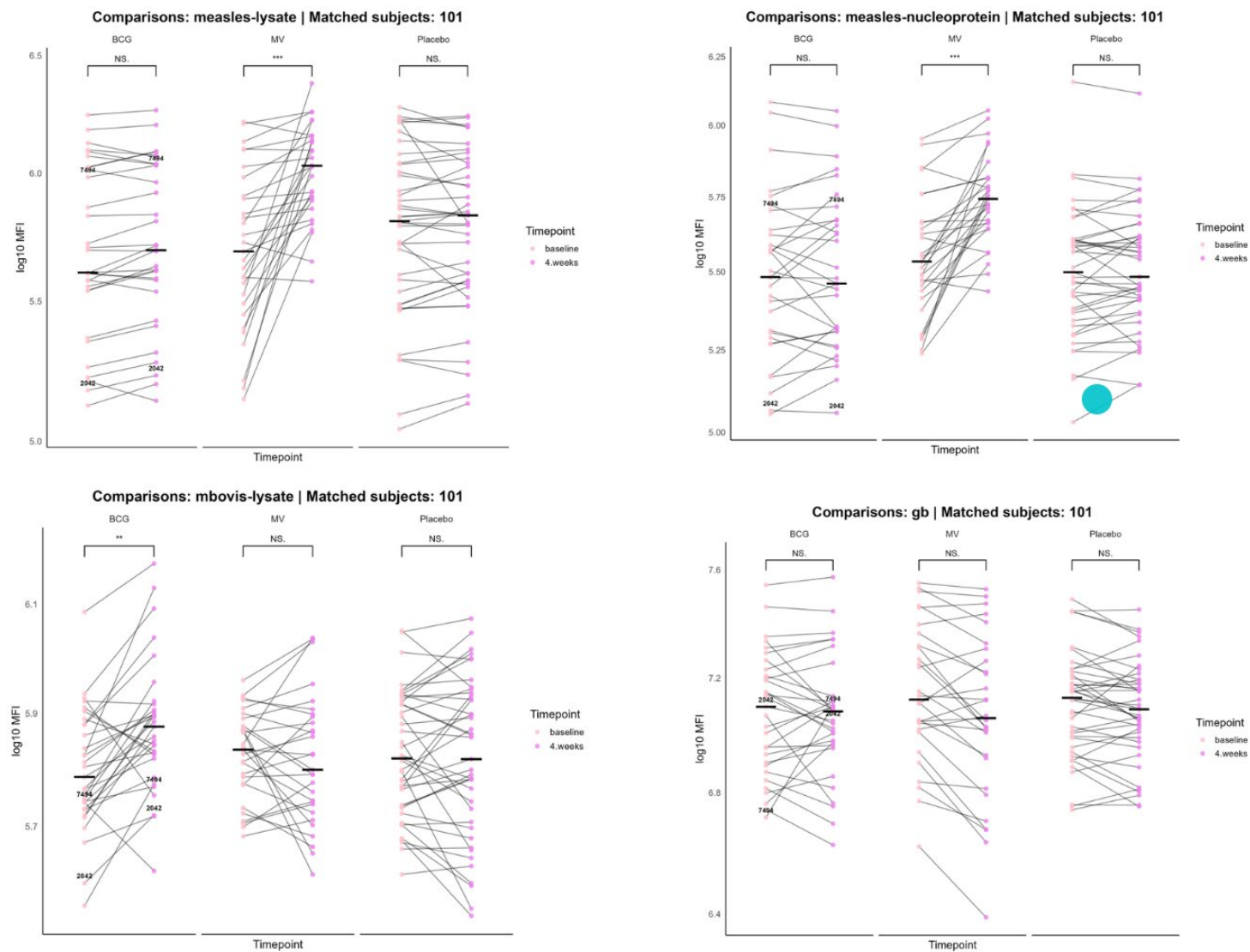
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495 **Figure 2: Antigen analysis panel by randomisation group: fold change from baseline to four**
496 **weeks**



497
498 *Abbreviations: BCG, Bacille Calmette-Guérin; M, Mycobacterium; CMV, Cytomegalovirus; MV,*
499 *measles vaccine.*

501 **Figure 2b: Comparison of paired immune responses (baseline vs 4th week)**

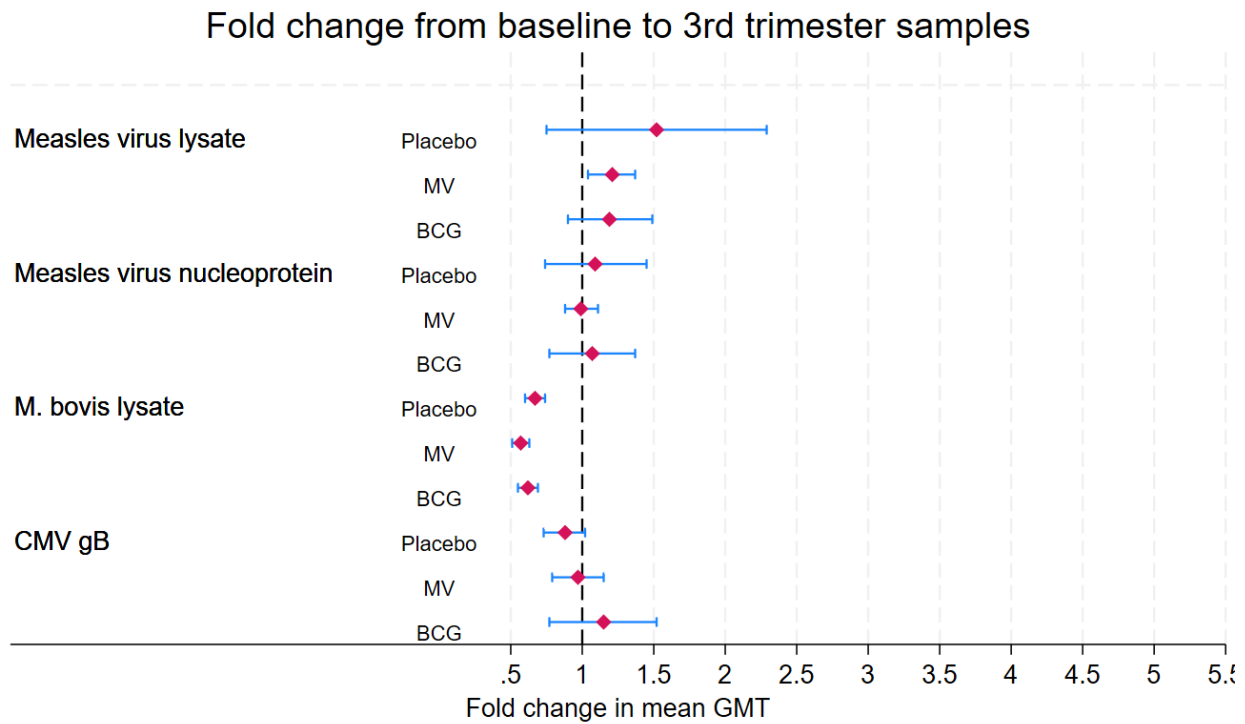


502

503 *** Significance level

504

505 **Figure 3: Antigen analysis panel by randomisation group: fold change from baseline to third**
506 **trimester of pregnancy**

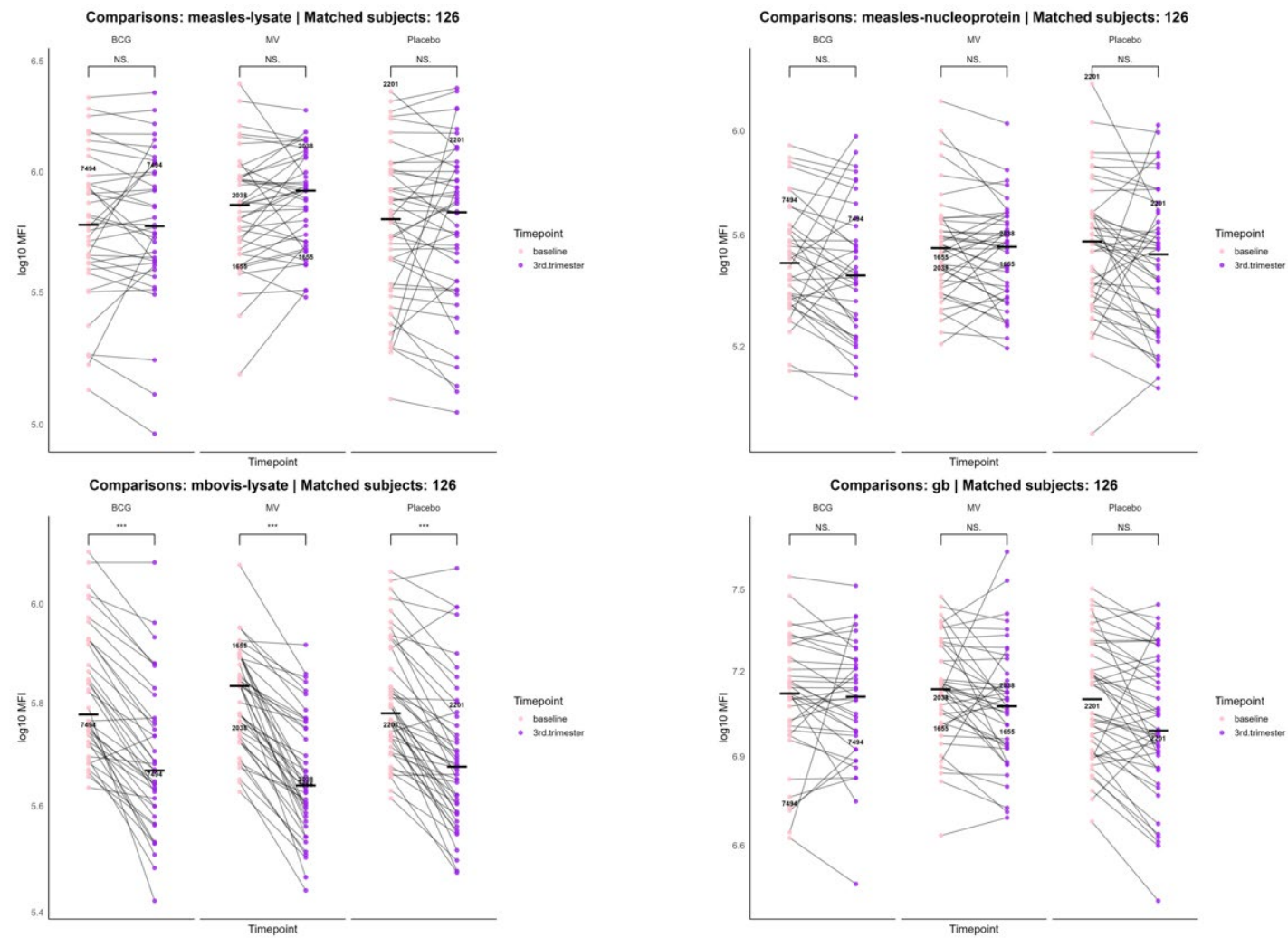


507

508 *Abbreviations: BCG, Bacille Calmette-Guérin; M, Mycobacterium; CMV, Cytomegalovirus; MV,*
509 *measles vaccine.*

510

511 **Figure 3b: Comparison of paired immune responses (baseline vs third trimester)**



512

513 *** Significance level

514

515 **Table 1: Antigens used in the serological evaluation**

Antigen	Pathogen	Purpose
Measles virus lysate	Measles virus	Measles bulk antigens
Measles virus nucleoprotein	Measles virus	Associated with viral encapsidation
M. bovis lysate	M. bovis	M. bovis (BCG) bulk antigens
CMV gB	Cytomegalovirus	Control antigen (non-vaccine antigen)

516

517 **Table 2: Baseline participant characteristics**

Variable	Placebo (N=162)	MV (N=150)	BCG (N=150)	P-value of MV vs. placebo	P-value of BCG vs. placebo
Sociodemographic characteristics					
Mean age at enrolment	26 (22–30)	26 (23–29)	26 (23–30)	1.00	0.99
Woman lives together with husband % (n)	60.5 (98/162)	60.7 (91/150)	64.7 (97/150)	0.98	0.45
Enrolled during rainy season (June–November)	74.1 (120/162)	77.3 (116/150)	77.3 (116/150)	0.50	0.50
Mean Body mass index (BMI)	23 (20–27) [162]	25 (20–28)	24 (20–26)	0.44	0.99
Pregnancy History					
Births					
One birth	52.5 (85/162)	53.3 (80/150)	53.3 (80/150)	0.31	0.06
Two births	40.7 (66/162)	42.7 (64/150)	42.0 (63/150)		
Three or more births	6.8 (11/162)	4.0 (6/150)	4.7 (7/150)		
Last child born had died/stillbirth % (n)	6.8 (11/162)	5.3 (8/150)	8.0 (12/150)	0.59	0.68
Last child born had died % (n)	3.7 (6/162)	3.3 (5/150)	4.0 (6/150)	1.00	1.00
Last child stillbirth	3.1 (5/162)	2.0 (3/150)	4.0 (6/150)	0.72	0.76
Mean time since last pregnancy ended (months)	22 (17–24)	21 (16–24)	20 (15–24)	0.76	0.12
Vaccination history					
Has at least one BCG scar % (n)	78.4 (127/162)	83.3 (125/150)	78.7 (118/150)	0.27	0.95
Recent vaccination (last four weeks) % (n)	1.2 (2/162)	0.0 (0/150)	0.0 (0/150)	0.50	0.50

Risk factors at enrolment (health-related)						518
Chronic disease % (n)	0.0 (0/162)	1.3 (2/150)	0.7 (1/150)	0.23	0.48	519
Medical consultation (last three months) % (n)	1.2 (2/162)	2.7 (4/150)	1.3 (2/150)	0.43	1.00	520
Baseline antibody levels	Log10 mean level (95% CI)					521
Measles virus lysate	3.46 (3.41–3.51)	3.47 (3.42–3.51)	3.46 (3.42–3.51)	0.86	0.74	522
Measles virus nucleoprotein	3.23 (3.20–3.27)	3.25 (3.21–3.28)	3.23 (3.20–3.27)	0.90	0.78	
<i>M. bovis</i> lysate	2.50 (2.48–2.52)	2.50 (2.48–2.52)	2.50 (2.48–2.51)	0.52	0.72	523
CMV gB	2.26 (2.24–2.28)	2.26 (2.23–2.29)	2.27 (2.24–2.29)	0.85	0.78	
						524

525

526 *Abbreviations: BCG, Bacille Calmette-Guérin; M, Mycobacterium; CMV, Cytomegalovirus; MV, measles vaccine.*

527 *# p-values calculated with chi2 test or Fisher's exact test (for factors with cells with less than six observations, approx. 5% of observations*
528 *within each randomization group) for categorical factors. P-values calculated with Kruskal-Wallis rank-sum test for continuous factors.*

529

530 **Table 3: Factors associated with having an antibody response to measles lysate above the median at four weeks post-randomisation**

Variable	Univariable analysis			Multivariable analysis		
	% above median change in Ab at four weeks (n/N)	Univariable binomial regression risk ratio (RR) with 95% CI	p-value	% above median change in Ab at four weeks (n/N)	Multivariable RR (95% CI)	p-value
Randomisation group						
Placebo	29 (12/41)	Reference		29 (12/41)	Reference	
MV	76 (22/29)	2.59 (1.54–4.35)	0.01	76 (22/29)	2.05 (1.26–3.33)	0.01
BCG	52 (16/31)	1.76 (0.98–3.17)	0.06	52 (16/31)	1.45 (0.83–2.50)	0.20
Baseline measles lysate antibody levels						
Baseline level below median	69 (35/51)	Reference		69 (35/51)	Reference	
Baseline level above median	30 (15/50)	0.44 (0.28–0.69)	0.01	30 (15/50)	0.53 (0.34–0.81)	0.01
Mean age at enrolment above median	46 (23/50)	0.87 (0.58–1.29)	0.49			
Woman lives with husband (yes)	49 (37/75)	0.99 (0.63–1.54)	0.95			
Enrolled during rainy season(yes)	44 (7/16)	0.86 (0.48–1.57)	0.63			
BMI above median (yes)	49 (24/49)	0.98 (0.66–1.45)	0.92			
Pregnancy history						
Previous births						
One birth	60 (24/40)	reference				
Two births	38 (14/37)	0.63 (0.39–1.02)	0.06			
Three or more births	50 (12/24)	0.83 (0.52–1.34)	0.45			
Last born child died (yes)	50 (1/2)	1.01 (0.25–4.10)	1.00			
Last child stillbirth (yes)	50 (2/4)	1.01 (0.37–2.75)	0.98			
Mean time since last pregnancy (months)	54 (27/50)	1.20 (0.81–1.78)	0.37			
Vaccination history						
Has at least one BCG scar (yes)	53 (40/75)	1.39 (0.82–2.36)	0.23			

531

532

533 **Table 4. Factors associated with having an antibody response to *M. bovis* lysate above the median at four weeks post-randomisation**

Variable	Univariable analysis			Multivariable analysis		
	% above median change in Ab at four weeks (n/N)	Univariable binomial regression risk ratio (RR) with 95% CI	P-value	% above median change in Ab at four weeks	Multivariable RR (95% CI)	P-value
Randomisation group						
Placebo	34 (14/41)	Reference		34 (14/41)	Reference	
MV	45 (13/29)	1.31 (0.73–2.36)	0.36	45 (13/29)	1.31 (0.74–2.32)	0.35
BCG	74 (23/31)	2.17 (1.35–3.49)	0.01	74 (23/31)	2.10 (1.33–3.34)	0.01
Baseline <i>M. Bovis</i> antibody levels						
Baseline level below median	59 (30/51)	Reference				
Baseline level above median	40 (20/50)	0.68 (0.45–1.02)	0.07			
Mean age at enrolment above median	50 (25/50)	1.02 (0.69–1.51)	0.92			
Woman lives with husband (yes)	51 (38/75)	1.10 (0.69–1.76)	0.70			
Enrolled during rainy season (yes)	31 (5/16)	0.59 (0.28–1.25)	0.17			
BMI above median (yes)	61 (30/49)	1.59 (1.06–2.40)	0.03	61 (30/49)	1.52 (1.05–2.20)	0.03
Previous births						
One birth	55 (22/40)	Reference				
Two births	49 (18/37)	0.88 (0.57–1.36)	0.58			
Three or more births	42 (10/24)	0.76 (0.44–1.31)	0.32			
Last born child died (yes)	0 (0/2)	1.00 (.-)	.			
Last child stillbirth (yes)	100 (4/4)	1.00 (.-)	.			
Mean time since last pregnancy above median (months)	50 (25/50)	1.02 (0.69–1.51)	0.92			
Vaccination history						
Has at least one BCG scar (yes)	47 (35/75)	0.81 (0.54–1.22)	0.31			

Supplementary material

Supplementary table 1: Association between baseline characteristics and antibody levels to measles virus lysate.

Variable	Antibody above median (N=231)	Antibody below median (N=231)	p-value
Sociodemographic characteristics			
Mean age at enrolment	27 (23-30)	26 (23-30)	0.64
Woman lives together with husband % (n)	64.9 (150/231)	58.9 (136/231)	0.18
Enrolled during rainy season (June-November)	78.4 (181/231)	74.0 (171/231)	0.27
Mean Body mass index (BMI)	24 (20-26)	24 (20-27)	0.72
Pregnancy history			
Births			
One birth	47.6 (110/231)	58.4 (135/231)	
Two births	47.6 (110/231)	35.9 (83/231)	0.04
Three or more births	4.8 (11/231)	5.6 (13/231)	
Last child born had died/stillbirth % (n)	4.8 (11/231)	8.7 (20/231)	0.09
Last child born had died % (n)	3.0 (7/231)	4.3 (10/231)	0.46
Last child stillbirth	1.7 (4/231)	4.3 (10/231)	0.17#
Mean time since last pregnancy ended (months)	21 (15-24)	21 (16-24)	0.65
Vaccination history			
Has at least one BCG scar % (n)	79.7 (184/231)	80.5 (186/231)	0.82
Recent vaccination (last four weeks) % (n)	0.9 (2/231)	0.0 (0/231)	0.50#
Risk factors at enrolment (health related)			
Chronic disease % (n)	0.9 (2/231)	0.4 (1/231)	1.00#
Medical consultation (last three months) % (n)	1.7 (4/231)	1.7 (4/231)	1.00

Fischer's exact test.

Supplementary table 2: Association between baseline characteristics and antibody levels to *Mycobacterium bovis* lysate.

Variable	Antibody above median (N=231)	Antibody below median (N=231)	p-value
Sociodemographic characteristics			
Mean age at enrolment	26 (23-30) [231]	26 (22-30) [231]	1.00
Woman lives together with husband % (n)	61.9 (143/231)	61.9 (143/231)	1.00
Enrolled during rainy season (June-November)	72.7 (168/231)	79.7 (184/231)	0.08
Mean Body mass index (BMI)	24 (20-27) [231]	24 (20-27) [231]	0.92
Pregnancy history			
Births			
One birth	50.2 (116/231)	55.8 (129/231)	0.13
Two births	45.9 (106/231)	37.7 (87/231)	
Three or more births	3.9 (9/231)	6.5 (15/231)	
Last child born had died/stillbirth % (n)	5.2 (12/231)	8.2 (19/231)	0.08
Last child born had died % (n)	2.2 (5/231)	5.2 (12/231)	0.14
Last child stillbirth	3.0 (7/231)	3.0 (7/231)	1.00
Mean time since last pregnancy ended (months)	22 (16-25) [231]	20 (16-22) [231]	0.00
Vaccination history			
Has at least one BCG scar % (n)	77.5 (179/231)	82.7 (191/231)	0.16
Recent vaccination (last four weeks) % (n)	0.4 (1/231)	0.4 (1/231)	1.00#
Risk factors at enrolment (health related)			
Chronic disease % (n)	0.4 (1/231)	0.9 (2/231)	1.00#
Medical consultation (last three months) % (n)	1.3 (3/231)	2.2 (5/231)	0.72#

Fischer's exact test.

1 **Impact of pre-pregnancy BCG or measles vaccination on pregnancy outcomes: a nested study**
2 **within a randomised trial in Guinea-Bissau**

3

4 **Short running title**

5 **Pre-pregnancy** Vaccination and Pregnancy Outcomes in Guinea-Bissau

6

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20 **Abstract**

21 **Background:** Vaccination is one of the most effective public health interventions. Beyond disease-
22 specific protection, vaccines may exert non-specific effects (NSEs) on health outcomes. In Guinea-
23 Bissau, maternal and neonatal mortality remain high, and it has been hypothesized that pre-
24 pregnancy vaccination could benefit fertility and pregnancy outcomes.

25 **Methods:** We conducted a placebo-controlled randomised trial at the Bandim Health Project in
26 Bissau, Guinea-Bissau. A total of 2,860 HIV-negative women of fertile age (15–35 years), who had
27 previously given birth, were randomized 1:1:1 to receive placebo, measles vaccine (MV), or BCG.
28 Participants were followed through structured phone calls and home visits. The outcomes included
29 pregnancy incidence, miscarriage, stillbirth, and early neonatal death (within a week). Analyses
30 focused on the first pregnancy after enrolment. Incidence rates, risk ratios, and hazard ratios were
31 estimated using Cox and binomial regression models.

32 **Results:** Five years into the study, of 2,781 women included in the analysis, 1,177 pregnancies were
33 registered, of which 1,062 first pregnancies were analysed for the present paper. Pregnancy
34 incidence was 15.9 per 100 person-years of risk, with no significant differences between groups
35 (placebo: 16.2; MV: 16.5; BCG: 14.9). Rates of failed pregnancy (miscarriage and stillbirth) and early
36 neonatal death did not differ significantly across groups, but early neonatal deaths tended to be
37 higher in the BCG group. Of registered pregnancies, the rate of miscarriage or stillbirth was 78/1000.
38 The early neonatal mortality rate was 36/1,000 live births, higher than national estimates, likely
39 reflecting underreporting in official statistics.

40 **Conclusion:** Maternal vaccination with MV or BCG before conception does not pose safety concerns
41 regarding fertility or pregnancy outcomes. A signal in relation to BCG and neonatal mortality
42 warrants further follow-up. Failed pregnancies and early neonatal mortality remain major problems
43 in Guinea-Bissau.

44 **Keywords:** BCG, fertility, Guinea-Bissau, measles vaccine, non-specific vaccine effects, pre-
45 pregnancy vaccination, pregnancy outcomes.

46

47 INTRODUCTION

48 Vaccination is one of the most cost-effective and successful interventions, saving millions of lives
49 each year (1). As vaccine use has increased over the past 100 years, the evidence has shown that
50 the immunological reaction to vaccination is more complex than initially thought, including that
51 vaccines can have broader non-specific effects (NSEs) aside from the protection against the targeted
52 disease (2–4).

53 Despite significant improvements in maternal and child health in Guinea-Bissau, the country
54 continues to face numerous challenges (5). Several key epidemiological factors contribute to
55 Guinea-Bissau's high burden of stillbirth and neonatal mortality. Infectious diseases, such as malaria,
56 in pregnancy remains widespread and significantly increases the risk of foetal loss (5). In addition,
57 hypertensive disorders of pregnancy, including pre-eclampsia and eclampsia, are highly prevalent
58 and strongly associated with adverse maternal and perinatal outcomes across sub-Saharan Africa
59 (6,7). These determinants, combined with gaps in antenatal and obstetric care, contribute to the
60 high levels of maternal and neonatal mortality in the country (8–10).

61 The prevalence of stillbirth is estimated to be 30/1,000 births and neonatal mortality 34/1,000 live
62 births (11). There is thus a long way to reaching WHO's Sustainable Development Goal of reducing
63 stillbirths and neonatal mortality to below 12 deaths/1,000 births (12).

64 Studies have shown that maternal immunity, can contribute to improved maternal health and may
65 influence early-life immune responses in the offspring (13–16). For both measles vaccine and BCG,
66 it has been observed, that the overall health effects of these vaccines are more pronounced in
67 children whose mothers had pre-existing immunity to the same vaccines. For example, children who
68 received an early measles vaccine (MV) in the presence of maternal antibodies (MatAb) had 50%
69 (20–68%) lower all-cause mortality between 4 months and 5 years of age compared with children
70 who simultaneously received early MV but had no MatAb (15), suggesting that children could
71 benefit in several ways from being born by mothers with high pre-pregnancy MatAb. Furthermore,
72 in a Danish trial, BCG vaccination to newborns whose mothers had been BCG vaccinated was
73 associated with increased benefits in the form of a 35% (95% CI: 6–55%) reduction in hospital
74 admission, an effect which was not seen in children of BCG-unvaccinated mothers (16). This led to
75 the hypothesis that providing these vaccines to women of childbearing age, thus priming them

76 towards the antigens that their future offspring would receive and enhancing the maternal immune
77 level, could be a potential tool to amplify the benefits of the same vaccines in their children and
78 help reduce infant mortality and morbidity.

79 For this reason, we conducted a placebo-controlled randomised trial where non-pregnant women
80 of fertile age were randomised 1:1:1 to receive either placebo, MV or BCG. The vaccines are
81 contraindicated in pregnancy due to theoretical safety concerns. We present data based on 5 years
82 of follow-up on the impact of pre-pregnancy vaccination on fertility, pregnancy outcomes, and the
83 health of the child within the first seven days of life. Follow-up of the cohort is still ongoing.

84 **METHODS**

85 **Setting**

86 The present study was conducted at the Bandim Health Project (BHP, www.bandim.org), in Bissau,
87 Guinea-Bissau. The BHP's Health and Demographic Surveillance System (HDSS) in urban Bissau
88 includes six districts in the capital, covering approximately 100,000 individuals.

89 **Trial design, participants and Sampling**

90 The present study took place within an ongoing randomised trial, initiated in May 2021, which aimed
91 to allocate at least 2,400 women 1:1:1 to placebo, MV or BCG. Inclusion criteria were being HIV-
92 negative, non-pregnant, of fertile age (15 to 35 years old), having given birth one to three times,
93 with the last pregnancy ending at least 12 months ago (or less if the last child had died). The criterion
94 regarding previous children was to ensure that we enrolled women, who had previously been able
95 to become pregnant and were likely to conceive again.

96 Based on HDSS census data, an annual delivery incidence of around 15% was expected, meaning
97 that more than 300 births would naturally occur among the 2,400 enrolled women between the 5th
98 and 10th trimesters after enrolment. With this projected number of pregnancies and births, the
99 study anticipated having a sufficient sample size of 100 mother–infant pairs per randomisation
100 group to assess the primary outcomes of the main trial, namely measles antibody responses and the
101 induction of trained innate immunity in the offspring.

102 In the present study, we followed all enrolled women with available information on pregnancy and
103 delivery outcomes to assess the safety in relation to fertility and pregnancy outcomes.

104 **Enrolment and randomisation of women into the randomised trial**

105 Eligible women were identified through the BHP HDSS databases. A home visit was conducted by a
106 trial assistant, who explained the study. Provided they were interested in participating, women were
107 invited to a nearby health centre for a more detailed explanation. Following oral and written
108 informed consent, eligible women were randomised and vaccinated according to the randomisation
109 allocation.

110 **Follow-up**

111 After inclusion, women were followed in the following ways:

112 **Morbidity and registration of pregnancies:** Initially enrolled women were contacted by telephone
113 every two months. From September 2023, this interval was changed to every 4 months due to a
114 heavy workload. The objective of the morbidity follow-up was to collect information about the
115 general health status of the participants and to detect new pregnancies.

116 **Pregnancy confirmation:** Once a pregnancy was registered during a phone interview or by the trial
117 assistant, a home visit was conducted where the pregnancy was confirmed through a pregnancy
118 test or a previous documented medical examination.

119 **Pregnancy follow-up visits:** Depending on the week of gestation when the pregnancy was
120 registered, two to three additional home visits were performed to monitor the pregnancy and
121 update the woman's status. At these visits, the assistant asked to see the pregnancy card. If the card
122 was seen it was noted if the woman had received antimalarial treatment (IPTp).

123 **Determination of the gestational age:** To estimate the gestational age, we utilised multiple data
124 sources, with priority based on reliability and availability of information. We applied the following
125 prioritisation:

- 126 1. Obstetric ultrasound (sonography). Considered the most accurate method, especially in the
127 first trimester.
- 128 2. Uterine fundal height measurement. Used when ultrasound was not available, measured
129 either by medical personnel at antenatal care visits and documented on the pregnancy card,
130 or measured by our physician/nurse.
- 131 3. In case both ultrasound and fundal height was not available, we calculated an average of the
132 following available dates (up to five):
 - 133 ○ Estimated date of delivery (EDD): Obtained from the woman's pregnancy card.
 - 134 ○ Date of the first day in the last menstrual period (LMP): Collected during both
135 morbidity calls and pregnancy follow-up.
 - 136 ○ Self-reported gestational age: The gestational age reported directly by the pregnant
137 woman during morbidity follow-up and pregnancy follow-up. This estimate is based
138 on the woman's own perception of the duration of her pregnancy, always expressed
139 in months.

140 In cases where miscarriage or stillbirth occurred and the pregnancy was not previously identified,
141 an interview was carried out to estimate the gestational age at the time of the event.

142 After the pregnancy was concluded, a follow-up visit was conducted to update the woman's status
143 and the status of the child.

144 **Outcomes**

145 Outcomes in the present study included the incidence of pregnancy, miscarriage (failed pregnancy
146 occurring before 24 weeks of gestation) (17–19), stillbirth (defined as babies born with no signs of
147 life beyond 24 weeks of gestation), and early neonatal deaths (defined as the death of a live-born
148 infant within the first 7 days of life) (20,21).

149 **Statistical analyses**

150 We focused on the first pregnancy after inclusion in the present study. Consequently, pregnancy
151 follow-up of women was censored at the time of the first registered pregnancy.

152 The baseline characteristics by randomisation group were analysed separately for all women
153 included in the trial, and for women with confirmed pregnancies. Background characteristics are
154 presented as percentages for categorical variables, while continuous variables are presented as
155 mean $\pm$ standard deviation by randomisation group. Assessment of differences in proportion among
156 the randomization groups were carried out using chi-squared or Fisher's exact test. Distributions of
157 continuous variables were compared using the Kruskal-Wallis rank-sum test.

158 The incidence of pregnancy was expressed as the number of events per 100 person-years of follow-
159 up, and comparisons between groups were made using the hazard ratio (HR) with 95% confidence
160 interval estimated using a Cox proportional hazards model with time since enrolment as the
161 underlying time scale. Results were presented overall and stratified by year of follow-up in the trial.

162 The early neonatal death rate was calculated per 1,000 live-born neonates. Comparisons between
163 groups were also done by Cox analysis yielding HRs (with 95% confidence interval). Children were
164 considered at risk from the day of birth and children that were born alive but died on the same day
165 were included in the analysis by adding 0.01 days of follow-up to the exit variable.

166 The proportional hazards assumption was assessed visually and tested with Schoenfeld residuals.
167 We found no violations of proportionality assumption (Supplementary Figure 1-3).

168 For failed pregnancy (stillbirth + miscarriage), the rate of pregnancy loss was calculated per 1,000
169 pregnancies using binomial regression providing Risk Ratios (RRs) (with 95% confidence interval) for
170 comparisons between randomisation groups.

171 **Ethical approval**

172 The trial was approved by the National Committee for Ethics in Health Research of Guinea-Bissau
173 (CNES), reference number **043/CNES/INASA/2019**, and the trial was pre-registered with
174 ClinicalTrials.gov trial ID [NCT04899765](https://clinicaltrials.gov/ct2/show/study/NCT04899765). As part of the study protocol, participating women received
175 a range of health services, including free consultations, pregnancy follow-up, basic medications, and
176 counselling, in line with national health and ethical standards. These services were also made
177 available to women who chose not to participate in the trial.

178 Data were analysed with Stata 19 software.

179 **RESULTS**

180 From May 2021 to February 2026, a total of 2,860 participants were randomised equally between
181 the three intervention groups: placebo (n=953), MV (n=954), and BCG (n=953). Of these, 16 were
182 not included in the analysis as they withdrew consent (placebo: 3; MV: 5; BCG: 8) and 63 women
183 were excluded as they had no follow-up after the day of enrolment (placebo: 19; MV: 19; BCG: 25)
184 (Figure 1). The analysis therefore included 2,781 (97%) women: 931 in the placebo group, 930 in the
185 MV group, and 920 in the BCG group.

186 By the end of the follow-up on 11 February 2026, a total of 1,177 women confirmed pregnancies
187 had been registered (Figure 1), distributed among the placebo (n=407), MV (n=401) and BCG
188 (n=369) group. A total of 115 second pregnancies were excluded (placebo: 44; MV: 38; BCG: 33) in
189 the present analysis; thus, 1062 first pregnancies were included in the analysis of pregnancy
190 outcomes: placebo (n=363), MV (n=363) and BCG (n=336). At the time of writing, 997 pregnancies
191 had ended, 338 in the placebo group, 338 in the MV group and 321 in the BCG group. Approximately
192 11% were reported as home births.

193 **Baseline characteristics of all trial participants and of woman who became pregnant after** 194 **inclusion**

195 Baseline characteristics were evenly distributed between the randomisation arms, with the mean
196 age of the participants being 27 (Interquartile range: 23-30) years. Most women lived together with
197 their husband (approximately 63% across all arms). Pregnancy history before enrolment was also
198 comparable, with around 52% of women having had one birth, 31% two births, and 17% three or
199 more births. A proportion reported adverse outcomes in their last pregnancy, including stillbirths
200 (≈2%) and child deaths (≈4%). The mean time since the last ended pregnancy was approximately 22
201 months (Tables 1 and 2).

202 **Pregnancy incidence**

203 The overall first pregnancy incidence was 15.9 per 100 Person-Years of Risk (PYRs), without
204 significant differences between randomisation groups (placebo: 16.2 (363/815938 person days);
205 MV: 16.5 (363/804120 person days) and BCG: 14.9 (336/825249 person days))(Table 3).

206 **Main pregnancy outcomes**

207 Table 4 presents the main pregnancy outcomes among the randomisation groups, including early
208 neonatal death, and pregnancy failure (miscarriages and stillbirths). The data is presented overall
209 and for singleton deliveries only.

210 Regarding failed pregnancy (miscarriages and stillbirths), the overall rate was 78/1000 pregnancies.
211 No statistically significant differences were observed between the three groups: MV vs. placebo: RR
212 = 1.12 (0.67-1.88) and BCG vs. placebo: RR = 1.05 (0.62-1.79).

213 For early neonatal death, the observed rates were placebo: 25/1,000 live births; MV 31/ 1,000 births
214 and BCG 53/1,000 births. The risk ratio (RR) for MV compared to placebo was 1.26 (0.50-3.19), while
215 for BCG versus placebo it was 2.15 (0.92-5.00).

216 In the analysis restricted to singleton pregnancies (Table 4), the rate of early neonatal death was 26
217 per 1,000 live births in the placebo group, 10 per 1,000 in the MV group and 45 per 1,000 in the BCG
218 group, corresponding to a MV vs. Placebo RR of 0.38 (0.10-1.42) and a BCG vs. Placebo RR of 1.74
219 (0.72-4.17).

220 **Effects by year of follow-up**

221 When analysing the main pregnancy outcomes across years of follow-up, the pregnancy incidence,
222 the incidence of failed pregnancies and early neonatal deaths showed variation across time (Table
223 5). No systematic pattern was observed across the years of follow-up.

224 **The use of malaria prophylaxis in pregnancy**

225 Among women who had terminated their pregnancy, a subset had information on the use of IPTp
226 from one or more pregnancy visits.

227 Subgroup analyses revealed that pregnancy failure rates were very low among women who had
228 received IPTp: Placebo 5/1,000 (1/204), MV 14/1,000 (3/218), and BCG 21/1,000 (4/191) compared
229 with those who did not receive IPTp: Placebo 58/1,000 (6/103), MV 41/1,000 (5/122), and BCG
230 18/1,000 (2/113). Numbers were too small to indicate any differential effect of BCG or MV by IPTp
231 status.

232 **DISCUSSION**

233 The main results did not show statistically significant differences between the randomisation
234 groups, indicating no effect of the interventions on fertility and pregnancy outcomes. A tendency
235 for increased early neonatal mortality in the BCG group did not show any systematic association
236 with time since vaccination, as would have been anticipated if it was a biological effect, but warrants
237 further follow-up.

238 **Strengths and limitations**

239 Among the strengths include the randomised trial and a high retention rate, with 97% of participants
240 retained in the analysis after randomisation (Supplementary table 1), which strengthens the internal
241 validity of the study. The randomisation was well-balanced between the groups in terms of
242 sociodemographic characteristics, pregnancy history, vaccination history, and health risk factors.
243 The follow-up was structured with follow-up phone calls every (two or) four months after inclusion
244 and every two months after pregnancy registration, which allowed detailed data collection and early
245 pregnancy detection. This enabled the monitoring of pregnancy incidence and evaluation of the
246 impact of vaccine effects. Furthermore, we enrolled women that had previously given birth, for
247 which reason the women have better understanding of the different scenarios that characterise
248 pregnancy, regarding symptoms, risks and care.

249 Among the limitations are that although women of childbearing age were included, the results may
250 not be generalizable to all women, especially those with different sociodemographic characteristics
251 or without previous pregnancies (nullipara). The data regarding the time to pregnancy and
252 pregnancy outcomes were based on self-reported data, sometimes without the possibility of
253 confirmation by ultrasound or other medical documentation, which could result in recall bias.

254 The trial was not originally designed to evaluate the impact of vaccination in women on fertility or
255 pregnancy outcomes. Therefore, the findings related to fertility and pregnancy outcomes should be
256 interpreted with caution.

257 The study excluded HIV-positive women but did not include systematic assessment of important
258 determinants of pregnancy outcomes in Guinea-Bissau, such as malaria infection during pregnancy
259 or hypertensive complications.

260 **Consistency with previous studies**

261 Our findings are consistent with previous evidence showing that both BCG and MV can be safely
262 administered to adults, though a signal in relation to BCG vaccine and early neonatal death should
263 be followed up (22–24).

264 This study, along with other studies, shows that pregnancy loss remains common and represents a
265 significant public health burden in West Africa, especially in Guinea-Bissau (11,25). Pregnancy loss
266 is often more common than neonatal and infant deaths but frequently not reported (26,27).

267 According to the literature, pregnancy loss due to miscarriage is often associated with the woman's
268 general health status, working conditions, trauma, lack of adequate health care, and exposure to
269 infectious diseases (28,29). Therefore, the number of pregnancy losses observed in this study is not
270 surprising, especially considering that Guinea-Bissau still suffers from high infectious disease
271 pressure while there are at the same time significant limitations in the health infrastructure
272 including access to prenatal care (30,31).

273 The incidence of pregnancy failure was 78/1000 pregnancies, but the rate of stillbirth did not
274 contribute a lot to this, as it was 19/1000 pregnancies, lower compared to the UNICEF estimate for
275 stillbirth (31/1,000 births) (11). The lower risk in the present study may be related to the fact that
276 we did not enrol HIV-positive women and that the risk of stillbirth is highest in the first pregnancy
277 (32,33). Also, women who lived within the BHP HDSS, were found at home and agreed to
278 participate, may have had a lower risk *a priori*. Furthermore, official statistics may suffer from
279 deficiencies in the classification and monitoring of miscarriages and stillbirth (34,26). Our study
280 carried out active pregnancy monitoring, therefore likely providing more accurate information.

281 Although this study only included early neonatal deaths, the mortality rates observed in this age
282 group were 36/1000, which is higher than the national estimate for Guinea-Bissau when considering
283 overall neonatal mortality (including both early and late neonatal deaths) (34/1000) (11,35). We did
284 not specifically ask for early neonatal deaths in previous pregnancies, thus, we cannot compare
285 internally within the cohort. Any difference from the national estimate may reflect underreporting
286 in official statistics. In Guinea-Bissau, many vital events, including neonatal deaths, occur at home
287 without medical attention and are often not reported to health authorities or civil registration
288 systems (36).

289 **Interpretation and implications**

290 This study confirms that pregnancy loss and neonatal mortality remain important public health
291 burdens in Guinea-Bissau.

292 This randomized trial did not identify statistically significant differences in fertility or pregnancy
293 outcomes between women allocated to MV, BCG, or placebo prior to pregnancy. However, the
294 cohort will continue to be followed to elucidate if a safety signal in relation to BCG vaccine is
295 sustained. The absence of beneficial effects at this stage suggests that such interventions should not
296 be expected to improve pregnancy outcomes in this context.

297 The study contributes valuable evidence to counter misconceptions and conspiracy theories about
298 vaccines affecting fertility, which are prevalent in some African settings (37,38).

299 From a public health perspective, the findings emphasise that maternal vaccination strategies
300 should continue to be guided by their primary purpose - protection against infectious diseases and
301 enhancement of infant immunity (39,40) - rather than on any expectations of effects on fertility or
302 pregnancy outcomes.

303 A UNDP report from the same group of women made during the study confirmed widespread
304 adoption of preventive measures: 92% of pregnant women received at least one IPTp dose (average
305 of three), and 89% reported sleeping under bed nets. These findings highlight that IPTp coverage
306 and bed net use are high, reducing malaria's impact on pregnancy outcomes (41).

307 The small number of women with IPTp information limits the ability to detect significant differences
308 between randomized groups by IPTp status. The much lower rate of pregnancy failures among IPTp
309 recipients could to a large extent be due to healthy user effect and healthy survivor effects, but
310 malaria remains a leading cause of pregnancy failure in Africa, particularly in Guinea-Bissau (5).

311

312 **CONCLUSION**

313 In this study, we found no significant effects of pre-pregnancy MV or BCG vaccination on fertility,
314 pregnancy outcomes, or early neonatal mortality risk. The findings in relation to fertility provide
315 valuable reassurance against misconceptions and conspiracy theories linking vaccines to infertility,
316 which are prevalent in African settings. A signal for potentially increased risk of early neonatal
317 mortality in the BCG group warrants further follow-up.

318 Pregnancy failures and neonatal mortality remain major public health challenges in Guinea-Bissau.
319 The incidence observed in this study highlights the importance of active surveillance, as official
320 statistics often underestimate or misclassify adverse pregnancy outcomes.

321

322

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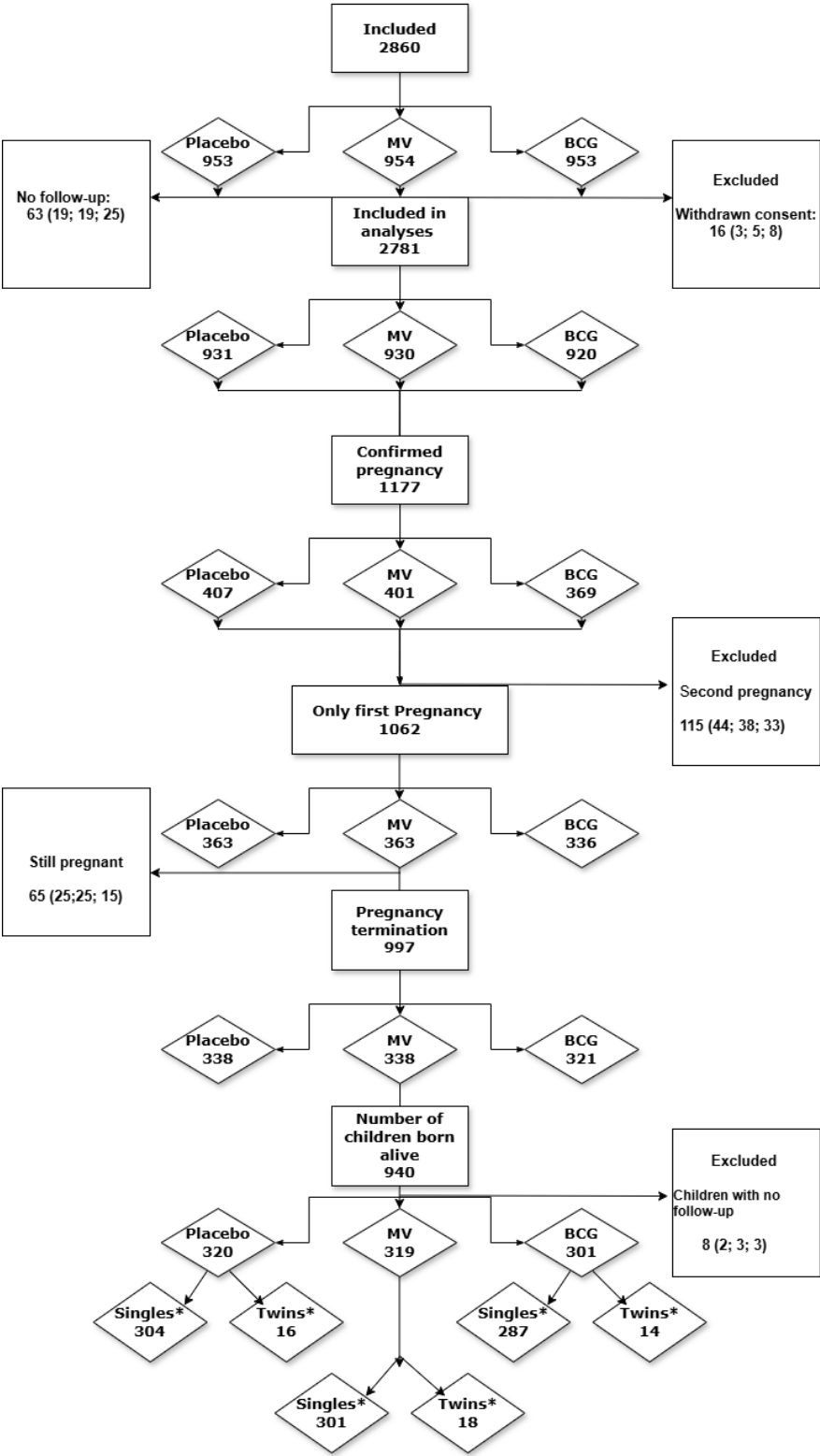
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461 **Figure 1. Flow chart of inclusions**



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463 **Singles = singletons; Twins = Twins or more**

464 **Table 1: Baseline characteristics of trial participants.**

Variable	Placebo (N=931)	MV (N=930)	BCG (N=920)	p-value	
				MV vs placebo	BCG vs placebo
Sociodemographic characteristics					
Mean age at enrolment	27 (23-30)	27 (23-30)	27 (23-30)	0.98	0.91
Woman lives together with husband % (n)	63.9 (595/931)	63.4 (590/930)	61.6 (567/920)	0.81	0.31
Enrolled during rainy season (May-November)	54.2 (505/931)	54.2 (504/930)	54.2 (499/920)	0.96	0.98
Mean Body mass index	24 (20-27) [931]	24 (20-27) [929]	24 (20-27) [920]	0.51	1.00
Pregnancy History					
Births					
One birth	51.8 (482/931)	51.7 (481/930)	52.4 (482/920)		
Two births	32.4 (302/931)	30.4 (283/930)	30.5 (281/920)	0.39	0.58
Three or more births	15.7 (146/931)	17.8 (166/930)	17.1 (157/920)		
Last child born had died/stillbirth % (n)	5.7 (53/931)	6.5 (60/930)	6.0 (55/920)	0.50	0.80
Last child born died postpartum % (n)	3.5 (33/931)	4.4 (41/930)	3.7 (34/920)	0.35	0.86
Last child stillbirth % (n)	2.1 (20/931)	2.0 (19/930)	2.3 (21/920)	0.87	0.85
Mean number of months since last pregnancy ended	22 (14-27) [931]	21 (14-26) [930]	22 (14-27) [920]	0.96	0.83
Vaccination history					
Has at least one BCG scar % (n)	73.9 (688/931)	73.3 (682/930)	73.6 (677/920)	0.75	0.85
Recent vaccination (last 4 weeks) % (n)	0.4 (4/931)	0.2 (2/930)	0.3 (3/920)	0.69	1.00
Risk factors at enrolment (health related)					
Chronic disease % (n)	0.4 (4/931)	0.9 (8/930)	0.4 (4/920)	0.39	1.00
Medical consultation (last 3 months) % (n)	6.2 (58/931)	4.8 (45/930)	5.3 (49/920)	0.19	0.40

Table 2: Baseline characteristics of women who became pregnant after enrolment in trial.

Variable	Placebo (N=363)	MV (N=363)	BCG (N=336)	p-value	
				MV vs placebo	BCG vs placebo
Sociodemographic characteristics					
Mean age at enrolment	27 (24-30)	27 (23-30)	27 (23-30)	0.90	0.77
Woman lives together with husband % (n)	78.2 (284/363)	78.8 (286/363)	77.1 (259/336)	0.86	0.77
Enrolled during rainy season (May-November)	54.5 (198/363)	55.9 (203/363)	53.9 (181/336)	0.71	0.86
Mean Body Mass Index	24 (20-27) [363]	24 (21-28) [362]	24 (20-26) [336]	0.88	0.48
Pregnancy History					
Births					
One birth	44.6 (162/363)	44.1 (160/363)	44.3 (149/336)		
Two births	36.1 (131/363)	36.9 (134/363)	36.9 (124/336)	0.97	0.97
Three or more births	19.3 (70/363)	19.0 (69/363)	18.8 (63/336)		
Last child born had died/stillbirth % (n)	9.1 (33/363)	6.6 (24/363)	8.9 (30/336)	0.21	0.95
Last child born had died % (n)	5.5 (20/363)	4.7 (17/363)	4.8 (16/336)	0.58	0.68
Last child stillbirth	3.6 (13/363)	1.9 (7/363)	4.2 (14/336)	0.17	0.68
Mean number of months since last pregnancy ended	22 (14-28) [363]	23 (15-31) [363]	23 (14-31) [336]	0.81	0.93
Vaccination history					
Has at least one BCG scar % (n)	74.4 (270/363)	71.3 (259/363)	74.4 (250/336)	0.36	0.99
Recent vaccination (last 4 weeks) % (n)	0.8 (3/363)	0.6 (2/363)	0.0 (0/336)	1.00	0.25
Risk factors at enrolment (health related)					
Chronic disease % (n)	0.8 (3/363)	0.3 (1/363)	0.9 (3/336)	0.62	1.00
Medical consultation (last 3 months) % (n)	5.8 (21/363)	5.2 (19/363)	5.4 (18/336)	0.74	0.81

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469 **Table 3. Pregnancy incidence among enrolled women by randomisation group**

	Placebo	MV	BCG	HR from Cox model	
	[pregnancies/women]	[pregnancies/women]	[pregnancies/women]	(95% confidence interval)	
	[363/931]	[363/930]	[336/920]	MV vs Placebo	BCG vs Placebo
Incidence rate per 100 years (events / total days)					
Overall	16.2 (363/815938)	16.5 (363/804120)	14.9 (336/825249)	1.01 (0.87-1.17)	0.91 (0.79-1.06)

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471

472 **Table 4: Pregnancy outcome and early neonatal deaths by randomisation group.**

	Placebo	MV	BCG	HR from Cox model (95% confidence interval)	
	Mortality rate per 1,000 children born alive (events / total years of follow-up) [number of children]			MV vs placebo	BCG vs placebo
Early neonatal death					
Overall	25.0 (8/2192) [320]	31.3 (10/2165) [319]	53.2 (16/2025) [301]	1.26 (0.50-3.19)	2.15 (0.92-5.00)
Singletons	26.3 (8/2080) [304]	10.0 (3/2082) [301]	45.3 (13/1941) [287]	0.38 (0.10-1.42)	1.74 (0.72-4.17)
	Incidence rate per 1000 pregnancies (events / number of pregnancies)			MV vs placebo	BCG vs placebo
Failed pregnancies					
Overall					
Failed pregnancy#	74 (25/338)	83 (28/338)	78 (25/321)	1.12 (0.67-1.88)	1.05 (0.62-1.79)
Stillbirth	18 (6/338)	21 (7/338)	19 (6/321)	1.17 (0.40-3.44)	1.05 (0.34-3.23)
Miscarriages	56 (19/338)	62 (21/338)	59 (19/321)	1.11 (0.61-2.02)	1.05 (0.57-1.95)
Singletons					
Failed pregnancy#	73 (24/329)	82 (27/329)	77 (24/313)	1.21 (0.62-2.39)	1.00 (0.49-2.04)
Stillbirth	15 (5/329)	21 (7/329)	19 (6/313)	0.94 (0.24-3.75)	1.55 (0.43-5.60)
Miscarriages	58 (19/329)	61 (20/329)	58 (18/313)	1.31 (0.60-2.86)	0.81 (0.33-1.94)

473 *# Combined incidence of stillbirths and miscarriages.*

474 Note: This table only includes pregnancies that have been recorded as terminated by either a failed pregnancy or the delivery of a child.

475 **Table 5. Pregnancy incidence, pregnancy outcomes and early neonatal deaths by randomisation group and year of follow-up**

	Placebo	MV	BCG	HR from Cox model (95% confidence interval)	
				MV vs Placebo	BCG vs Placebo
Pregnancy Incidence rate per 100 years (events / total days)					
By year of follow-up (since enrolment) [total women]					
Overall	16.2 (363/815938) [931]	16.5 (363/804120) [930]	14.9 (336/825249) [920]	1.01 (0.87-1.17)	0.91 (0.79-1.06)
1st year	16.68 (146/319683) [931]	17.41 (152/318965) [930]	15.57 (136/319103) [920]	1.05 (0.83-1.31)	0.93 (0.74-1.17)
2nd year	21.90 (145/241854) [785]	20.41 (135/241536) [778]	18.39 (123/244261) [784]	0.93 (0.74-1.18)	0.84 (0.66-1.07)
3rd year	10.87 (46/154602) [526]	15.77 (64/148219) [524]	12.54 (55/160151) [550]	1.45 (1.00-2.12)	1.16 (0.78-1.71)
4th year	10.78 (24/81350) [320]	5.23 (11/76871) [297]	8.19 (19/84781) [326]	0.48 (0.24-0.99)	0.76 (0.42-1.39)
5th year	3.96 (2/18449) [149]	1.97 (1/18529) [145]	6.46 (3/16953) [145]	0.51 (0.05-5.47)	1.60 (0.28-9.29)
Failed pregnancy Incidence rate per 1,000 pregnancies (events / total women)					
Overall	74 (25/338)	83 (28/338)	78 (25/321)	1.12 (0.67-1.88)	1.05 (0.62-1.79)
1st year	66 (9/136)	74 (11/148)	102 (13/127)	1.03 (0.37-2.84)	1.55 (0.58-4.10)
2nd year	60 (12/199)	60 (12/199)	32 (6/188)	1.22 (0.44-3.36)	0.40 (0.10-1.53)
3rd year	53 (4/76)	58 (5/86)	76 (6/79)	1.77 (0.33-9.44)	1.28 (0.22-7.53)
4th year	0 (0/30)	0 (0/22)	0 (0/27)	--	--
5th year	0 (0/4)	0 (0/1)	0 (0/10)	--	--
Early neonatal death Incidence rate per 1000 children (events / total days) [total children]					
Overall	25.0 (8/2192) [320]	31.3 (10/2165) [319]	53.2 (16/2025) [301]	1.26 (0.50-3.19)	2.15 (0.92-5.00)
1st year	41.7 (3/485) [72]	31.7 (2/415) [63]	61.2 (3/315) [49]	0.78 (0.13-4.63)	1.51 (0.31-7.39)
2nd year	32.3 (5/1048) [155]	29.8 (5/1124) [168]	54.9 (9/1086) [164]	0.94 (0.27-3.23)	1.74 (0.72-4.17)
3rd year	0.0 (0/428) [62]	41.7 (3/482) [72]	30.3 (2/443) [66]	--	--
4th year	0.0 (0/200) [30]	0.0 (0/144) [21]	52.6 (1/126) [19]	--	--
5th year	0.0 (0/31) [5]	. (./.) [0]	111.1 (1/55) [9]	--	--

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478 **Supplementary table 1: Months of follow-up of women included in the study by randomisation**479 **group.**

Months follow-up	Placebo group % (n/N)	MV group % (n/N)	BCG group % (n/N)
2	98 (930/950)	98 (929/949)	97 (920/945)
4	97 (925/950)	97 (922/949)	96 (911/945)
6	97 (920/950)	97 (918/949)	96 (904/945)
8	95 (899/950)	95 (901/949)	94 (890/945)
10	93 (885/950)	94 (889/949)	94 (884/945)
12	90 (854/945)	91 (862/945)	90 (849/940)
14	91 (839/918)	92 (841/917)	90 (822/911)
16	89 (789/891)	90 (800/890)	88 (776/886)
18	89 (761/859)	89 (765/859)	88 (754/855)
20	85 (698/826)	86 (709/827)	86 (708/823)
22	86 (683/790)	88 (692/789)	87 (683/787)
24	83 (626/750)	85 (638/751)	83 (621/745)
26	85 (612/720)	87 (623/720)	85 (605/714)
28	80 (549/682)	83 (567/683)	81 (551/678)
30	82 (526/638)	84 (534/636)	81 (516/635)
32	80 (460/576)	80 (460/574)	80 (459/572)
34	79 (430/544)	79 (430/544)	79 (428/541)
36	74 (356/483)	73 (353/485)	74 (355/482)
38	77 (335/437)	77 (337/440)	77 (336/436)
40	72 (276/386)	71 (275/389)	73 (281/385)
42	74 (250/339)	73 (249/342)	74 (250/339)
44	67 (207/310)	66 (205/311)	69 (212/306)
46	68 (184/270)	63 (170/270)	70 (186/265)
48	62 (128/206)	59 (123/209)	64 (131/204)
Any follow-up	98 (931/950)	98 (930/949)	97 (920/945)

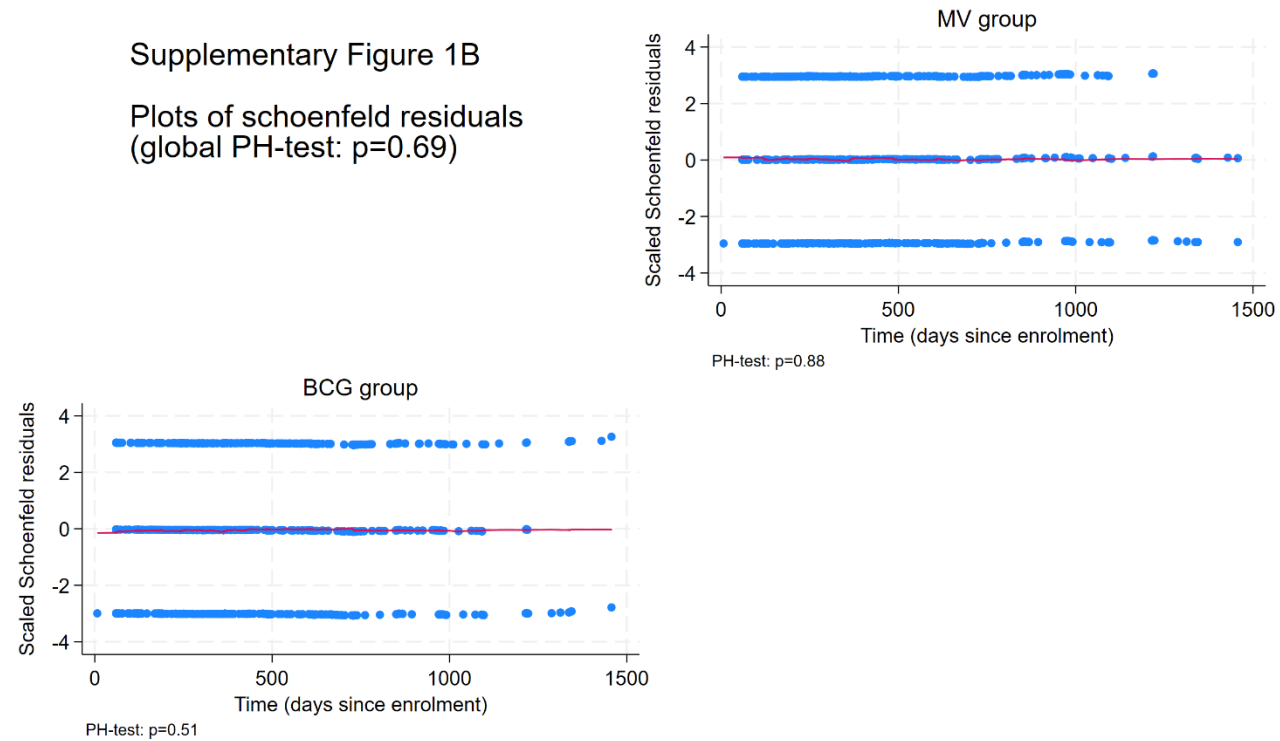
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483 **Supplementary Figure 1:**
484 **Assessment of the proportional hazards assumption for pregnancy incidence: Schoenfeld residuals by intervention group (MV and**
485 **BCG)**

Supplementary Figure 1B
Plots of schoenfeld residuals
(global PH-test: $p=0.69$)



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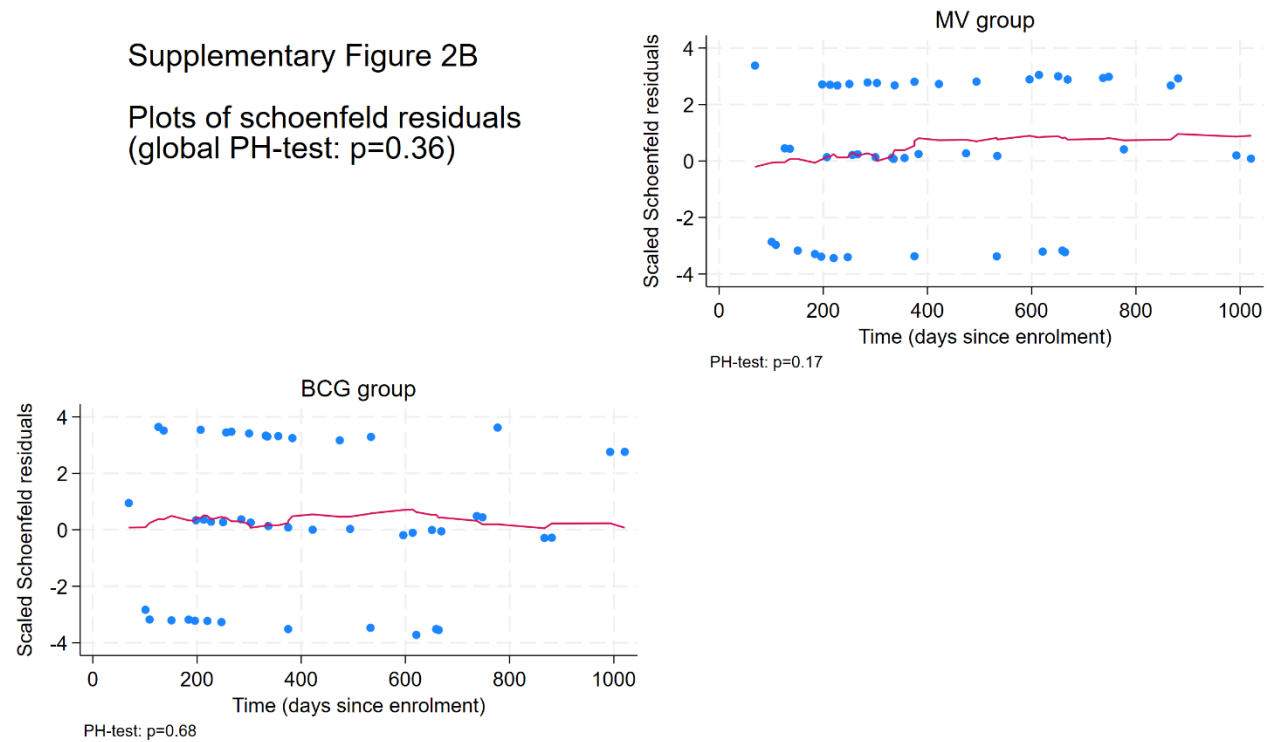
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491 **Supplementary Figure 2:**
492 **Assessment of the proportional hazards assumption for pregnancy failure (miscarriage and stillbirth): Schoenfeld residuals by**
493 **intervention group (MV and BCG)**

Supplementary Figure 2B

Plots of schoenfeld residuals
(global PH-test: $p=0.36$)

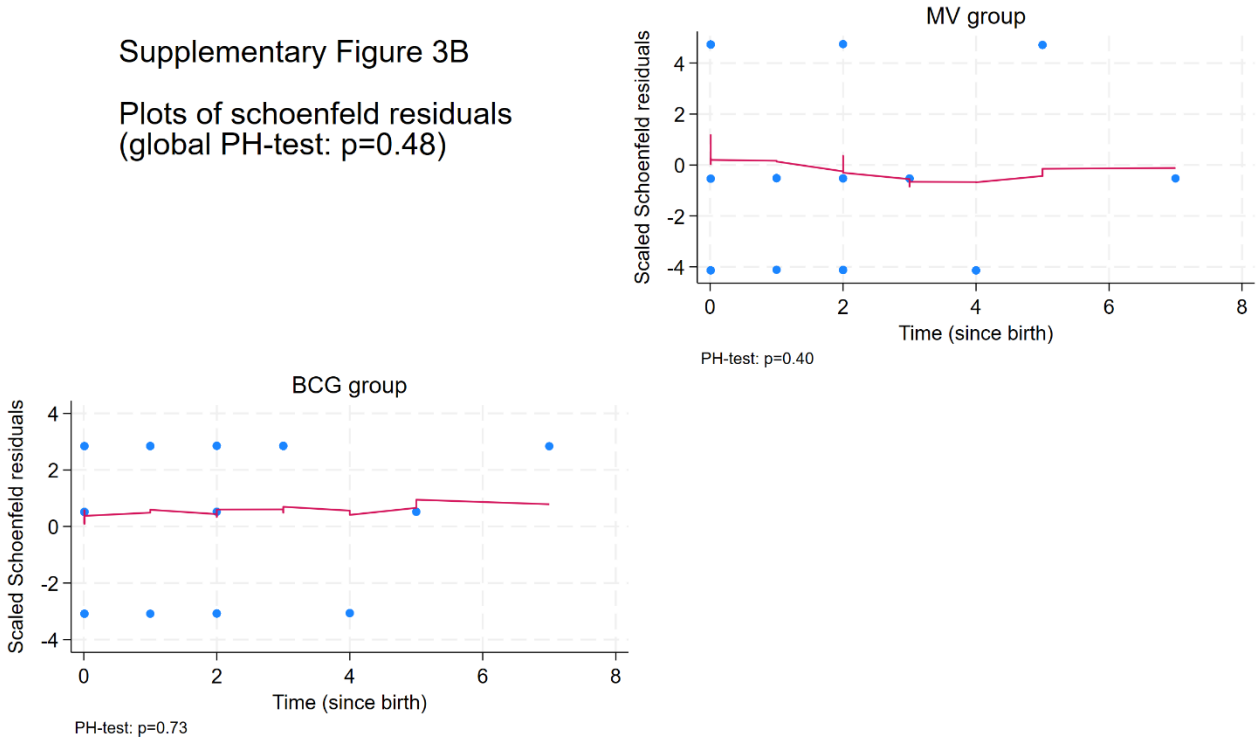


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498 **Supplementary Figure 3: Assessment of the proportional hazards assumption for early neonatal mortality: Schoenfeld residuals by**
499 **intervention group (MV and BCG)**



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