

Evaluating the effects of polio vaccines on general child health in Guinea-Bissau

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Evaluating the effects of polio vaccines on general child health in Guinea-Bissau

Colophon

This thesis was submitted to the Graduate School of Health Sciences at the University of Southern Denmark on 30th of June 2023.

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ABBREVIATIONS

BCG	Bacille Calmette-Guerin vaccine
BHP	Bandim Health Project
bOPV	Bivalent oral polio vaccine
CI	95% confidence interval
C-OPV	Campaign with oral polio vaccine
cVDPV	Circulating vaccine-derived poliovirus
DTP(1-3)	Diphtheria-tetanus-pertussis vaccine
F/M	Female/male
HDSS	Health and demographic surveillance site
Hib	Haemophilus influenzae type b vaccine
HNSM	National Hospital Simão Mendes
HR	Hazard ratio
INDEPTH	The International Network of field sites with continuous Demographic Evaluation of Populations and Their Health
IPTW	Inverse probability of treatment weighting
IPV	Inactivated polio vaccine
MUAC	Mid-upper-arm-circumference
MR	Mortality rate
MRR	Mortality rate ratio
MV	Measles vaccine
NSE	Non-specific effects
OPV(1-3)	Oral polio vaccine
OPV0	Oral polio vaccine scheduled at birth
PCV	Pneumococcal conjugate vaccine
Penta(1-3)	Pentavalent vaccine (diphtheria-tetanus-pertussis-hepatitis B-haemophilus influenzae type b)
PS	Propensity score
PYRS	Person-years at risk
RCT	Randomised controlled trial
Rota	Rotavirus vaccine
RR	Relative risk
tOPV	Trivalent oral polio vaccine
YF	Yellow fever vaccine
VA	Verbal autopsy
VAPP	Vaccine-associated paralytic polio

VAS Vitamin A Supplementation
WHO World Health Organisation
WP(1-3) Wild poliovirus

PREFACE

In the spring of 2018, Bandim Health Project posted a job as a Research Assistant based in Bissau, Guinea-Bissau. From the moment I read the job post, I knew I would not let the opportunity pass. For me, it was a unique opportunity to work within global health and more specifically maternal and child health, combining field work and evaluation of health interventions. And it certainly has been a unique experience; from arriving in Bissau, learning the language and the culture, to meeting my husband - all while conducting research.

There are many people who has been part and formed my journey along this experience. First, I feel very lucky and privileged to have had Ane as my supervisor. Your drive, ambitions and curiosity set a high standard for conducting research and combined with your 24/7 presence and support it provides great motivation and inspiration. Likewise, Peter and Christine, your very down-to-earth mentality, your eagerness to create and grab opportunities and inviting others to join, when for example writing a book chapter, really contributes to a team feeling despite often a physical distance. Furthermore, none of this work would have been possible without the local BHP assistants in Bissau. Their openness to receive and teach us many students about Guinea-Bissau both in verbal and non-verbal form has given me precious insights and experiences both for my work and my life and is a big part of why I today still experience Bissau as my (and my family's) second home. Returning to Denmark, I want to thank my colleagues and friends at OPEN for opening your arms and letting me be part of your work environment in both a scientific and personal manner. Second, I want to thank the rest of my Bandim colleagues/friends for caring, supporting and co-running and -jumping at the stadium in Bissau, or just making sure Bobbi was well looked after. Last, I want to thank my husband, daughter, and family for just being there, being honest and supportive, and always trying to put a smile on my face although it sometimes has been further away than ever.

When people ask me what my PhD investigates, it is very hard to keep it short, but PhD courses, in particular, have taught me to keep it short in the interest of capturing and holding people's attention. So, I say "the effect of polio vaccines on general child health in Guinea-Bissau". However, to me, this is merely the endpoint. All the prior steps, including field work, supervision, data management, research communication and presentation, leading to this endpoint have

been crucial for both my professional and personal development, and for how I, today, read, conduct, interpret and reflect on research.

Formed by a practical understanding of data and its methods on the ground in Guinea-Bissau, the three quantitative studies included in this dissertation have been conducted. However, this have not been without bumps on the way – but I have tried to make the most of these bumps and grab them as opportunities. To mention the most influential bump - Covid-19 came along, and we were all “sent home” (to Denmark). But an opportunity arose for being part of conducting a randomised trial from the get-go – and I grabbed it. It indeed, was a huge task, both professionally and personally; assessing the effect of distributing cloth face masks on Covid-19-like illness during a pandemic, takes hard(er) work, (more) resources, and acknowledgement of strength of weaknesses. However, being surrounded by an optimistic, ambitious, and supportive team of colleagues, the research output for me was quite unique, and I would do it all over again – though with more insights and scepticism on the prerequisites for conducting large-scale health interventions.

Back to the three studies included in this dissertation; they also represent opportunities. With one randomised trial and two natural experiments, they reflect on one side, how research can be planned thoroughly – from design, data collection and the statistical methods. On the other side, they reflect how reality can “plan” the unplanned opportunities for research – if you are aware of and open to them, and of course are so fortunate to have a well-established basis, such as a health and demographic surveillance system, for exploiting them. Hence, this thesis should not merely be a contribution to the evidence base on the effect of polio vaccines on general child health. It should also demonstrate how the effects can be measured in various ways – each by cautiously accounting for the reality by which the data is formed.

LIST OF SCIENTIFIC CONTRIBUTIONS NOT INCLUDED IN THIS THESIS

Nanque LM, Jensen AM, Diness AR, Cabral C, Nielsen S, Cawthorne D, Martin JSD, Ca, EJC, Jensen K, Martins CL, Rodrigues A, Fisker A. Effect of Distributing Locally Produced Cloth Facemasks on COVID-19-Like Illness and All-Cause Mortality – a Cluster-Randomised Controlled Trial in Urban Guinea-Bissau. Preprint: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4307646

Varma A, Thyssen SM, Martins JSD, **Nanque LM**, Jensen AKG, Fisker AB. Overall effect of a campaign with measles vaccine on the composite outcome mortality or hospital admission: A cluster-randomized trial among children aged 9-59 months in rural Guinea-Bissau. *International journal of infectious diseases* : IJID : official publication of the International Society for Infectious Diseases 2023; 134: 23-30.

Nanque LM, Fisker AB. Maximising the lessons learned from trial data after emergency use listing of a novel oral polio vaccine. *The Lancet*. 2023;401(10371):83-5.

Medeiros MM, Ingham AC, **Nanque LM**, Correia C, Stegger M, Andersen PS, et al. Oral polio revaccination is associated with changes in gut and upper respiratory microbiomes of infants. *Frontiers in Microbiology*. 2022;13.

Fisker AB, Martins JSD, **Nanque LM**, Jensen AM, Ca EJC, Nielsen S, et al. Oral Polio Vaccine to Mitigate the Risk of Illness and Mortality During the Coronavirus Disease 2019 Pandemic: A Cluster-Randomized Trial in Guinea-Bissau. *Open Forum Infectious Diseases*. 2022;9(9).

Aaby P, Nielsen S, Fisker AB, **Pedersen LM**, Welaga P, Hanifi SMA, et al. Stopping Oral Polio Vaccine (OPV) After Defeating Poliomyelitis in Low- and Middle-Income Countries: Harmful Unintended Consequences? Review of the Nonspecific Effects of OPV. *Open Forum Infectious Diseases*. 2022;9(8).

Pedersen LM, Benn CS. Vaccinationer. Forebyggende sundhedsarbejde. 7 ed. Copenhagen: Munksgaard; 2021.

Varma A, Jensen AKG, Thyssen SM, **Pedersen LM**, Aaby P, Fisker AB. Research protocol of two concurrent cluster-randomized trials: Real-life Effect of a CAMPAign with Measles Vaccination (RECAMP-MV) and Real-life Effect of a CAMPAign with Oral Polio Vaccination (RECAMP-OPV) on mortality and

morbidity among children in rural Guinea-Bissau. BMC Public Health. 2019;19(1):1506.

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01 LIST OF MANUSCRIPTS INCLUDED IN THIS THESIS

Paper 1

Nanque LM, Varma A, Thyssen SM, Benn CS, JSD Martins, Jensen AKG, Correia C, Möller S, AVDB Biggelaar, Aaby P, Fisker AB. Effect of a campaign with oral polio vaccine on mortality and hospital admission: a cluster-randomised trial among children aged 0-8 months in rural Guinea-Bissau.

Paper 2

Nanque LM, Rodrigues AM, Möller S, Umbasse P, Fisker AB. OPV shortage in 2004: Effect of not giving OPV with DTP on admission and at-hospital mortality among children attending outpatient consultations.

Paper 3

Nanque LM, Careme M, Djana Q, Vedel JO, Aaby P, Fisker AB. IPV introduction in the routine vaccination programme in 2016 and subsequent reintroduction: a natural experiment.

02 ENGLISH SUMMARY

Background

While OPV has been key in preventing and controlling polio, evidence also suggest that OPV has contributed to lowering child mortality by up to 15-30% in low-income countries through a non-specific effect (NSE) protecting against non-polio infections. However, the use of OPV carries a risk of vaccine-associated paralytic polio and vaccine-derived polioviruses spreading between children. Therefore, to eradicate poliovirus, the global plan is to replace OPV with the inactivated polio vaccine (IPV). IPV will ensure protection against polio. However, IPV induces a weaker mucosal immune response and does therefore not prevent transmission of poliovirus. Furthermore, limited evidence suggests that unlike OPV, IPV does not have beneficial NSEs. We therefore need real-life data on what may happen when OPV is replaced by IPV.

Aim

The aim of this thesis is to evaluate the potential implications of replacing OPV with IPV, specifically whether this may augment child morbidity and mortality in Guinea-Bissau due to depriving children of the benefit of OPV and exposing them to a potential negative effect of IPV.

Methods

Study 1. In a cluster-randomised trial, we used the Bandim Health Project's (BHP) health and demographic surveillance system (HDSS) to test the effect of campaign-OPV (C-OPV) vs no C-OPV on non-accidental mortality/hospital admission, consultation, illness, and growth among children aged 0-8 months. In Cox proportional hazards models with age as the underlying timescale, we compared rates of non-accidental mortality/hospital admission (composite outcome) during 12 months of follow-up by trial arm. Hazard ratios (HR) were estimated with 95% confidence intervals (CI) using cluster-robust standard errors to account for intra-cluster correlation. The effects of C-OPV on growth, illness, and consultation were estimated using linear and log-binomial regression models to obtain mean growth differences and relative risks (RR) with 95% CIs.

Study 2. During an OPV-shortage in 2004, we investigated the effect of not having received OPV with diphtheria-tetanus-pertussis (DTP) vs DTP+OPV on the risk of hospital admission/at-hospital mortality (severe morbidity) among children aged 6 weeks to 14 months attending outpatient consultations. Using

BHP's urban HDSS, including registry of all outpatient consultations and admissions at the National Hospital Simão Mendes (HNSM), we assessed crude and adjusted estimates of risk of severe morbidity in log-binomial regression models using propensity score matching and cluster-robust standard errors to account for children who had more than one consultation.

Study 3. In August 2016, IPV was introduced in Guinea-Bissau, to be given alongside the 3rd pentavalent vaccine (penta), and due to an IPV-shortage in May 2017, IPV was re-introduced in January 2019. Using the BHP's urban HDSS, including data from health centres and the HNSM, we compared rates of outpatient consultations and hospital admissions between children who received penta3+OPV3+IPV vs penta3+OPV3. Outcome rates between receipt of penta3 and age 9 months were compared overall and by sex in Cox proportional hazards models with age as the underlying timescale. HRs were estimated with 95% CIs adjusted for weight-for-age z-score. Furthermore, HR for consultation was adjusted for the underlying consultation rates, and HR for hospitalisation and mortality was adjusted for season of penta3 vaccination.

Results

In study 1, we found no marked overall effect of C-OPV vs no C-OPV on non-accidental mortality/hospital admission (HR: 0.87, 95% CI 0.68-1.12), reported illness (RR: 0.95, 95% CI 0.84-1.09), risk of consultation (RR: 0.86, 95% CI 0.70-1.06) nor growth among children aged 0-8 months in rural Guinea-Bissau. However, interactions were observed with timing of OPV at birth (OPV0) for the composite outcome ($p=0.04$), driven mainly by a differential effect on mortality.

In study 2, we did not find an increased risk of severe morbidity among children attending outpatient consultations who received DTP-only vs DTP+OPV as the most recent vaccine during the OPV-shortage in 2004 (RR: 0.85, 95% CI 0.60-1.21). However, children attending outpatient consultations who received DTP-only appeared to have a lower risk of admission due to measles infection.

In study 3, contrary to our hypothesis, children who received IPV with penta3+OPV3 had no increased risk of non-accidental consultation, hospital admission and mortality compared with children who only received penta3+OPV3 (consultation: HR 0.94, 95% CI 0.86-1.03; hospital admission: HR 0.88, 95% CI 0.56-1.37; mortality: HR 0.47, 95% CI 0.15-1.48). However, IPV administration may be associated with an increase in the female/male mortality ratio.

Conclusion

The beneficial effect of OPV was less than anticipated, and no difference in risk of consultation, hospital admission and mortality was observed after co-administration of IPV with penta3+OPV3. However, potential interactions such as with

sex, timing of OPV0, and time since vaccination need to be explored further in studies assessing the effect of OPV vs IPV. We recommend to further clarify the overall and sex-differential effect of OPV vs IPV using real-life data before replacing OPV with IPV to ensure that a polio free world does not deprive children the possibility of better health and survival.

03 DANSK RESUMÉ

Baggrund

Den oral poliovaccine (OPV) har været essentiel for at forebygge og kontrollere forekomsten af polio. Studier tyder dog på, at OPV også har bidraget til at sænke børnedødeligheden med op til 15-30% i lav- og middelindkomstlande gennem såkaldte non-specifikke effekter, der kan beskytte det polio-vaccinerede barn mod andre infektioner end polio. OPV indebærer dog også en risiko for vaccine-associeret polio og spredning af vaccinstammevirus. For at udrydde polio, har verdenssamfundet derfor lagt en plan om at erstatte OPV med den inaktiverede poliovaccine (IPV). Ligesom OPV sikrer IPV beskyttelse mod polio, men grundet dens inaktiverede form, fremkalder den et svagere immunrespons, og kan derfor ikke forebygge spredningen af poliovirus. Derudover er der endnu få studier, der har undersøgt effekten af IPV på den generelle børnesundhed, men de få udførte studier tyder på, at IPV ikke har samme gavnlige non-specifikke effekter som OPV. Verden har derfor brug for studier, der uden for laboratorierne og ved brug af virkelighedens data, tester hvad der sker med den generelle børnesundhed når OPV erstattes af IPV.

Formål

At evaluere de potentielle implikationer af at erstatte OPV med IPV, og specifikt om dette vil øge børnesygelighed og -dødelighed i Guinea-Bissau, ved at fratage børn de fordelagtige effekter af OPV og udsætte dem for en potentiel negativ effekt af IPV.

Metoder

Studie 1. Ved brug af Bandim Health Project's (BHP) rurale sundheds- og demografiske overvågningssystem (HDSS), opsatte vi et cluster-randomiseret studie, der testede effekten af en kampagne-OPV (C-OPV) vs ingen C-OPV på dødelighed/hospitaliseringer, konsultationer, sygelighed og vækst blandt børn i alderen 0-8 mdr. I Cox Proportional Hazards modeller med alder som underliggende tidsskala, sammenlignede vi rater af dødelighed/hospitaliseringer i randomiseringsgrupperne gennem 12 måneders opfølgning. Hazard ratios (HR) blev estimeret med 95% sikkerhedsintervaller (CI) ved brug af cluster-robust standard errors for at tage højde for intra-cluster korrelation. Effekten af C-OPV på konsultationer, selvrapporteret sygdom og vækst blev estimeret ved brug af

log-binomial og lineær regressionsmodeller, for at udlede en relativ risiko (RR) og den gennemsnitlige væksthorskel med 95% CIs.

Studie 2. I løbet af en periode i 2004 manglede der OPV i Guinea-Bissau.

Denne periode brugte vi til at undersøge effekten af ikke at have modtaget OPV sammen med difteri-tetanus-kighoste (DTP) vs DTP+OPV som seneste vaccine på risikoen for hospitalisering/hospitalsdød alvorlig sygdom blandt børn i alderen 6 uger til 14 mdr, som søgte ambulante konsultation. Ved brug af data fra BHP's urbane HDSS, som også inkluderer registrering af alle konsultationer og hospitaliseringer på det nationale hospital Simão Mendes (HNSM), målte vi ujusterede og justerede estimer for risikoen for svær sygdom. Disse blev estimeret gennem log-binomial regressionsmodeller efter propensity score matching, og med brug af cluster-robust standard errors for at tage højde for børn med flere end én konsultation.

Studie 3. I august 2016 blev IPV introduceret i Guinea-Bissau med anbefalingen om, at den blev givet sammen med den tredje pentavalente vaccine (penta).

Grundet mangel på IPV i maj 2017, blev IPV reintroduceret i januar 2019. Ved brug af BHP's urbane HDSS, som også inkluderer data fra sundhedscentre og HNSM, sammenlignede vi raterne af konsultationer og hospitaliseringer blandt børn som modtog penta3+OPV3+IPV vs penta3+OPV3. Raterne i perioden mellem penta3 vaccination og alder 9 mdr blev sammenlignet overordnet og afhængig af køn i Cox Proportional Hazards modeller med alder som den underliggende tidsskala. HRs blev estimeret med 95% CIs og justeret for vægttil-alder. Ydermere blev HR for konsultationer justeret for de underliggende konsultationsrater, og HR for hospitalisering og mortalitet blev justeret for sæson for penta3 vaccination.

Resultater

I studie 1 fandt vi ingen overordnet effekt af C-OPV vs ingen C-OPV på risikoen for død/indlæggelse (HR: 0.87, 95% CI 0.68-1.12), konsultation (RR: 0.86, 95%CI 0.70-1.06), selvrapporeret sygdom (RR: 0.95, 95% CI 0.84-1.09) og vækst blandt børn i alderen 0-8 mdr i rurale Guinea-Bissau. Derudover modificerede timing af OPV ved fødslen (OPV0) effekten af C-OPV for det kompositte udfald ($p=0.04$). Denne interaktion var primært drevet af en forskellig effekt på dødelighed.

I studie 2, fandt vi ingen øget risiko for alvorlig sygdom blandt børn, der søgte ambulante konsultation og som i løbet af perioden med OPV-mangel, havde modtaget kun-DTP vs DTP+OPV som den seneste vaccine (RR: 0.85, 95% CI 0.60-1.21). Derimod viste det sig, at børn, der søgte ambulante konsultation og som havde modtaget kun-DTP havde en lavere risiko for hospitalisering grundet mæslinger.

I modsætning til vores hypotese i studie 3, fandt vi ingen øget risiko for konsultation (HR 0.94, 95% CI 0.86-1.03), hospitalisering (HR 0.88, 95% CI 0.56-1.37) og død (HR 0.47, 95% CI 0.15-1.48) for børn, der havde modtaget penta3+OPV3+IPV vs børn, der havde modtaget penta3+OPV3. Dog kan IPV vaccination være associeret med en øget pige/dreng mortalitetsratio.

Konklusioner

Den gavnlige effekt af OPV var mindre end forventet, og vi fandt ingen forskel i risikoen for konsultation, hospitalisering og død efter IPV givet med penta3+OPV3. Køn, timing af OPV0, og tid siden vaccination blev dog identificeret som mulige effektmodifikatorer, og det er derfor nødvendigt at udforske disse nærmere i fremtidige studier, der evaluerer effekten af OPV vs IPV. Vi anbefaler at afklare effekten af OPV vs IPV yderligere før OPV erstattes med IPV. Dette bør gøres ved brug virkelighedens data, som afspejler de reelle effekter og interaktioner, der er nødvendige at kende for at kunne sikre, at en verden fri af polio ikke fratager børn muligheden for bedre sundhed og overlevelse.

04 INTRODUCTION

04.01 Poliomyelitis

Poliomyelitis, henceforth referred to as polio in this dissertation, is a highly infectious disease, which has been around since prehistoric times. In the late 19th and early 20th century frequent epidemics made polio a serious threat to life, and by the mid-20th century polio could be found all over the world[1].

Poliovirus is transmitted through the oral-faecal route and is therefore a great threat in areas with poor hygiene and sanitation. Polio exists naturally as three types of wild poliovirus (WP); type 1, 2, and 3. Immunity against one of the three types does not confer immunity to another type[2]. Initial symptoms of polio can be fever, fatigue, headache, vomiting, stiffness and pain in neck and limbs[3]. However, in most cases, polio causes no or only mild symptoms[3]. In one in 200 symptomatic infections, poliovirus enters the blood stream and invades the central nervous system causing irreversible paralysis and/or death due to immobilisation of the respiratory muscles[4].

There exists no cure for polio. However, two different polio vaccines have been developed: the oral polio vaccine (OPV) and the inactivated polio vaccine (IPV), to prevent transmission and outbreaks of polio. Furthermore, as polio infections are often asymptomatic or only cause mild symptoms[5], it can be difficult to identify an outbreak in the initial phase. Therefore, to prevent and control poliovirus from spreading 'silently', it is crucial to attain herd immunity (Figure 1). The threshold for herd immunity is disease specific as it depends on how contagious the disease is. For polio, the threshold is 80%[6]. Hence, eight out of 10 must be vaccinated against polio to protect persons, who are not vaccinated.

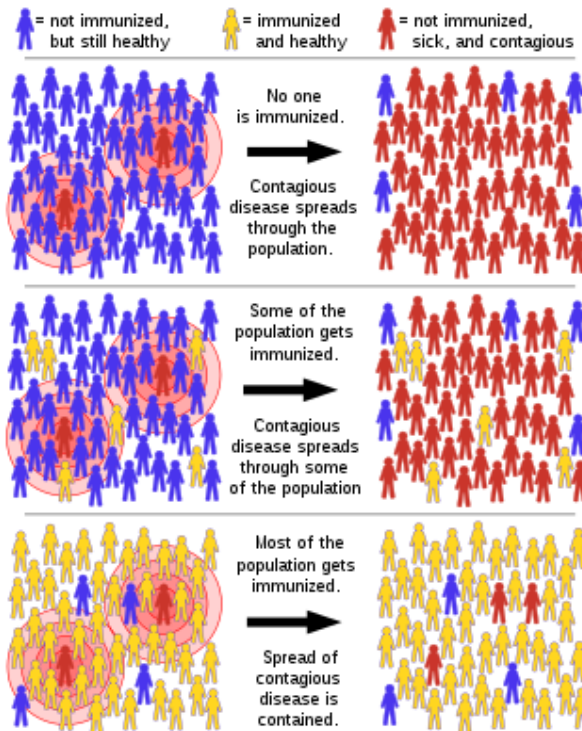


Figure 1. Illustration of herd immunity. Illustration Source: National Institute of Allergy and Infectious Diseases (https://commons.wikimedia.org/wiki/File:Herd_immunity.svg). All three boxes show an infectious disease outbreak. In the top part, the outbreak occurs in a community where no one is vaccinated; in such case, a contagious disease can spread freely in the community. In the middle, the outbreak occurs in a community in which some are vaccinated; in such case, a contagious disease infects most unvaccinated persons. In the bottom part, almost all are vaccinated, in such case spread of the infectious disease is prevented, also to unvaccinated persons.

04.02 Polio vaccines: OPV and IPV

OPV was developed in 1961 by Dr Albert Sabin[2]. Different types of OPV exists, containing one (monovalent: mOPV), two (bivalent: bOPV) or all three poliovirus types (trivalent: tOPV). Common for all types of OPV is that they contain live-attenuated poliovirus(es). Once vaccinated with OPV, the vaccine virus replicates in the gut, and thus induces an immune response including good mucosal immunity against poliovirus in the vaccinated child[2]. In addition, poliovirus is shed in the faeces of the vaccinated child and is thereby able to immunise other children in vicinity. Due to this so-called 'passive immunisation' of

unvaccinated children and its ability to interrupt transmission, OPV is the predominant vaccine used in communities with low vaccination coverage and in controlling polio outbreaks[2]. Other important advantages are that the OPV is inexpensive (US \$0.11-\$0.19 per dose)[7] and easy to administer by two oral drops. However, disadvantages also exist. With further cycles of replication in non-immune populations, a mutated virus can become circulating (circulating vaccine-derived poliovirus: cVDPV)[5]. In extreme cases (2-4 per 1 million infants) this can lead to vaccine-associated-paralytic polio (VAPP)[5].

IPV was developed in 1955 by Dr Jonas Salk[8]. IPV consists of all three poliovirus types in an inactivated form. IPV vaccination induces an immune response in which antibodies to all three types of polioviruses are produced in the blood thus protecting the vaccinated child against polio[8]. As IPV consists of inactivated poliovirus it carries no risk of VAPP. However, IPV is not able to prevent transmission of poliovirus as it induces only low levels of mucosal immunity[8]. Thus, if a child vaccinated with IPV is exposed to wild poliovirus, the virus can still replicate and shed in the faeces and spread to other children. Furthermore, IPV is expensive (US \$1.25-\$3.1 per dose)[9] compared to OPV (see price in section above), and requires trained health personnel as it is administered by injection.

Both OPV and IPV induce strong protection against polio, and the current use of OPV and IPV in combination seem to complement each other well[10].

04.03 OPV ‘switch’ and ‘swap’

In 1988, the World Health Assembly declared the commitment of the WHO to eradicate polio by the year 2000[11]. To achieve this goal, the Global Polio Eradication Initiative was formed by six core partners: WHO, Rotary International, the US Centers for Disease Control and Prevention, the United Nations Children’s Fund, Bill & Melinda Gates Foundation, and GAVI Alliance. Eradicating polio turned more challenging than expected. In 2013, the first strategic report was published, including among other objectives, a withdrawal of OPV by first switching from tOPV to bOPV (types 1 and 3) (the ‘switch’), and second, replacing OPV with IPV (the ‘swap’)[12].

04.03.01 The ‘switch’

Between 2006 and 2016, type 2 cVDPV (cVDPV2) accounted for more than 94% of polio cases caused by cVDPV and 40% of VAPP[13]. In 2016, shortly after eradication of WP2 and the global ‘switch’ from tOPV to bOPV[14] (Figure

2), the risk of cVDPV and VAPP were therefore markedly lowered. As part of the switch from tOPV to bOPV, it was recommended that all children receive one dose of IPV together with the third dose of bOPV (OPV3), which was co-administered with the third pentavalent vaccine (penta3: diphtheria-tetanus-pertussis-hepatitis B-haemophilus influenzae type b (Hib)) and the third pneumococcal conjugate vaccine (PCV) at 14 weeks of age[5]. However, due to major supply constraints during the initial phase of the switch, a global shortage of IPV was in effect from May 2017[15]. In Guinea-Bissau, this shortage lasted until January 2019.

04.03.02 The ‘swap’

In August 2020, the African region was certified free of wild poliovirus[16], but WP1 remains endemic in Afghanistan and Pakistan. Furthermore, the risk of cVDPV persists due to low immunisation rates, poor hygiene, and high population densities. Although, the global number of cVDPV2 detected in humans declined from 286 cases in 2020 to 112 cases in 2022, cVDPV2 remains the biggest threat in the African region and the key challenge for polio eradication[17].

To eliminate the risk of cVDPV and VAPP completely, OPV will be stopped and replaced by IPV once WP1 has been eradicated and the absence of all three types of cVDPV has been validated[18]. The phase-out of bOPV in the routine vaccination programme is planned for 2028, and the complete phase-out of all OPV and swap to exclusive use of IPV is currently planned for 2030+[18] (Figure 2).

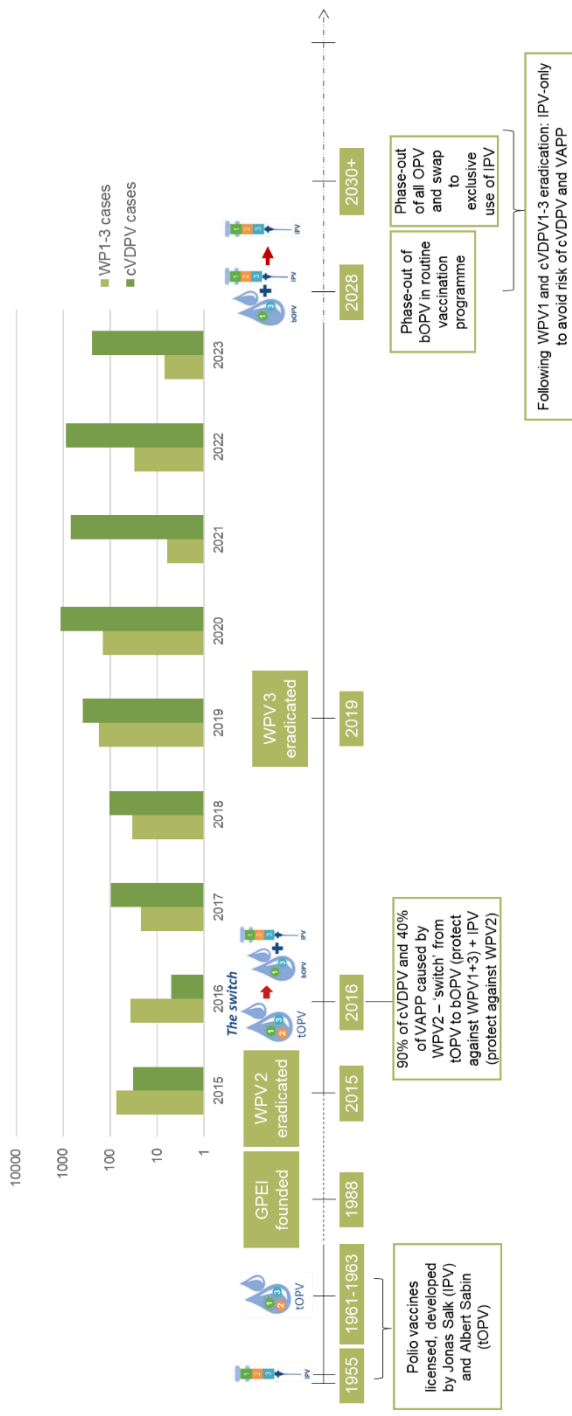


Figure 2. Timeline of initiatives related to the global polio eradication initiative (1955-2030+) including graph illustrating cases of wild poliovirus 1-3 (WP1-3) and circulating vaccine-derived poliovirus (cVDPV) from 2015-2023.

Data source World Health Organization (<https://extranet.who.int/polis/public/CaseCount.aspx>), last updated 09/07/2023.

04.04 WHO routine vaccination recommendations in Guinea-Bissau

OPV was introduced together with diphtheria-tetanus-pertussis (DTP) into the child routine vaccination programme in 1981 in Guinea-Bissau[19].

The routine vaccination programme was not the same across all three studies described in this thesis. During the time of data collection for Paper 2, collected during 2003-2004, the routine vaccination programme recommended Bacillus Calmette Guerin (BCG) and OPV at birth, DTP and OPV at 6, 10, and 14 weeks as well as booster doses at 18 months of age, and measles vaccine (MV) at 9 months of age (Figure 3).

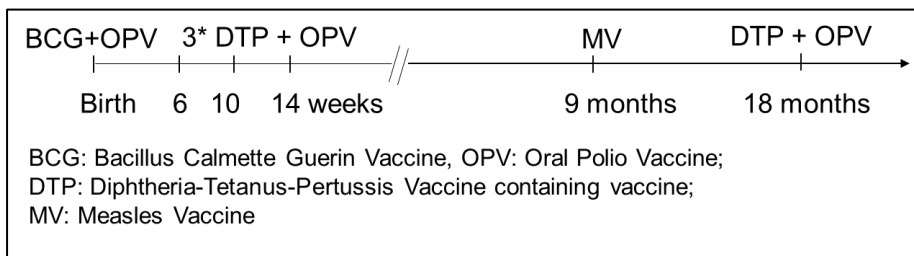


Figure 3. Routine vaccination programme in Guinea-Bissau 2002-2007.

During the data collection period for Paper 1 and 3, collected during 2015-2020, Guinea-Bissau had received support from GAVI to replace DTP with penta and introduce yellow fever vaccine (YF) to be co-administered with MV[20]. Furthermore, rotavirus vaccine (Rota) and PCV was introduced in 2015[21] (Figure 3).

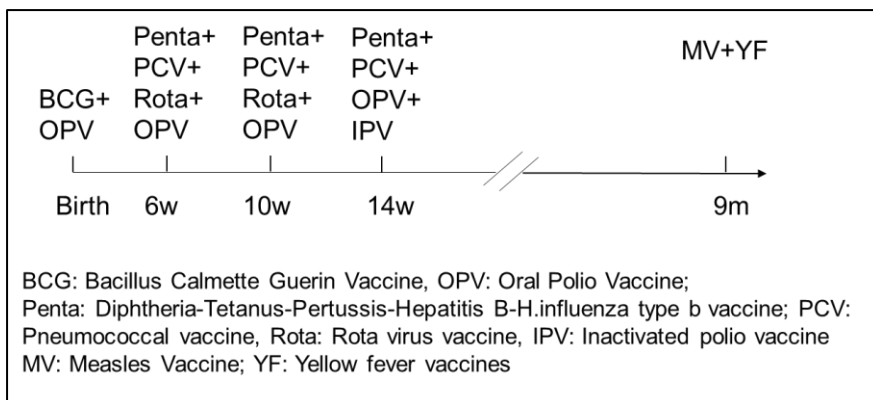


Figure 4. Routine vaccination programme in Guinea-Bissau 2016.

04.05 OPV campaigns

As part of the global effort to eradicate polio, many campaigns with OPV (C-OPV) (\pm Vitamin A Supplementation (VAS)) have been conducted globally since the beginning of the 1990s up until today. These campaigns are most often so-called mass immunisation campaigns, which as a supplement to the childhood routine vaccination programme, aim at vaccinating all children under five years of age with two OPV doses. Therefore, a campaign is commonly conducted in two vaccination rounds, one month apart. Children are eligible for participation independent of their previous OPV vaccination status as the idea is to catch children who are unvaccinated/partially vaccinated and boost immunity in already vaccinated children.

In Guinea-Bissau, the first national C-OPV was conducted in 1998[22], and except from the period from 2006-2009 when there were no C-OPVs conducted, C-OPVs have been conducted in response to outbreaks every year or second year since 1998 (data from WHO correspondence).

04.06 Non-specific effects of vaccines

Non-specific effects of vaccines (NSEs) refer to effects on general health beyond protection against the disease(s) targeted by the vaccine. Vaccines described in this dissertation can be divided into live vaccines and non-live vaccines[23]. The live vaccines, such as BCG and MV, have been found to induce protection against unrelated diseases. In a WHO commissioned review published in 2016, the NSEs of the live vaccines, BCG and MV, were found to be associated with a 40-50% lower mortality[24]. In contrast, the non-live vaccines, such as DTP, penta, hepatitis B and H1N1 influenza vaccine have been found to induce susceptibility to unrelated infections, in particularly for girls[23]. Furthermore, the WHO commissioned review, found DTP to be associated with a 38% higher mortality[24]. These observations are supported by numerous observational studies and randomised trials (RCTs), primarily from low-income settings[23]. Furthermore, these studies have found that NSEs are strongest for the most recent vaccination received, and that boys and girls are affected differently.

04.06.01 OPV and IPV

The role of OPV and IPV as part of both the routine vaccination programme and as vaccination campaigns remain key towards preventing and controlling polio

and ultimately eradicating polio. However, evidence indicates that their NSEs may be very different[23].

04.06.02 NSEs of OPV

The NSEs of OPV have mostly been investigated in low- and middle-income countries (LMICS) in observational studies assessing the effect of C-OPV on mortality[25]. In Guinea-Bissau[22, 26-29], Bangladesh[30, 31], Burkina Faso[32], and Ghana[33] such studies have found beneficial effects of C-OPV on child survival, with up to 15-30% decline in overall child mortality (Table 1). Large cohort studies have also investigated the effect of subsequent C-OPV doses, which appeared to offer an additional protection against child mortality[26, 27, 30]. Thus, the numerous C-OPVs implemented in LMICS since the mid-1990s may have contributed markedly to lowering overall child mortality, which have declined around 60% from the 1990s until today[34].

The effect of routine-OPV (as part of the routine vaccination programme) has also been assessed in other study designs (Table 1). A randomised trial in Guinea-Bissau, assessing the effect of OPV vs no OPV given at birth (OPV0), found a 32% (0-55%) reduction in infant mortality[35] (Table 1). In addition, a small natural experiment comparing no-OPV0 vs OPV0 within a trial of early-BCG+VAS among low birthweight infants supports a beneficial effect of OPV0[36] (Table 1). Furthermore, due to vaccine shortages when DTP and OPV were introduced in Guinea-Bissau, natural experiments occurred which allowed for comparing routine-OPV with DTP. These studies have found a considerably higher mortality among children who received DTP-only than children who received DTP+OPV[19, 25, 37-39], indicating that OPV may have attenuated some of the possible negative NSEs found for DTP[40]. Furthermore, the in-hospital case fatality was found to be lower for children who received OPV-only versus OPV+DTP[25, 41].

Only few studies have examined the association between OPV and morbidity (Table 1). Beneficial effects of OPV on hospital admissions have been reported in studies from both low- and middle income countries[22, 32] and Denmark[42].

Furthermore, only few studies have investigated the disease-specific effect of OPV. However, OPV appears to be particularly protective against respiratory infections[42-45] and diarrhoea[46-48].

The immunological mechanisms behind OPV's potential beneficial NSEs are not yet well understood. However, current evidence suggests that live vaccines can both modify the adaptive immune response to unrelated infections and train the innate immune system[49-52]. OPV's effects may also be mediated via effects on the microbiome: a study found OPV at birth to be associated with higher excretion of the anti-microbial peptide cathelicidin LL37 in the stool 6 weeks post-vaccination[53] and in a sub-study nested in the cohort of Paper 1, OPV was associated with a healthier microbiome composition 2 months after re-vaccination, based on a more abundant and diversified bacterial community of Prevotellaceae and fewer pathogenic/opportunistic organisms[54].

04.06.03 NSEs of IPV

The evidence on NSEs of IPV is very limited, however, indicating possible negative NSEs of IPV. Two randomised trials from Finland and Bangladesh have compared the effect of OPV vs IPV. The Finish RCT found a 24% lower incidence of otitis media (incidence rate ratio: 0.76 (95% CI 0.59 to 0.94)[43], and the Bangladeshi RCT found a lower number of days with bacterial diarrhoea (5.9 and 6.7 days, respectively, $p=0.004$)[46].

For IPV, sex as an effect modifier has been explored in four RCTs of early-MV, where IPV was used as a comparator vaccine. Here, IPV was associated with a significantly higher mortality in girls than boys (female/male (F/M) mortality rate ratio (MRR) among IPV vaccinated: 1.52, 95% CI 1.02–2.28; F/M MRR among MV vaccinated: 1.01, 0.69–1.46)[55]. Furthermore, receiving IPV after MV has also been associated with increased mortality in girls[56].

The immunological mechanism behind IPV's potential NSEs also remains undefined; however, the survival pattern after IPV vaccination appear similar to that after DTP vaccination[55, 56], which like IPV is an inactivated vaccine. Furthermore, studies suggest that DTP may induce tolerance and increase susceptibility to unrelated infections[23, 57]. The mechanism behind IPV could therefore be similar to that for DTP.

Table 1. Overview of studies assessing non-specific effects of OPV.

Study (data collection year), year published	Study design; Adjustment; Type of OPV	Age group	Outcome estimate (95% CI)
Studies of campaigns with OPV (or OPV-only) on mortality and/or morbidity			
Guinea-Bissau (1998), 2005[22]	Observational study comparing C-OPV participants vs non-participants in two C-OPVs Adjusted for MUAC, ANC, twinning status, mother's schooling and measles vaccination Type of OPV used: tOPV	Age <5 years Age <6 mth (only adjusted for age)	<i>Mortality</i> C-OPV participants vs non-participants: MR 0.81 (0.54-1.21) <i>Hospitalisation</i> C-OPV participants vs non-participants: RR 0.27 (0.10-0.76)
Guinea-Bissau (2002-2014), 2018[26] A part of [27]	Observational study. Comparing time after vs before C-OPVs in participant from seven RCTs in urban Bissau Adjusted for age and season Types of OPV used: mOPV, bOPV, and tOPV	0-35 mth	<i>Mortality</i> After- vs before C-OPV: MRR 0.81 (0.68-0.95)
Guinea-Bissau (2002-2014), 2021[27]	Observational study. Comparing time after vs before- C-OPV using 13 years of observational data from the BHP urban HDSS Adjusted for age and season, and other health campaigns Types of OPV used: mOPV, bOPV, and tOPV	1 day-35 mth	<i>Mortality</i> After- vs before C-OPV: MRR 0.75 (0.67-0.85)

Bangladesh (2004-2019), 2021[30]	Observational study. Comparing time after vs before C-OPV; 15 years of observational data from the Chakaria HDSS in rural Bangladesh Adjusted for age and period and other health campaigns Type of OPV not reported	1 day-35 mth	<i>Mortality</i> After- vs before C-OPV: HR 0.69 (0.52-0.90)
Burkina-Faso (2012-2015), 2018[32]	Observational study. Comparing time after vs before any C-OPV within an RCT of early-MV. Adjusted for age, season and sex Type of OPV not reported	4-35 mth	<i>Severe morbidity (Hospitalisation/death)</i> After- vs before C-OPV: HR 0.64 (0.44-0.94)
Guinea-Bissau (2002-2003), 2017[58]	Observational study. Comparing time after vs before any C-OPV Adjusted for age and season Type of OPV used: tOPV	0-11 mth	<i>Mortality</i> After- vs before C-OPV: HR 0.90 (0.69-1.17)
Guinea-Bissau (2011-2015), 2021[59]	Observational study. Comparing time after vs before any C-OPV within a cluster-RCT of MV-for-all vs Restrictive-MV (MV between 9-11 mth only, according to national policy) Adjusted for age, region, and vaccination coverage Types of OPV used: bOPV and tOPV	9-35 mth	<i>Mortality</i> After- vs before C-OPV: HR 0.81 (0.45-1.45)
Guinea-Bissau (2020-2021), 2022[60]	Cluster-RCT of OPV vs no OPV during Covid-19 Adjusted for age and residential area	Adults >50 years	<i>Mortality/ Admission/ Consultation for infection</i> C-OPV vs no C-OPV: HR 0.97 (0.79-1.18) <i>Mortality</i>

	Type of OPV used: bOPV		<p>C-OPV vs no C-OPV: HR 0.96 (0.59-1.55)</p> <p><i>Admission</i> C-OPV vs no C-OPV: HR 0.76 (0.49-1.17)</p> <p><i>Recorded consultation</i> C-OPV vs no C-OPV: HR 0.99 (0.79-1.25)</p> <p><i>Symptoms of infection</i> C-OPV vs no C-OPV: HR 1.10 (1.03-1.17)</p>
Studies of routine-OPV (co-administered with other vaccines) and the effect on mortality and/or morbidity			
Guinea-Bissau (2008-2011), 2015[35]	<p>RCT comparing OPV0+BCG vs BCG-only.</p> <p>Mortality estimate adjusted for age Morbidity estimates adjusted for sex.</p> <p>Type of OPV not reported</p>	<p>follow-up: age 12 mth</p> <p>1 mth follow-up</p>	<p><i>Mortality</i> OPV0+BCG vs BCG-only: HR 0.83 (0.61-1.13)</p> <p>Censoring at first national C-OPV: HR 0.68 (0.45-1.00)</p> <p><i>Reported illness</i> OPV0+BCG vs BCG-only: IRR 0.99 (0.76-1.28)</p> <p><i>Reported consultation</i> OPV0+BCG vs BCG-only: IRR 1.16 (0.91-1.47)</p>

Guinea-Bissau (2007-2008), 2012[36]	Natural experiment comparing no-OPV0 vs BCG+OPV0 within a trial of early-BCG+VAS among low birth-weight infants Adjusted for age, sex and admission to neonatal nursery Type of OPV used: tOPV	12 mth follow-up	<i>Mortality</i> OPV0 vs No OPV0: HR 0.55 (0.28-1.08), original estimate (1.83, 95% CI 0.93-3.61) inverted
Guinea-Bissau (1980-1983), 2017[19] Cohort overlaps with [38]	Natural experiment. Mortality associated with most recent vaccination(s). Adjusted for age Type of OPV used: tOPV	3-5 mth	<i>Mortality</i> DTP-only: HR 10.0 (2.61-38.6) DTP+OPV: HR 3.52 (0.96-12.9)
Guinea-Bissau (1981-1984), 2018[39]	Observational study. Comparing DTP (\pm OPV) vs DTP-unvaccinated (OPV-only) Adjusted for weight-for-age z-score Type of OPV used: tOPV	6-35 mth	<i>Mortality</i> OPV-only vs DTP \pm OPV: HR 0.27 (0.06-1.12), original estimate (3.76, 95% CI 0.89–15.83) inverted
Guinea-Bissau (1984-1987), 2004[37]	Observational study. Mortality associated with DTP-only and OPV+DTP as most recent vaccination(s). Adjusted for age and region (DTP-only); Adjusted for age and village (OPV+DTP) Type of OPV used: tOPV	2-8 mth	<i>Mortality</i> DTP-only: MR 5.09 (0.65-39.9) OPV+DTP: MR 1.91 (1.04-3.51)
Guinea-Bissau (1980-1983), 2020[38]	Observational study. Mortality associated with most recent vaccination(s). Adjusted for age Type of OPV used: tOPV	3-8 mth	<i>Mortality</i> DTP-only vs DTP+OPV: HR 3.38 (1.59-7.20)

Guinea-Bissau (2001-2002), 2004[41]	Natural experiment. Comparing OPV-only vs OPV+DTP (vaccination status at admission) among children hospitalised. Adjusted for age Type of OPV used: tOPV	0-59 mth (no MV)	<i>In-hospital case fatality</i> OPV-only vs OPV+DTP: CFR 0.29 (0.11-0.77)
Denmark (1997-1999), 2016[42]	Observational study comparing OPV vs DTP-IPV-Hib3 as most recent vaccine Adjusted for age, season and calendar year Type of OPV not reported	24-36 mth	<i>Hospitalisation</i> OPV vs DTP-IPV-Hib3: IRR 0.85 (0.77-0.95)
Studies assessing the effect of OPV on effect estimates for other vaccines (within studies testing the effect of other vaccinations)			
Guinea-Bissau (2003-2009), 2016[28]	Natural experiments of C-OPV in urban Guinea-Bissau during RCT of 1-dose vs 2-dose MV No adjustments Type of OPV used: tOPV	4.5-36 mth	<i>Mortality</i> Children who received no C-OPV before RCT-enrolment: MRR (2-dose/1-dose MV) 0.60 (0.42-0.85) Children who received C-OPV before RCT-enrolment: MRR (2-dose/1-dose MV) 1.16 (0.64-2.13)
Bangladesh (1986-1999), 2021[31]	Observational study. Comparing DTP with or after MV vs MV-only using observational data from the Matlab HDSS in rural Bangladesh Adjusted for religion, maternal age, maternal education, birth order, asset score, distance from hospital and year.	9-24 mth	<i>Mortality</i> DTP with/after MV vs MV-only before C-OPV: MRR 2.20 (1.31-3.70) DTP with/after MV vs MV-only after C-OPV (from C-OPV until age 2 years): MRR 0.73 (0.22-2.44)

	Type of OPV not reported		
Guinea-Bissau (1999-2006), 2018[61]	Observational study. Comparing MV vs MV-unvaccinated before C-OPV and after C-OPV	6-36 mth	<i>Mortality</i> MV vs No-MV before C-OPV: HR 0.74 (0.60–0.92)
Cohort overlaps with [58]	Adjusted for age and stratified by vil-lage cluster		MV vs No-MV after C-OPV: HR 0.85 (0.56–1.27)
	Type of OPV used: tOPV		

04.07 OPV vs IPV: Where do we stand?

Most of the evidence of the NSEs of OPV and IPV is currently based on observational studies assessing the risk of mortality and/or morbidity after OPV or studies assessing whether polio vaccination impact the effect of other vaccinations tested in an RCT (Table 1). These studies are conducted using different sources of data and methodological approaches and could therefore involve different potential bias structures influencing the quality of the evidence (Table 2). RCTs may be the gold standard for assessing the NSEs of OPV and IPV. However, such a study design is very costly. As an alternative, evidence of NSEs of OPV and IPV have been strengthened through triangulation, thus combining the evidence based on the data available[23, 25, 62].

Table 2. Overview of potential bias structures discussed in previous studies.

Potential bias structure	Bias implication	Examples of methodological assessments	Bias assessment
Healthy vaccinee bias: selection bias associated with health status/vaccination status[19, 22, 28, 36, 37, 39, 42]	Systematic error in an association or outcome, for example lower outcome rates for children who received most vaccines	Control for health indicators (e.g., MUAC, WAZ) [22, 36] No difference in birth weight between vaccinated vs unvaccinated[22] OPV/C-OPVs implemented as natural experiments not depended on child health status[19, 28, 36] No difference in outcome rate when OPV1 was given after MMR or vice	Vaccination status not a pure reflection of health status; effect of OPV unlikely to be explained by health vaccinee bias

		versa[42] or when comparing DTP-vaccinated with unvaccinated[37]	
Selection bias due to missing information (e.g., vaccination status) [19, 37, 39]	Systematic error in an association or outcome	Outcome rate in group with missing vaccination cards similar to the general mortality outcome rate	Exclusion of missing unlikely to influence estimate
Frailty bias[38]	Unvaccinated group become increasingly frail producing an outcome estimate in favour of the vaccinated	HR continued to be at ≥ 1 among older children	Frailty bias unlikely
Recall bias[22]	Differential misclassification causing over- or underestimation of association	Analysing outcome rate depending on interview respondent (e.g., mother vs other family member)	Recall bias less likely
Uncontrolled confounding[19, 31, 33, 36, 38, 41, 42] and time-varying confounding[26-28, 30]	Effect of C-OPV due to variables not adjusted for and or covariates varying over time; general change in mortality coinciding with timing of C-OPV	Use of same inclusion criteria throughout[28] Adjusted for major determinants of vaccination and child mortality[26, 27, 30, 31, 33, 36, 41, 42] Adjustment for season and or calendar time[26-28, 30] For RCTs; Adjusted intention-to-treat analyses	Uncontrolled confounding unlikely to explain pattern
Survival bias[30]	Risk of assessing successful outcomes and disregard failures if group assignment is based on retrospective data before time-zero; from reported date of birth rather than first seen alive [63]	Analysed using both the landmark approach (followed from first seen alive) and the retrospective approach (followed from reported date of birth) [30]	No survival bias
Unblinding[35]	Influencing behavior of participants (mothers) and/or trial management (fx. intervention, outcome assessment)	Blinding health workers and trial assistants	Unlikely to have affected results (see discussion of Paper 1, section 08.01.01)

04.08 A burning platform

From the perspective of polio eradication, the replacement of OPV with IPV seems justified. Novel types of OPV are also being developed and rolled out under emergency use listing to mitigate the risk of cVDPV2 outbreaks as IPV opposed to OPV is not able to interrupt transmission[64]. However, less recognised is the paucity of evidence and therefore the need to collect more data on what the effects on general child health will be when OPV is replaced by IPV. Regardless of how OPV and IPV exert their effects, there is a need to evaluate the implications of their NSEs and complement the evidence on their specific effects with data on their NSEs to be able to identify a potential problem and remedies to alleviate it.

04.09 Potential effect modifiers of NSEs

Potential effect modifiers may be just as important to recognise as the NSEs. The evidence on sex as a potential effect modifier is consistent, and the most tested. However, other potential effect modifiers may also be relevant. Due to limited studies on IPV, all effect modifiers mentioned below, except for sex, have been investigated in studies of OPV.

04.09.01 Sex

Both observational studies[27-31, 65-67] and RCTs[32, 35], which have indicated a beneficial effect of OPV on mortality, have also found a better effect in males[27, 29, 35, 65, 67] (Table 3).

04.09.02 Age

Age as an effect modifier has been investigated in many previous studies of OPV. The difference in age-intervals makes it difficult to compare these studies. However, in studies from Guinea-Bissau, Bangladesh and Ghana, which investigated the effect of C-OPV in broader age-intervals, a potentially larger effect was found in children older than 12 months[26, 27, 30] (Table 3). Studies testing narrower and/or other age-intervals have been less consistent and limited in part due to small subsamples[22, 41, 43].

04.09.03 Season

Only one large observational study assessing season as a potential effect modifier in the effect of OPV on mortality in after- vs before-C-OPV among children aged 1 day to 36 months old in Guinea-Bissau has been conducted (Table 3).

This study found a stronger effect of C-OPV in the rainy season (MRR 0.73, 95% CI 0.58–0.92) than in the dry season (MRR 0.90, 95% CI 0.71–1.14)[26].

04.09.04 Boosting and number of OPV doses

Boosting with OPV, understood as additional OPV doses, most likely also have beneficial effects on general child health. In three large observational studies from Guinea-Bissau and Bangladesh, OPV was consistently found to offer a benefit for child survival for each additional C-OPV dose[26, 27, 30] (Table 3). In addition, a Danish observational study found similar results for an additional routine-OPV dose on hospital admission (incidence rate ratio (IRR) (Table 3)[42]. C-OPV might also modify the effect of other health interventions when given before and/or after enrolment[28]. In an RCT investigating the effect of two- vs one-dose MV in Guinea-Bissau, the effect of two- vs one-dose MV was stronger when children had not received C-OPV before enrolment ($p=0.06$, test of same effect)[28].

04.09.05 Priming with OPV

OPV is recommended to be given at birth (OPV0) together with BCG in the routine vaccination programme (Figure 3+4). This may prime the immune system[53] and positively influence the immune response to subsequent OPV doses. Due to the routine vaccination programme, OPV0 has only been investigated as an effect modifier in a natural experiment, and until now, only within an RCT testing the effect of MV[28]. However, an RCT comparing BCG+OPV0 vs BCG-only found a lower risk of mortality for children enrolled within the first two days of life (HR 0.58, 95% CI 0.38-0.90)[35] (Table 3).

Table 3. Potential effect modifiers investigated in previous studies.

Study	Study design	Outcomes	Effect modifier(s) assessed	
Guinea-Bissau, 2018[26]	Comparing time after vs before C-OPVs in participant from seven RCTs in urban Bissau	All-cause mortality	Sex	Boys: MRR 0.74 (0.58-0.94) Girls: MRR 0.87 (0.70-1.07)
			Age	0-5 mth: MRR 0.95 (0.93–0.98) 6-11 mth: MRR 0.89 (0.85–0.94) <12 mth: MRR 0.83 (0.85-0.94)
			Season	Dry: MRR 0.90 (0.71–1.14) Rainy: MRR 0.73 (0.58–0.92)
			Additional OPV doses	Overall: MRR 0.87 (0.79–0.96) Boys: MRR 0.80 (0.70–0.91) Girls: MRR 0.94 (0.83–1.05)
			OPV type	mOPV: MRR 0.77 (0.56–1.08) bOPV: MRR 0.80 (0.57–1.12) tOPV: MRR 0.85 (0.71–1.02)
Guinea-Bissau, 2021[27]	Comparing time after vs before- C-OPV using 13 years of observational data from the BHP urban HDSS	All-cause mortality	Sex	Boys: MRR 0.73 (0.62–0.86) Girls: MRR 0.77 (0.65–0.92)
			Age	0-11mth: MRR 0.79 (.68–.91) 12-35mth: MRR 0.70 (.57–.86)
			Additional C-OPV doses	Overall: MRR 0.86 (.81–.92) C-OPV1: MRR 0.79 (.69–.90) C-OPV2: MRR 0.72 (.62–.83) C-OPV3: MRR 0.60 (.47–.77) C-OPV4: MRR 1.00 (.60–1.64)
			OPV type	mOPV: MRR 0.78 (0.61-1.00) bOPV: MRR 0.87 (0.71-1.08) tOPV: MRR 0.79 (0.71-0.89)
Bangladesh, 2021[30]	Comparing time after vs before- C-OPV 15 years of observational data from the Chakaria HDSS in rural Bangladesh	All-cause mortality	Sex	Boys HR 0.78 (0.54-1.13) Girls: HR 0.58 (0.37-0.89)
			Age	0-12mth: HR 0.77 (0.54-1.08) 12-35mth: HR 0.56 (0.35-0.90)
			Additional OPV doses	Overall: HR 0.92 (0.85-1.00)

Guinea-Bissau, 2005[22]	Observational study comparing C-OPV participants vs non-participants in two C-OPVs in 1998	All-cause mortality	Age	0-5mth: MR 0.56 (0.31–1.01) 6-11mth: MR 1.03 (0.43–2.45) 12-23mth: MR 0.83 (0.39–1.76) 24-35mth: MR 0.29 (0.14–0.60) 36-59mth: MR 1.79 (0.42–7.71)
Guinea-Bissau, 2004[41]	Natural experiment comparing OPV-only vs OPV+DTP	In-hospital case fatality	Age	0-5mth: CFR 0.49 (0.07–3.38) 6-11mth: CFR 0.62 (0.16–2.36) 12-23mth: CFR 0.32 (0.05–2.22)
Guinea-Bissau, 2012[36]	Natural experiment comparing no-OPV0 vs OPV0 within a trial of early-BCG+VAS among low birth-weight infants	All-cause mortality	Sex	Boys: HR 1.89 (0.82–4.36) Girls: HR 1.42 (0.69–2.95)
			Timing of OPV0	<i>post hoc</i> analysis: significant beneficial effect of receiving OPV during first two days of life
			Season	P=0.20 for interaction
Guinea-Bissau, 2015[35]	RCT comparing OPV0+BCG vs BCG-only	All-cause mortality	Sex	Boys: HR 0.72 (.47–1.10) Girls: HR 0.97 (.61–1.54)
			Age at enrolment	0-2d of life: HR 0.58 (.38–.90) ≥3 days: HR 1.26 (.79–2.00)
		Illness and health centre consultations	Sex	Illness: Males: IRR 1.16 (0.80-1.67) Females: IRR 0.84 (0.58-1.20) Consultations: Males: IRR 1.20 (0.87-1.66) Females: IRR 1.11 (0.78-1.58)
Denmark, 2016[42]	Observational study comparing OPV vs DTP-IPV-Hib3 as last vaccine	Risk of hospital admission (any infection and disease specific)	Sex	Any infection: Males: IRR 0.86 (.76–.99) Females: IRR 0.83 (.71–.98) Lower respiratory infections: Males: IRR 0.68 (.55–.85) Females: IRR 0.81 (.61–1.08)
			Additional OPV doses	OPV2: 0.93 (0.86-1.00) OPV3: 0.87 (0.79-0.96) OPV2 or OPV3: 0.92 (0.86-0.99)

05 AIM OF THE THESIS

The aim of this thesis is to evaluate the potential implications of replacing OPV with IPV, specifically whether this may augment child morbidity and mortality in Guinea-Bissau due to depriving children of the benefit of OPV and exposing them to a potential negative effect of IPV.

05.01 Specific hypotheses tested in papers

To test if providing OPV reduces child mortality and morbidity, and whether addition of IPV to the childhood routine vaccination programme augments child morbidity and mortality, a series of studies was conducted to assess the NSEs of OPV and IPV on childhood morbidity and mortality in Guinea-Bissau. The studies conducted tested the following hypotheses:

- A. Whether a campaign with OPV (C-OPV) vs no C-OPV reduces child mortality and morbidity by 25% (Paper 1 – Section 12.01).
- B. Whether not co-administering OPV with DTP increases hospital admissions and in-hospital mortality among children attending outpatient consultations (Paper 2 – Section 12.02).
- C. Whether introducing IPV together with the 3rd penta+OPV dose in the childhood routine vaccination programme has negative effects measured by increased (non-accidental) consultation, hospital admission, and mortality rates (Paper 3 – Section 12.03).

06 METHODS

The BHP runs an urban and a rural HDSS in Guinea-Bissau, West Africa (Figure 5). This thesis used the setup and data of the BHP rural HDSS to conduct a randomised trial testing the effect of C-OPV vs no C-OPV (Paper 1 – Section 12.01) (Figure 6). Furthermore, the data collected and organised by the BHP urban HDSS were used to conduct two natural experiments (Paper 2 – Section 12.02; Paper 3 – Section 12.03) (Figure 6).

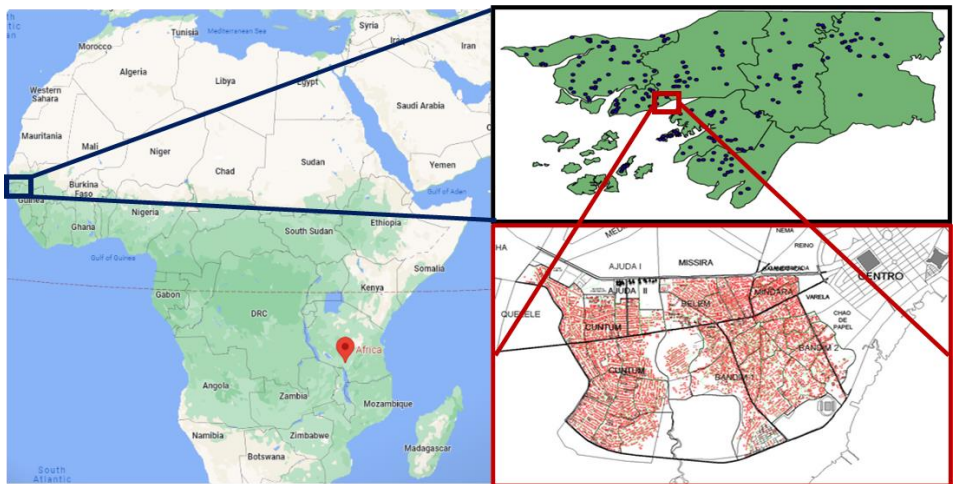
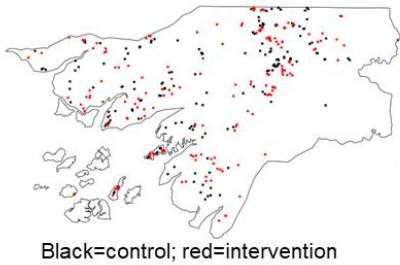


Figure 5. Geographical location of the Bandim Health Project's rural (framed in dark blue) and urban (framed in red) Health and Demographic Surveillance System.

Study 1. Effect of C-OPV vs no C-OPV on mortality and/or hospital admission among children aged 0-8mth in rural Guinea-Bissau

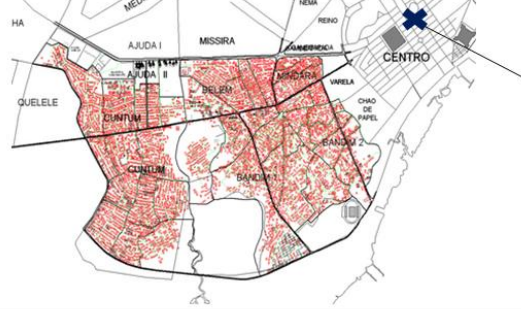


Exposure: randomisation (see map on the left)



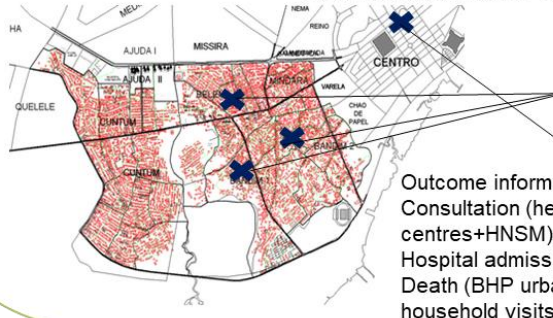
Outcome information: BHP rural HDSS household visits

Study 2. Risk of hospital admission/at-hospital death after DTP vs DTP+OPV among children attending outpatient consultations in urban Guinea-Bissau



Exposure and outcome information: The national hospital – Hospital Nacional Simão Mendes (HNSM)

Study 3. Risk of consultation, hospital admission and mortality after penta3+OPV3±IPV among children vaccinated at health centres in urban Guinea-Bissau



Exposure information: Urban health centres

Outcome information: Consultation (health centres+HNSM), Hospital admission (HNSM), Death (BHP urban HDSS household visits)



Figure 6. Overview of study designs in relation to the data source for exposure and outcome information.

06.01 The Bandim rural HDSS

The BHP rural HDSS collects routine data on maternal and child health in 222 village clusters across nine health regions of rural Guinea-Bissau[68]. Using the sampling method recommended by the Expanded Programme on Immunization for surveys of immunisation coverage, the village clusters constitute a representative sample of the rural population[68].

The routine data are collected by three field teams conducting household visits to all houses in the village clusters: bimonthly to children under 12 months of age in the three regions closest to the capital city Bissau, and biannually to older children, and children in the remaining regions (Table 4).

At the household visits, all women of reproductive age and children under the age of five years are followed as an open cohort. This means that girls reaching the reproductive age (13-15 years of age) and women migrating into the village will be followed from the day they are registered. Children of already-residing mothers are typically registered as foetuses, and thus followed from date of birth, whereas in-migrating children under the age of 5 years are followed from the date of registration. All women and children are followed until they move out of the village, die, or reach 5 years of age (children) (Table 4).

For the study using the rural setup in this thesis, data on the child was the primary focus. This data is collected in an interview with the mother/guardian of the child residing in the village. At the first visit after birth, the mother/guardian is asked whether it was a live birth, stillborn or an abortion and data on date of birth and sex is collected. Given that the child was liveborn, data is collected on date of birth, sex, breastfeeding status, vaccination status by inspection of vaccination card, mid-upper-arm-circumference (MUAC), BCG-scar, socio-economic factors, and whether the child is living with his/her mother. Except for BCG scar and MUAC, the data are collected independent of whether the child is present or absent/travelling. In addition, the assistant will ask whether the child has been ill and/or hospitalised since the last visit. If the child has been hospitalised, a hospitalisation form will be completed. Except for date of birth, sex and socioeconomic factors, the assistant collects the same information at the subsequent household visits.

06.02 The Bandim urban HDSS

The BHP urban HDSS covers six districts of the capital city Bissau and monitors the health and survival of around 100,000 people. Census visits are conducted

every 2-7 years, when funding allows. At these visits, all residing persons in the six districts are registered. For the more frequent routine visits registering children and pregnancies, women enter the population when registered as pregnant or as mother of a child below the age of 3 years (Table 4). Children are followed until the age of 3 years by quarterly household visits.

06.02.01 Hospitalisation form

Once a hospitalisation is registered in the rural HDSS, a hospitalisation form is used to collect information on duration, cause, date, and place of hospitalisation (Table 4).

In the urban HDSS, BHP assistants collect data on hospitalisation at the health centres and at the National Hospital Simão Mendes (HNSM) (Table 4). Thus, at the household visit the field assistant only collects information on whether the child was hospitalised since last visit and if so, where.

06.02.02 Verbal autopsy

If a child has died since the last visit, the rural and urban HDSS uses a verbal autopsy form to obtain additional information preceding the event. For the purpose of Paper 1, a specially trained field assistant conducted a verbal autopsy using the INDEPTH verbal autopsy to verify and elaborate on symptoms preceding a death[69].

06.02.03 BHP at health centres and at the National Hospital

BHP assistants are present at three urban health centres. To ensure we have data from all health centres in the six districts, data is collected with the help of local staff at the remaining urban health centres serving the study-area children. Through daily monitoring, assistants at health centre register all vaccines administered as well as outpatient consultations.

The HNSM serves both as the primary healthcare contact for children living in Bissau and as the national referral paediatric hospital. At the HNSM, BHP assistants are present both at the outpatient department, where all outpatient consultations are registered along with information on age, weight, and symptoms at the time of the consultation, and measurement of weight as well as maternal education and ethnicity. For children admitted to the paediatric ward, BHP assistants also register vaccination status from the child's vaccination card on date of

admission. At the paediatric ward, BHP assistants collect and verify information on diagnosis, length of stay and discharge status (“cured”, “recovering”, “dead” or “left against medical advice”) during update rounds twice a day.

Table 4. Overview of data collection in BHP urban/rural HDSS

BHP HDSS platform	Household visit cohort	Visit Frequency	Hospitalisation form	Verbal autopsy
Urban HDSS	Mothers and pregnant women and children aged 0-3 years	Quarterly	At household visit only asked if hospitalised and if yes, where	BHP form filled out at household visit
	Entire population Health facilities	Approximately every 2 years Vaccinations for all children Consultations for all children <5 years (health centers); <15 years HNSM	Forms filled out at health centres and HNSM	
Rural HDSS	Women of fertile age and children aged 0-5 years	Bimonthly to children aged <12mth in the three regions closest to Bissau; Biannually to children aged ≥12mth and in remaining regions	BHP form filled out at household visits	

06.03 Study designs

06.03.01 Randomised trial (Paper 1)

In Paper 1, we aimed to conduct the first cluster-randomised trial, RECAMP-OPV, assessing the effect of C-OPV vs no C-OPV on morbidity and mortality (Table 5) among children aged 0-8mth in rural Guinea-Bissau[70].

RECAMP-OPV was initiated in March 2017 using the setup of the BHP rural HDSS. The 222 village clusters were randomised 1:1 to an intervention or a control group based on externally generated numbers[70]. The randomisation was stratified by region and cluster-level pre-trial vaccination coverage.

Through the BHP rural HDSS routine household visits, all children aged 0-8 months were invited to the enrolment post where they were invited to participate and enrolled into RECAMP-OPV given that the mother/guardian of the child consented. The trial nurse then conducted a health check including a structured interview on symptoms, and measurement of temperature, mid-upper-arm-circumference (MUAC) (TALC insertion tape), and weight to nearest 20g (SECA 385 electronic scale). To mimic a national C-OPV there were few exclusion criteria (Paper 1 – Section 12.01).

Upon enrolment, children in the intervention group received one dose of OPV (two oral drops of a WHO prequalified standard bOPV). Children in the control group did not receive any vaccines on the date of enrolment. All children were eligible for the nationally recommended vaccines in the routine vaccination programme and vaccination campaigns.

RECAMP-OPV also tested the effect of an additional OPV dose. This was done by revisiting a subsample of the enrolled children 1-2 months after enrolment. During these visits, both children in the intervention and control group were visited, but only children in the intervention group received a second OPV dose. This subsample of children who received a revisit 1-2 months after enrolment also composed a sub-study in which we assessed the effect of C-OPV on the secondary outcomes; health centre consultation, self-reported illness, cause-specific morbidity (health centre consultation and self-reported illness, and growth).

The outcome of mortality, morbidity and growth was defined by the following outcome definitions.

Table 5. Definition of primary and secondary outcomes assessed in RECAMP-OPV (Paper 1 – Section 12.01).

Outcome	Definition	Data source
Primary outcome		
Non accidental mortality/hospital admission (composite outcome)	First hospital admission with overnight stay not due to accident or death due to any non-accident cause	BHP rural HDSS routine household visit, BHP HDSS hospitalisation form, BHP HDSS verbal autopsy, and INDEPTH Verbal Autopsy
Secondary outcomes		
Non-accidental mortality	Death due to any non-accident cause	BHP rural HDSS routine household visit, BHP verbal autopsy, and INDEPTH Verbal Autopsy
Non-accidental hospital admission	First hospital admission with overnight stay not due to accident	BHP rural HDSS household visit, BHP HDSS hospitalisation form, and INDEPTH Verbal Autopsy
Cause-specific mortality/hospital admission	Hospital admission with overnight stay or death due to: Malaria, respiratory infection, and/or gastrointestinal infection	BHP rural HDSS routine household visit, BHP HDSS hospitalisation form, and INDEPTH Verbal Autopsy
Health centre consultation	Any reported consultation in the month after enrolment	BHP rural HDSS routine household visit during interview with mother/guardian
Cause-specific health centre consultation	Any reported consultation in the month after enrolment due to: Malaria, respiratory infection, and/or gastrointestinal infection	BHP rural HDSS routine household visit during interview with mother/guardian
Self-reported illness	Any illness reported in the month after enrolment	BHP rural HDSS routine household visit during interview with mother/guardian
Cause-specific self-reported illness	Any illness reported during follow-up due to: Malaria, respiratory infection, and/or gastrointestinal infection	BHP rural HDSS routine household visit during interview with mother/guardian
Growth	Weight-for-age z-score at one month follow-up adjusted for baseline weight-for-age z-score	At enrolment post and at revisit 1-2 month after enrolment

Mid-upper-arm circumference at one month follow-up adjusted for baseline mid-upper-arm circumference	BHP rural HDSS routine household visit
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06.03.02 Natural experiments (Paper 2+3)

Both Paper 2 and Paper 3, tested the effect of OPV in natural experiments due to vaccine shortages. Natural experiments constitute a unique study opportunity for testing health interventions such as vaccines in the routine vaccination programme. In the perfect natural experiment, an outside factor determines the vaccination status of an individual. Thus, the self-selection for vaccination/non-vaccination which would otherwise commonly bias the comparison is no longer present. Hence, the variation resembles randomisation without interference from researchers by using naturally occurring variation in exposure to divide the study population into exposed and unexposed[71]. In the case of the routine vaccination programme, natural experiments also constitute an ethical design for evaluation of nationally recommended vaccines for which all children are eligible. However, the potential to infer causality between exposure and outcome in natural experiments is highly dependent on the ability to understand and define the contextual factors and the determinants of exposure to be able sufficiently control for potential confounding factors[71].

The natural experiment described in Paper 2 builds on data from a shortage of OPV in 2004. By using both data collected in relation to another study at that time[72] and data from the BHP urban HDSS collected at HNSM, the study investigates the risk of severe morbidity (Table 6) after vaccination with DTP-only vs DTP+OPV among children attending outpatient consultations at HNSM. Initially we wanted to include all children aged between 6 weeks and 24 months. However, a concurrent measles epidemic[73] and trial of two-dose-MV led us to exclude all MV-vaccinated children. Furthermore, as MV was recommended at 9 months of age (Figure 3), the age inclusion criteria was narrowed to 6 weeks to 14 months to avoid including a potentially highly selected population of older children (Paper 2 – Section 12.02).

Table 6. Definition of primary and secondary outcomes assessed in Paper 2 (Section 12.02).

Outcome	Definition	Data source
Primary outcome		
Hospital admission/at-hospital death	Hospital admission (recurrent event) or at-hospital death	Consultation questionnaire conducted in another study[72] verified through BHP urban HDSS data from HNSM
Secondary outcome		
Cause-specific hospital admission/at-hospital death	Hospital admission or at-hospital death due to: Respiratory infection, gastrointestinal infection, malaria, and/or measles	Consultation questionnaire conducted in another study[72]

The natural experiment described in Paper 3, assesses the effect of penta3+OPV3+IPV vs penta3+OPV3 on morbidity and mortality (Table 7) using data from the IPV introduction and subsequent re-introduction following the global IPV shortage. The starting point for this study was all children registered to have received penta3+OPV3±IPV at the urban health centres in Guinea-Bissau from one year before IPV introduction (August 1st, 2015) until 7 months into the IPV reintroduction (July 31st, 2019). As illustrated in Figure 4, penta3 is recommended at 14 weeks of age, and therefore only children who had received penta3(+OPV3±IPV) on or after 3 months of age were included.

Table 7. Definition of primary and secondary outcomes assessed in Paper 3 (Section 12.03).

Outcome	Definition	Data source
Primary outcome		
Non-accidental outpatient consultation	First outpatient consultation due to any non-accidental cause	BHP urban HDSS data from health centres and the outpatient department at HNSM
Non-accidental hospital admission	First hospital admission due to any non-accidental cause	BHP urban HDSS data from paediatric ward at HNSM
Secondary outcome		
Non-accidental mortality	Death due to any non-accidental cause	BHP urban HDSS routine household visit

06.04 Statistical analysis

Different statistical approaches were used to analyse the data used in the three papers depending on the study objective and the type and distribution of data. In all three papers, descriptive statistics were performed to enhance transparency and understanding of the study population and the statistical approach chosen for the comparative analyses. In the following, the studies will be described according to the statistical approach used.

06.04.01 Survival analysis

The Cox proportional hazards regression model allows for capturing the time-to-event when assessing the association between exposure and occurrence of an event of interest while adjusting for other risk factors[74]. Furthermore, censoring allows observations of individuals who have not experienced an event to contribute with risk time. This statistical approach was therefore applied in Paper 1 and Paper 3.

RECAMP-OPV: effect of C-OPV on mortality and/or hospital admission (Paper 1)

To assess the effect of C-OPV vs no C-OPV on the primary outcome as well as its separate components (Table 5), we calculated the rate of events per 1000 person-years-at-risk (PYRS), and compared the event rates between the intervention and control group in Cox-proportional hazards models allowing for different baseline hazards by region, pre-trial vaccination coverage, and sex[70].

Age was used as the underlying time scale to obtain age-adjusted estimates. Hazard ratios (HR) were estimated with 95% confidence intervals (CI) and cluster-robust standard errors allowing for intra-cluster correlation as children under HDSS surveillance were selected based on residence in a village cluster. The proportional hazards assumption was assessed using Schoenfeld residuals and log-log plots.

Potential effect modifiers were also assessed. Furthermore, when planning the study, VAS campaigns targeting children aged ≥ 6 months were conducted every 6 months, and we therefore anticipated that VAS campaigns would continue to be conducted every 6 months. However, this was not the case, and the analysis investigating VAS campaigns as an effect modifier by splitting observation time at 3 months of enrolment[70] was therefore replaced by an analysis investigating the effect of C-OPV during follow-up. The same principle of splitting observation time at 3 months of enrolment was applied.

Children contributed with risk time for up to 12 months or to death or first hospital admission, the next vaccination campaign for which the child was eligible, migration or end of study, whichever came first. All events due to accidents were censored.

Risk of consultation and hospital admission after penta3+OPV3±IPV (Paper 3)

Survival analysis using Cox proportional hazards models was also used to assess whether the risk of consultation and hospital admission was increased after penta3+OPV3+IPV (Paper 3 – Section 12.03). As this was a natural experiment other statistical adjustments than those in the randomised trial above were deployed. First, both crude and adjusted HRs were estimated with 95% CIs. Secondly, we adjusted for the weight-for-age z-score (WAZ)[75] at the time of penta3 vaccination in all analyses. Further adjustments made depended on the outcome of interest. For consultation, we observed large variations in consultation rates over time necessitating control for confounding conditioned by time-varying factors. The variations in consultation rates were in part caused by strikes and national vaccination campaigns diverting health personnel and resources and thus affecting access to healthcare and thus the risk of consultation in certain periods. Hence, to adjust for any potential confounding effect of access to healthcare, we adjusted for the underlying consultation rates (Paper 3 – Section 12.03). For hospital admissions, access to healthcare was not identified as a potential confounding factor as severely ill children were assumed to

be hospitalised independent of these strikes and campaigns (Paper 3, Supplementary material - Section 12.03). However, seasonal variation in morbidity, defined as month of penta3 vaccination, was adjusted for as it could affect both the exposure (significantly fewer children were IPV-vaccinated in the rainy season) and the outcome (the risk of hospital admission may be higher in the rainy season[76]).

In this analysis, children contributed with risk time from date of penta3 vaccination until 9 months of age, or to an event (consultation, hospital admission or death), the next vaccination campaign for which the child was eligible, or migration, whichever came first. Events due to accident were censored.

06.04.02 Log-binomial and linear regression models

The log-binomial regression model is appropriate to apply when the outcome is binary, and the outcome of interest is an adjusted relative risk[77]. This approach was therefore used in the sub-study described in Paper 1 (Section 06.03.01; Paper 1 – Section 12.01) and in Paper 2 (Section 06.03.02; Paper 2 - Section 12.02).

The linear regression model assumes a linear relationship between a categorical exposure variable and a continuous outcome variable and is therefore appropriate for assessing the difference in mean between two groups. This approach was therefore used to assess the effect on mean growth in the sub-study described in Paper 1 (Section 06.03.01; Paper 1 - Section 12.01).

RECAMP-OPV: effect of C-OPV on consultation and self-reported illness (Paper 1)

For the sub-study nested in RECAMP-OPV, log-binomial regression models were applied to assess the effect of C-OPV on consultation and illness. Relative risks (RR) and 95% CIs were obtained using cluster-robust standard errors, and the analyses were adjusted for the same stratification variables as in the primary analyses (region and pre-trial vaccination coverage), and sex[70].

RECAMP-OPV: effect of C-OPV on mean growth (Paper 1)

As part of the sub-study, mean growth, defined by WAZ and MUAC, from enrolment until the revisit 1-2 months after, was analysed in linear regression models

adjusting for the stratification variables and sex[70] and accounting for intra-cluster correlation by using cluster-robust standard errors.

06.04.03 Propensity score matching and inverse probability of treatment weight

Propensity score (PS) matching provides a method for adjusting for potential confounders in observational studies as potential confounding variables are balanced by matching the observations in the two study groups one-to-one based on similar propensity score values[78]. By double-adjustment; matching before running a regular adjusted regression analyses, it is possible to reduce confounding further if imbalance in covariates still exists after matching[79]. The data for Paper 2 was analysed using this approach as we had few events and many potential confounders we sought to adjust for.

Risk of severe illness after DTP vaccination (Paper 2)

In this natural experiment, confounding conditioned by individual factors was necessary to control for due to differences in WAZ, age and socioeconomic factors between the groups. Therefore, PS matching was used to balance potential confounding variables. Based on a logistic regression model including factors which were significantly different between the two groups in the unmatched sample as well as maternal education propensity scores for study group membership were estimated. Then, observations were matched one-to-one to nearest neighbour according to their propensity score without replacement using the Stata package psmatch2[80]. After PS matching, RRs were estimated in log-binomial regression models in which we accounted for children with more than one consultation by using cluster-robust standard errors. Furthermore, adjustments for age, period of consultation due to skewed distribution in groups over time, and WAZ were made.

Inverse probability of treatment weighted models (IPTW) and regular adjusted log-binomial regression models was also applied to evaluate the adjustments made to reduce confounding. Using IPTW, observations for children were assigned different weights depending on the probability of being DTP-only vaccinated given their characteristics at time of vaccination.

06.04.04

Effect modification

In all three Papers, potential effect modifiers identified in previous studies (Section 04.09) were investigated. This was done by including interaction terms and assessing whether the effect on the outcome measure varied across strata.

07 RESULTS

Below follows a brief summary of the results for each of the three studies conducted described by study objective. This will be done in the following order: first, the randomised trial comparing C-OPV vs no C-OPV; second, the natural experiment comparing DTP-only vs DTP+OPV among children attending outpatient consultations; third, the natural experiment comparing penta3+OPV3+IPV vs penta3+OPV3.

07.01 C-OPV vs no C-OPV

The rates of mortality/hospital admission, mortality and hospital admission were compared among 10,175 children aged 0-8 months who received C-OPV (5,288) or no C-OPV (4,887) in the period from March 2017 to March 2020 (Figure 7-9).

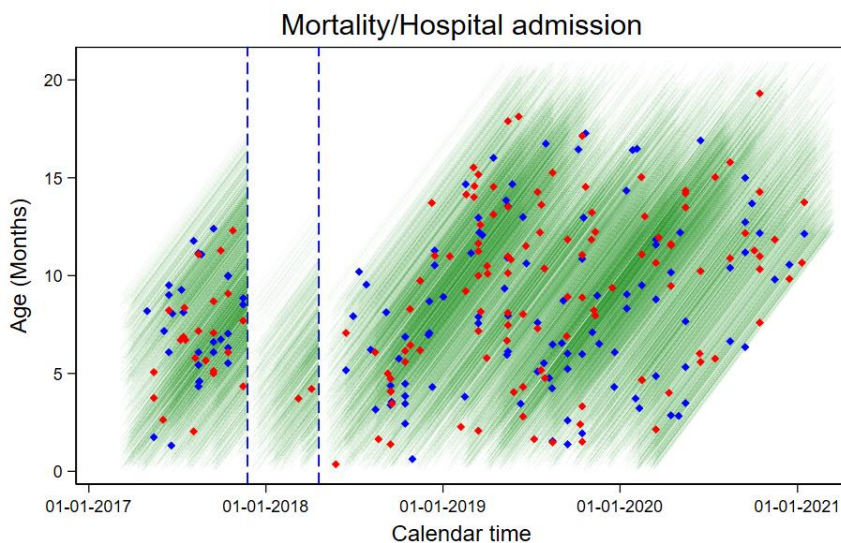


Figure 7. Lexis diagram illustrating the distribution of non-accidental deaths/hospital admissions according to calendar time and age in months. Blue indicates events in the C-OPV group and red indicates events in the no C-OPV group. The dotted lines marks censoring for national C-OPVs.

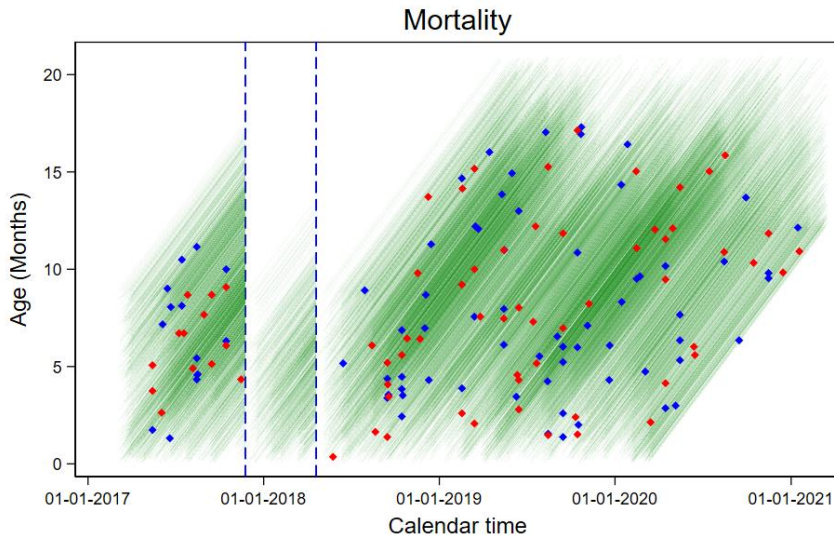


Figure 8. Lexis diagram illustrating the distribution of non-accidental deaths according to calendar time and age in months. Blue indicates events in the C-OPV group and red indicates events in the no C-OPV group. The dotted lines marks censoring for national C-OPVs.

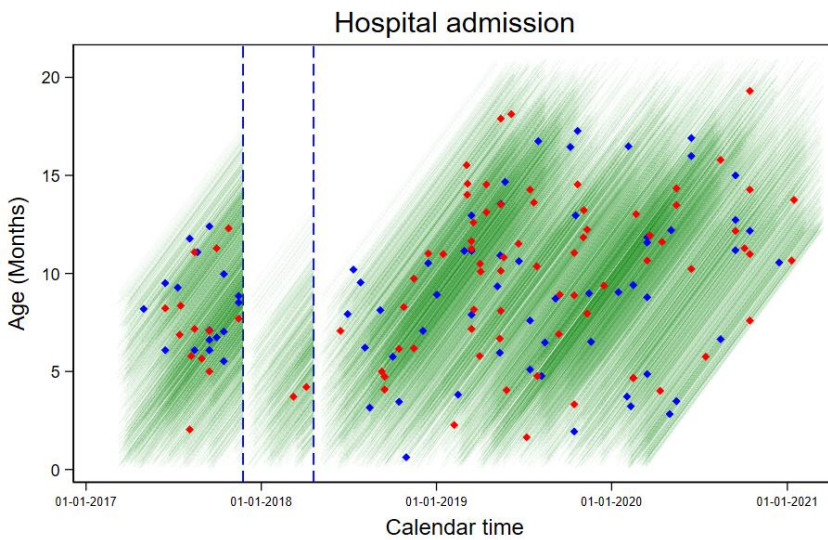


Figure 9. Lexis diagram illustrating the distribution of non-accidental hospital admissions according to calendar time and age in months. Blue indicates events in the

C-OPV group and red indicates events in the no C-OPV group. The dotted lines marks censoring for national C-OPVs.

For the children who received C-OPV compared with children who received no C-OPV, we found no significantly reduced risk of mortality/hospital admission (HR: 0.87, 95% CI 0.68-1.12), mortality (HR: 1.03, 95% CI 0.76-1.42) or hospital admission (HR: 0.82, 95% CI 0.59-1.15) (Figure 10).

Based on the Schoenfeld residuals and log-log plots, we found no indications of violation of the proportional hazards assumption for any of the outcomes. Furthermore, no cause-specific effect of C-OPV on the pre-specified cause-categories; malaria, respiratory and gastrointestinal infections were identified. The results were robust to sensitivity analyses conducted.

Timing of OPV0, significantly modified the effect of C-OPV on mortality/hospital admission ($p=0.04$) (Figure 10). Among the children who received OPV0 within the first 14 days of birth, C-OPV significantly reduced the risk of mortality/hospital admission by 34% (HR: 0.66, 95% CI 0.46-0.94), but among the children who received OPV0 after 14 days from birth, C-OPV did not reduce the risk of mortality/hospital admission (HR: 1.22, 95% CI 0.80-1.88) (Figure 10).

When we stratified the analysis by sex, the rate of mortality/hospital admission was lower for boys who received C-OPV (32.8 per 1,000 PYRS) compared with boys who received no C-OPV (43.4 per 1,000 PYRS) (Figure 10+11). The HR comparing mortality/hospital admission of boys in the intervention group with boys in the control group was 0.78 (95% CI 0.58-1.06). No difference in risk of mortality/hospital admission was observed between girls who received C-OPV compared with girls who received no C-OPV (HR: 1.00, 95% CI 0.69-1.43) (Figure 10+11). However, no significant interaction was found between C-OPV and sex ($p=0.27$ for same effect). The sex-differential tendencies were strongest for mortality.

The analysis of the effect of C-OPV during the course of follow-up, revealed that the effect of C-OPV on mortality/hospital admission was significantly different before and after 3 months after enrolment ($p=0.02$ for same effect) (Figure 10). We therefore explored the effect of C-OPV during follow-up by applying monthly cut-offs in observation time. From this analysis, we observed the strongest differential effect when the observation time was split at 4-5 months (Paper 1, Supplementary Material - Section 12.01). When conducting the analyses separately on mortality and hospital admission, we found that the change in the

effect of C-OPV over time was mainly driven by a differential effect on hospital admission (Paper 1, Supplementary Material - Section 12.01).

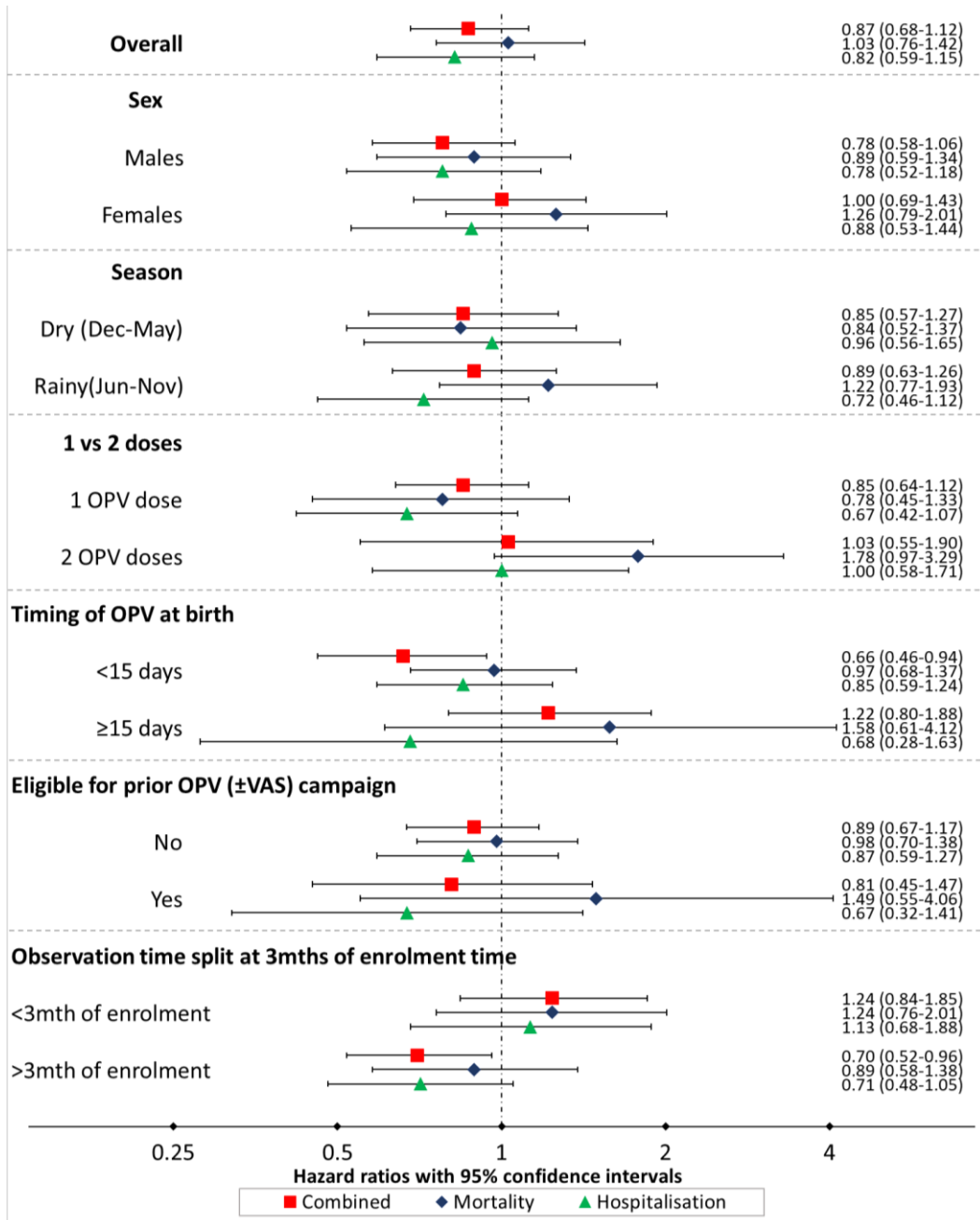
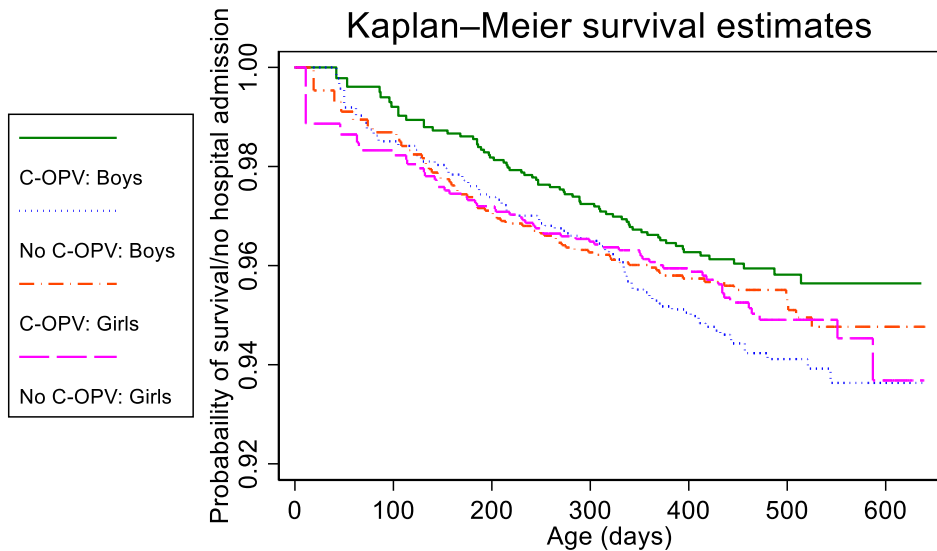


Figure 10. Age-adjusted hazard ratios of the effect of C-OPV for primary and secondary outcomes and estimates stratified by potential effect modifiers investigated.



Number at risk		0	100	200	300	400	500	600
sexx = 1/group = Inter	0	1063	1895	1937	1496	660	98	
sexx = 1/group = Control	1	1002	1793	1836	1413	640	124	
sexx = 2/group = Inter	1	1006	1853	1941	1533	705	119	
sexx = 2/group = Control	0	960	1663	1736	1313	581	72	

Figure 11. Kaplan-Meier survival estimates of mortality/hospital admission among boys and girls who received C-OPV and no C-OPV.

07.01.01 Sub-study results: health centre consultation, self-reported illness and growth

Among the 10,175 children enrolled in RECOMP-OPV, 3,993 (2,099 intervention/1,894 control) children were revisited at home at a median of 58 days after enrolment to conduct a morbidity interview. Through these revisits, information on consultation and self-reported illness was obtained for 89% and 91% of the children, respectively (Paper 1, Supplementary Material - Section 12.01).

The absolute risk of consultation was lower among the children who received C-OPV (15%: 281/1,867) compared with children who received no C-OPV (17%: 287/1,675), however not significantly (RR: 0.86, 95% CI 0.70-1.06) (Figure 12). No difference between the intervention and control group was observed when asking about self-reported illness (RR: 0.95, 95% CI 0.84-1.09) (Figure 12).

The risk of consultation and self-reported illness appeared to be independent of whether the event was due to fever, gastrointestinal, respiratory and/or non-gastrointestinal symptoms (Paper 1, Supplementary Material - Section 12.01). No significant effect modifiers were found for consultation and self-reported illness (Figure 12).

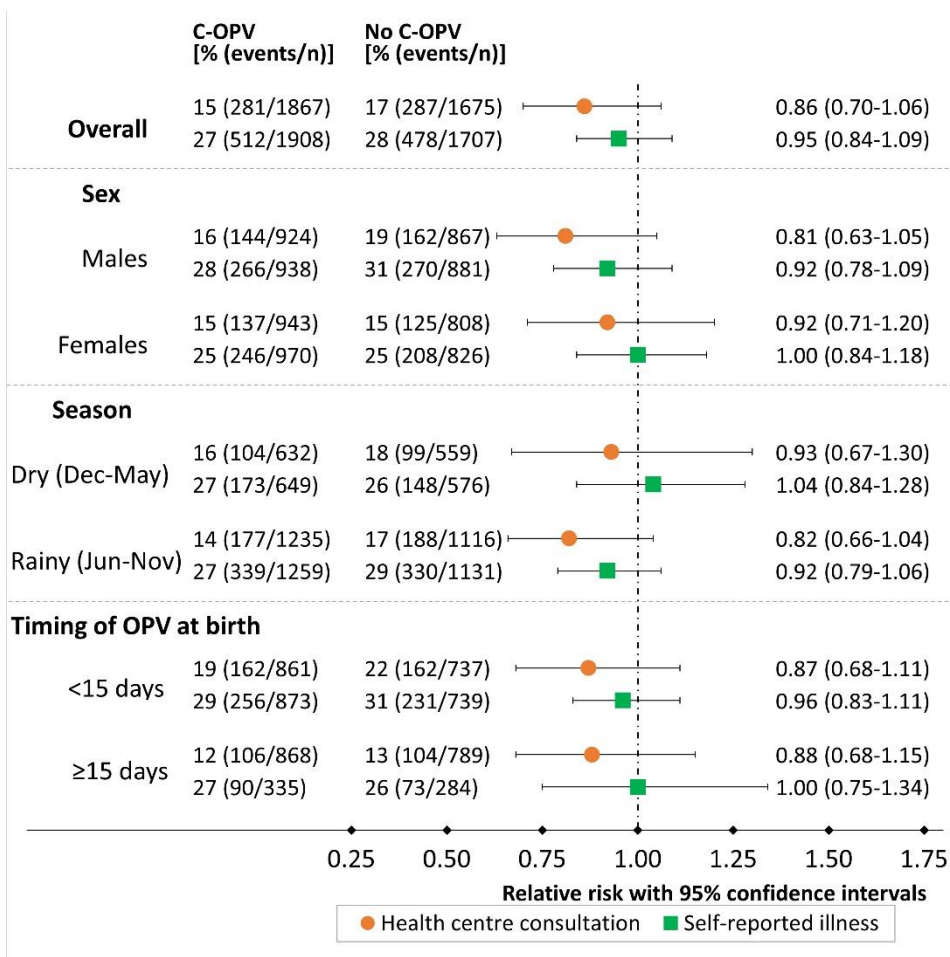


Figure 12. Relative risk of health centre consultation and illness, overall and for potential effect modifiers investigated.

At the revisits MUAC was also measured. A total of 3,156 children (1,667 intervention/1,489 control) who were present at the household visit had their MUAC measured both at enrolment and at the revisit (Paper 1, Supplementary Material - Section 12.01). Mean MUAC growth did not differ by sex although

MUAC growth was statistically significant reduced for only girls who received C-OPV at enrolment ($p=0.10$ for interaction) (Figure 13).

Among the 3,993 children revisited, 1,774 children (910 intervention/864 control) were revisited at a median of 32 days (IQR: 29-41 days) after enrolment (31 days intervention/32 days control) for weighing and to provide a second C-OPV one month after the C-OPV given at enrolment. At these revisits, 85% of the children were home and sent to our mobile health post and weighed (Paper 1, Supplementary Material - Section 12.01). No difference in WAZ growth was observed although weight gain tended to increase in boys who received C-OPV at enrolment ($p=0.14$ for interaction) (Figure 13).

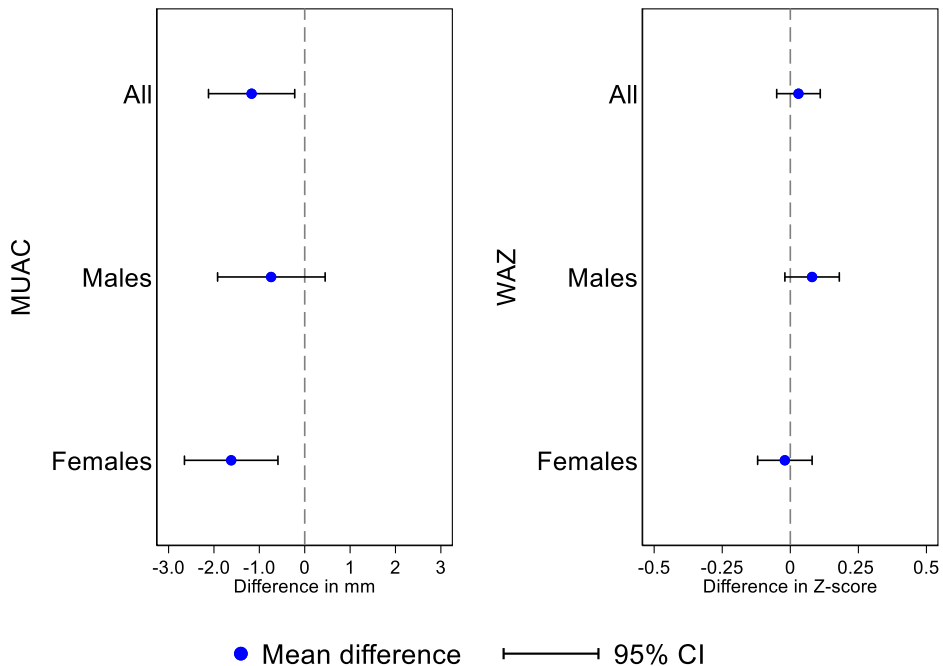


Figure 13. Difference in mean mid-upper-arm-circumference (MUAC) and weight-for-age z-score (WAZ) between C-OPV and no C-OPV children, overall and by sex (Paper 1, Supplementary Material - Section 12.01).

Abbreviations: CI=Confidence interval. Among children present at follow-up with complete information on MUAC.

When exploring the risk of consultation for the children referred to the post and thus revisited at a median of 32 days of enrolment, we found a 32% lower risk of consultation in the C-OPV group (RR 0.68, 95% CI 0.50-0.92) compared with

the no C-OPV group. This effect differed by sex ($p=0.04$ for interaction) with a beneficial effect for boys (RR 0.51, 95% CI 0.34-0.78) but not for girls (RR 0.92, 95% CI 0.62-1.38) (Figure 14, Paper 1, Supplementary Material - Section 12.01).

Using the sub-study sample of children who were revisited, we also conducted three sensitivity analyses where we estimated the effect of C-OPV while minimising the effect of other vaccinations. These analyses restricted the sample to children with; 1) confirmed non-receipt of vaccines during follow-up (sensitivity A); and 2) no scheduled vaccines during follow-up (Sensitivity B). In the third sensitivity analysis, we adjusted for the length of follow-up. The results were robust to these sensitivity analyses (Figure 14).

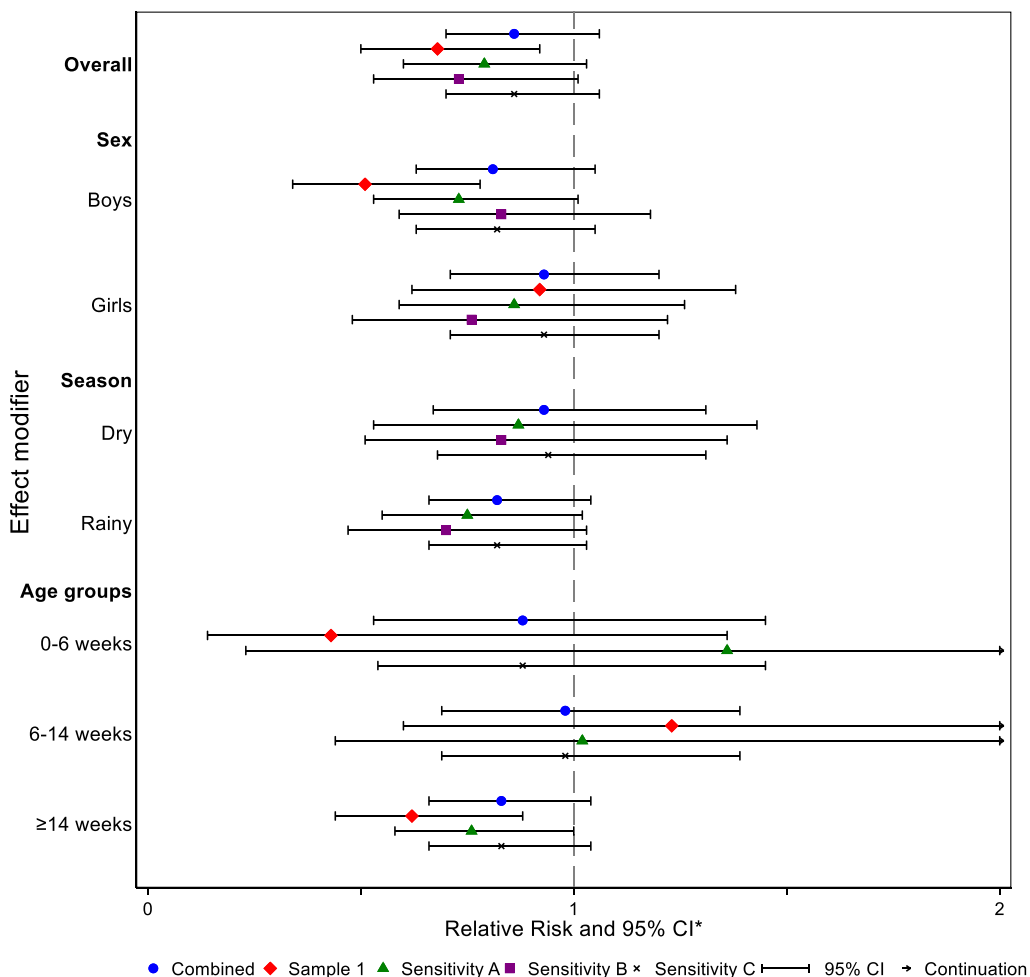


Figure 14. The estimated effect of C-OPV vs no C-OPV in different subsamples within the sample of children, who were revisited at 1-2 months after enrolment in RECAMP-OPV. Combined: All children revisited 1-2 months after enrolment in RECAMP-OPV. Sample 1: Sample restricted to children revisited at a median of 32 days after enrolment. Sensitivity A: Sample restricted to children who, documented by a vaccination card seen at the revisit, had received no routine nor national campaign vaccines since enrolment. Sensitivity B: Sample restricted to children aged <9 months at the revisit, who were not scheduled to receive any vaccines during follow-up, i.e., had received the full routine immunisation schedule up to 14 weeks of age before enrolment and were not eligible for national campaign vaccines during the period from enrolment until revisit. Sensitivity C: Including all children revisited, estimated effect adjusted for length of follow-up.

07.02 DTP-only vs DTP+OPV

A total of 10,146 children aged 6 weeks to 24 months attended outpatient consultations at HNSM between start-May 2003 and end-May 2004 (Paper 2 – Section 12.02). Among these children, we excluded all children who had presented no vaccination card, and who received the most recent vaccine >6 months before consultation, and who were previously vaccinated with MV, and who were older than 15 month. After this, 2,982 children aged 6 weeks to 14 months of age were included; 341 with DTP-only and 2,641 children with DTP-OPV as the most recent vaccine(s) received.

A total of 340 children from the DTP-only group were matched 1:1 with children in the DTP+OPV group based on a propensity score defined by covariates which significantly differed between the groups (age, period of consultation, area of residence, presence of ceiling, number of DTP doses, and eligibility for C-OPV and campaigns with VAS), and covariates which could potentially increase precision (maternal education and WAZ). Imbalances in age, area of residence, and number of DTP doses remained after matching.

A total of 104 hospital admissions (47 DTP-only/57 DTP+OPV), and no at-hospital deaths were observed. Contrary to our hypothesis, the risk of hospital admission tended to be lower for children who had received DTP-only (14%: 47/340) than for children who had received DTP+OPV (17%: 57/340). When comparing the risks and adjusting for age, period of consultation and WAZ the RR was 0.85 (95% CI 0.60-1.21) (Figure 15).

When analysing the relative risk by cause-specific events, the children who received DTP-only appeared to have a lower risk of hospital admission due to measles infection (Paper 2 – Section 12.02).

No potential effect modifiers explored significantly modified the risk of hospital admission/at-hospital death after DTP-only among children attending consultations at HNSM. Results were robust to the sensitivity analyses using other statistical approaches (Figure 15). Results also remained unaltered when we restricted the time period and adjusted for age in other ways (Paper 2, Supplementary Material - Section 12.02).

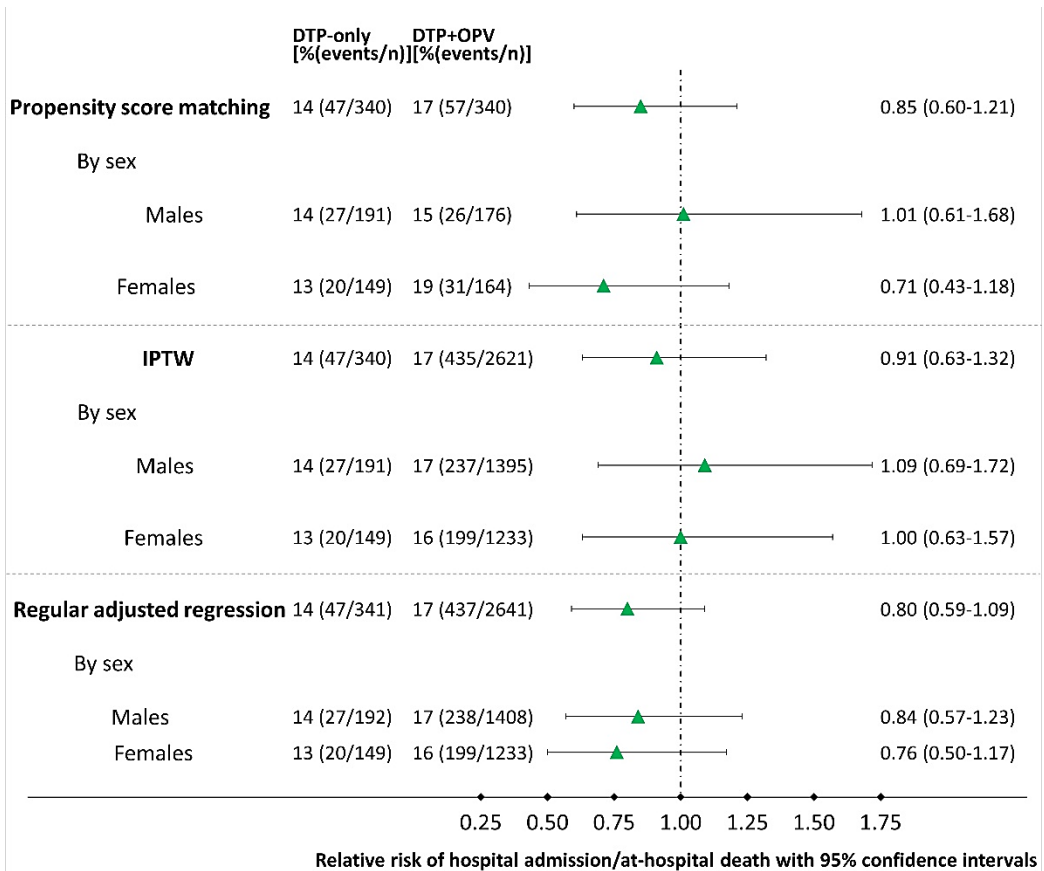


Figure 15. Relative risk of hospital admission/at-hospital death after DTP-only vs DTP+OPV as last vaccine(s), overall and by sex among children attending outpatient consultations, in the different statistical models applied. Abbreviations: DTP=Diphtheria-tetanus-pertussis vaccine; OPV=Oral polio vaccine; IPTW=Inverse probability of treatment weights. Note: to reach convergence in the IPTW model analysing the effect dependent on sex, age was adjusted for in quartiles instead of as a continuous variable.

07.03 IPV vs no-IPV

During the period of no IPV, IPV introduction, IPV shortage and the subsequent IPV reintroduction, 5,978 children residing in the BHP urban HDSS area and who were between the age of 3-8 months received penta3 at the urban health centres in Guinea-Bissau (Paper 3 – Section 12.03). Among these, 2,083 children received IPV together with their third dose of penta+OPV whereas 3,834 children received only penta3+OPV (Paper 3 – Section 12.03).

In this natural experiment, significantly more children in the penta3+OPV3 group were vaccinated in the rainy season and eligible for national C-OPVs both before and after penta3 vaccination, and the median follow-up time was therefore also shorter in this group compared with the group of children who received penta3+OPV3+IPV.

Contrary to our hypothesis, the crude rate of consultation was significantly lower among children who received IPV together with penta3+OPV3 (1.8 consultations per person-year) compared with children who received only penta3+OPV3 (2.1 consultations per person-year) (HR 0.87, 95% CI 0.80-0.94). However, this may largely have been driven by a differential access to consultation services: when we adjusted for the underlying consultation rates, the hazard ratio was 0.94 (95% CI 0.86-1.03) (Figure 16). Furthermore, the risk of consultation seemed to be lower among girls who received IPV with penta3+OPV3 than among boys ($p=0.06$ for same effect) (Figure 16). No statistically significant differences in the risk of hospital admission and mortality were found between the two groups, overall and by sex (Figure 16).

Based on the Schoenfeld residuals and log-log plots, we found no indications of violation of the proportional hazards assumption for any of the outcomes. The results were robust to sensitivity analyses conducted.

When we explored the change in risk of consultation and hospital admission over time, it appeared that the potential protective effect of co-administering IPV with penta3+OPV3 was only evident during the first 1-4 months after vaccination (Paper 3, Supplementary Material - Section 12.03). The differences in outcome rates within and between the groups over time were, however, small.

In the analyses exploring the F/M HRs by study group, females who received IPV co-administered with penta3+OPV3 tended to have a lower risk of consultation and hospital admission compared with boys (Paper 3, Supplementary Material - Section 12.03). The female and male rates according to IPV status in

Guinea-Bissau (no IPV, introduction, shortage, reintroduction) revealed that the outcome rates varied greatly across the study period for both boys and girls. Nonetheless, the tendency of a decline in risk of consultation and hospital admission for girls compared to boys, was also observed when we analysed the F/M HRs by IPV status while the opposite effect was seen for mortality (Figure 17). Furthermore, for mortality, the sex-differential effect became more pronounced when we included all children who received penta3+OPV3±IPV (F/M HR 4.02, 95% CI 1.29-12.47) (Paper 3 – Section 12.03, data not shown).

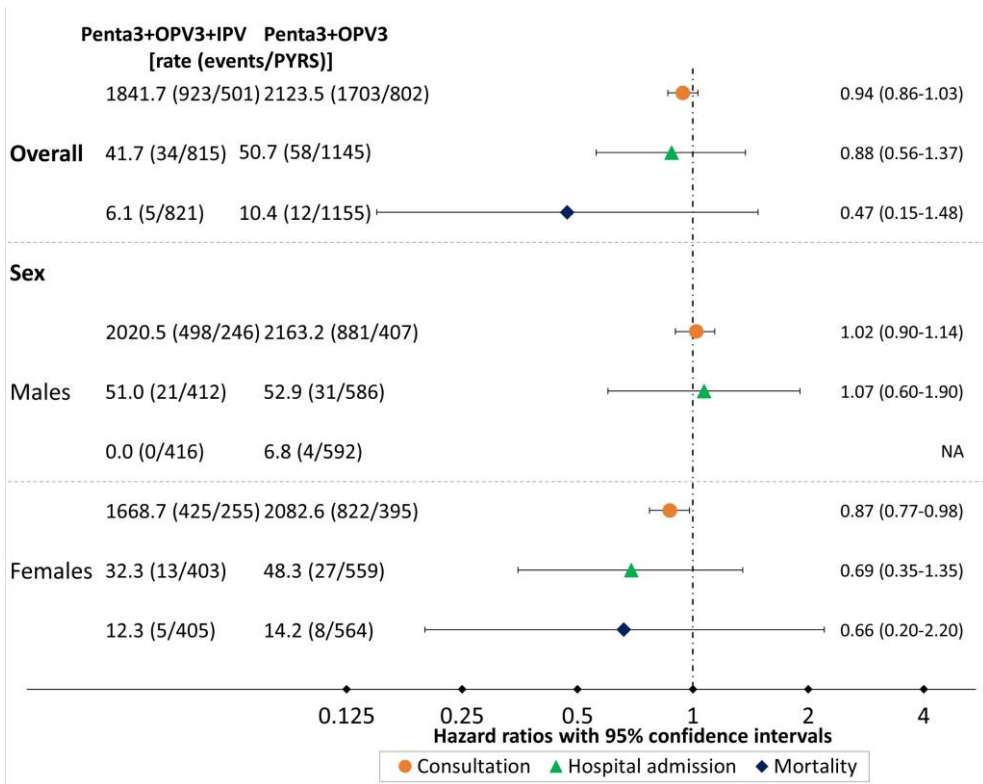


Figure 16. Age-adjusted hazard ratios of the risk of consultation, hospital admission and mortality after penta3+OPV3+IPV vs penta3+OPV3 adjusted for weight-for-age z-score (WAZ) and a) the underlying consultation rates (consultation) or b) month of penta3 vaccination (hospital admission and mortality), overall and by sex. Rate is shown per 1,000 PYRS.

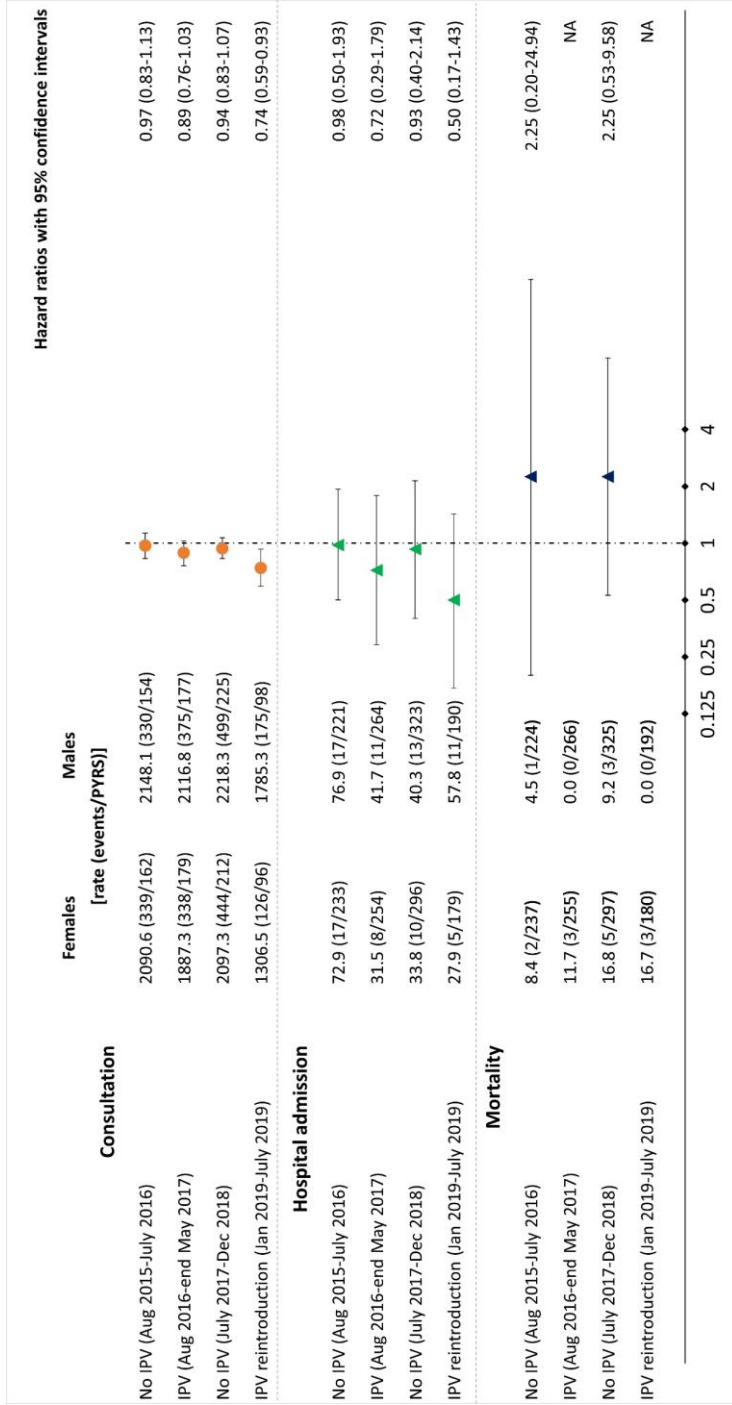


Figure 17. Overall age-adjusted female/male hazard ratios with 95% confidence intervals, by IPV status during the study period, adjusted for weight-for-age (WAZ) and a) the underlying consultation rates (consultation) or b) month of penta3 vaccination (hospital admission and mortality). Rate are shown per 1,000 PYRS.

08 DISCUSSION

This dissertation aimed to assess the NSEs of OPV and IPV on child health in Guinea-Bissau to provide insights into the implications of replacing OPV with IPV, and whether this may augment child morbidity and mortality. In summary, the overall results were less strong than anticipated and some were in contradiction to our hypothesis. Nonetheless, the results indicated NSEs of both OPV and IPV.

First, we found no effect of C-OPV vs no C-OPV on morbidity/mortality and growth. However, timing of OPV0, was identified as a potential effect modifier. Furthermore, sex and time since vaccination may also have modified the effect of C-OPV, though this did not reach statistical significance in Paper 1. Second, utilising a natural experiment to assess if no co-administration of OPV with DTP was associated with a higher risk of severe morbidity among children attending outpatient consultations, our hypothesis was contradicted (Paper 2). These results were robust to sensitivity analyses using different statistical approaches. Third, in Paper 3, we assessed the risk of consultation, hospital admission and mortality utilising a natural experiment in relation to the IPV introduction in 2016. Contrary to our hypothesis, results did not confirm a higher risk of any of the three outcomes after penta3+OPV3+IPV vs penta+OPV3. Sex was however, identified as a potential effect modifier.

The evaluation of OPV vs IPV in this dissertation was based on studies separately assessing the effect of OPV and IPV. This also included different study designs for assessing the NSEs of OPV and IPV on child health, and these demonstrated the strengths and limitations of using real-life data. As a starting point for the discussion, these strengths and limitations will therefore be discussed.

08.01 Strengths and limitations

The basis for all three studies conducted were the data from the BHP HDSS sites in Guinea-Bissau. This routine data is collected by trained and supervised fieldworkers, who are experienced in collecting data at household and health facilities, and the BHP holds a good reputation among the local population. This contributes to the quality and reliability of the data. The regular HDSS household visits and data collection at health centres and the HNSM allowed for

inclusion of information on various background and confounding factors, for which some are updated regularly (e.g., age, MUAC, WAZ, vaccination status). The data collection routines also made it possible to obtain information on timing of an event, such as birth, illness, health centre consultation, hospital admission and death, with high precision.

In addition, the BHP HDSS sites provided a unique opportunity for conducting and setting up a cluster-randomised trial in a feasible manner as well as exploiting the opportunities for natural experiments as unplanned research opportunities while being able to include information on potential confounders and changes in exposure.

08.01.01 Cluster-randomised trial

In RECAMP-OPV, the intervention was designed as a campaign to allow for assessment of the effect of C-OPV in a cluster-randomised trial design. Hence, no children were deprived of routine vaccinations as OPV was given as a supplement to the routine vaccination programme. The design of the trial also tried to mimic a national C-OPV by providing a second C-OPV a month after the first C-OPV to children in the intervention group. The results are therefore relevant for national C-OPVs in Guinea-Bissau. However, only children aged 0-8 months were eligible whereas normally children aged <5 years are eligible for the national C-OPVs. Thus, the results may not be generalisable to older age groups participating in national C-OPVs.

The trial included children in villages randomly assigned to C-OPV or no C-OPV, and the risk of confounding by background characteristics was therefore limited. Utilising the BHP rural HDSS we were able to feasibly obtain a large sample size. Furthermore, with the described features of OPV shedding in faeces and OPVs ability to immunise other children in close vicinity (Section 04.02), a potential beneficial effect could spread to children who received no C-OPV. Hence, a design with individual randomization would be expected to dilute any difference in effects between the groups. However, the randomisation by village-cluster limited such contamination and thus dilution of effects between the intervention and control group.

Certain statistical considerations strengthened the results. By stratifying the randomisation of village-clusters by region and pre-trial vaccination coverage, we were able to balance the intervention and control group on baseline factors, such as ethnicity, cultural norms, and access to healthcare, which could be

predictive of the outcome measure[81]. Furthermore, the cluster-randomised design implies that the individual-level data within each cluster is correlated[82]. However, this was accounted for by using cluster-robust standard errors.

The fact that RECOMP-OPV was an unblinded trial and thus did not use a placebo vaccine to blind the participants could have affected the results. If mothers/guardian anticipated a beneficial effect of C-OPV, they could have been less likely to recall milder illness or less prone to seek healthcare. Thus, making it less likely to observe any potential negative NSEs of C-OPV. However, within the sub-study population, there was no marked difference in the proportions having sought consultation among the children for whom illness episodes were reported (data not shown). Furthermore, as field workers were supervised and morbidity information was collected using standard questionnaires, the potential impact of unblinding likely had a limited effect.

As per-protocol, multiple factors were tested as effect modifiers[70]. We did not adjust for multiple testing as secondary and exploratory analyses were conducted to assess patterns. Therefore, conclusions based on p-values and CIs should be interpreted cautiously in relation to the interaction analyses as this increases the risk of type 1 errors.

Finally, the rate of mortality/hospital admission was much lower than expected, and this might have lowered the power to identify our primary endpoint as significant. Although we did estimate the effect on the separate components of the composite outcome, the trial might have benefitted from being powered to assess the effect on one of the separate outcome components: either mortality or hospital admission as the primary outcome rather than a composite outcome since the effect on different subcomponents differed.

08.01.02 Natural experiments

The two natural experiments provided a unique opportunity to study the potential effects of the vaccines in a study design, which would have been otherwise unethical. Exactly because it is unethical to manipulate exposure to health interventions which are recommended and presumably beneficial, these natural experiments can contribute with important and unique information. However, natural experiments as all other observational studies, are also more susceptible to bias and confounding, and it is therefore crucial to understand the processes determining exposure variation[83].

Unlike the RECAMP-OPV trial, the risk of confounding by individual factors posed a challenge in the natural experiment in Paper 2. Thus, the assumption of exchangeability for measuring causal inference was violated[84]. PS matching was therefore chosen as a tool to strengthen exchangeability between the groups. Matching the groups 1:1 on the propensity score strengthened exchangeability between the groups, but differences in important predictors of the outcome, e.g., median age and area of residence, remained. Furthermore, due to an unanticipated small proportion on children having received DTP-only, exchangeability was strengthened in exchange for loss of statistical power. Although we also adjusted for potential confounders in the regression analyses, residual confounding due to inexact matching or unmeasured confounding may have distorted the association between vaccination status and severe morbidity.

The robustness of estimates was assessed using different statistical models; IPWT and regular multivariable regression analysis. Like PS matching, IPWT is a known tool for estimating causal inference in observational studies by assigning weights according to the specified confounder variables so that the ratios of the confounding factor are comparable between the groups[84]. The same propensity score models were used in PS matching and IPTW and contributed to consistency in model specification. Furthermore, results appeared robust using different statistical approaches. On the other hand, the consistency in model specification could also mean that the same residual confounding was induced in all models. Thus, providing similar results due to the same confounding included in the different statistical models.

The study population in Paper 2 was restricted to children attending outpatient consultations – a population which may be worse off than the general population as they sought health care. Therefore, the study population may not be representative of the general population and whether DTP(\pm OPV) has affected the health status of the child prior to consultation cannot be concluded based on our study. Hence, this would have implications for the external validity of the study results. Furthermore, after matching, age and residence in study area remained statistically significant between the two groups; the DTP-only were slightly younger, and a larger proportion of them resided in the BHP study area. We did adjust for age in the main analyses; however, we did not adjust for area of residence. This could lead to a geographical selection in favour of the DTP-only group as children residing in the BHP area may be better off in terms of mortality[85] and in-hospital mortality[65] compared to children outside the BHP study area. On the other hand, we observed no difference in the outcome estimate

after adjusting for area of residence thus indicating that geographical selection may be limited.

Other limitations might be related to the quality of information collected on vaccination status as well as hospitalisation status. However, given that we only included children with a seen vaccination card at date of consultation we assume this to be limited.

In Paper 2, we adjusted for WAZ as a proxy for the child's health status and therefore potentially able to increase precision. However, it can be discussed whether using post-vaccination measurements of weight and age to adjust for WAZ defined as a pre-vaccination predictor have introduced information bias. It can also be discussed whether WAZ should have been considered as an intermediate variable; vaccination affects the child's health status (and therefore WAZ), which affects the child's risk of severe illness. However, in prior studies assessing the effects of DTP+OPV we have not observed effects mediated via WAZ: In a previous observational study of mortality after early-DTP (before 2 months of age) within an RCT of BCG vs no-BCG to low birthweight infants, the negative association between early-DTP and mortality became stronger when adjusted for nutritional status[86], though nutritional status was a strong predictor of mortality. Furthermore, no difference in outcome estimates were observed when excluding WAZ from the PS model and/or from the adjustment (data not shown). This could indicate that the influence of WAZ on risk of severe illness after DTP-only vs OPV+DTP among children attending outpatient consultations might be limited.

In Paper 3, the allocation of children to either penta3+OPV3+IPV or penta3+OPV3 happened seemingly more random. Thus, the distribution of background factors was more balanced at baseline compared to Paper 2. However, confounding by time due to differential access to healthcare posed a challenge. As data on health personnel strikes and vaccination campaigns were not available at the time of analysis, we adjusted for the underlying consultation rates (consultation) and season of vaccination (hospital admission and mortality). However, our adjustment may have been insufficient.

Right censoring due to exposure to OPV campaigns was more prevalent in the penta+OPV3 group compared to the penta3+OPV3+IPV group resulting in more incomplete follow-up. However, the censoring due to OPV campaigns was non-informative and thus unrelated to the study, and should therefore not introduce

bias[88]. Supporting this, estimates remained unaltered when we did not censor for OPV campaigns.

08.02 Paucity of evidence

The formulation of the hierarchy of evidence has been in place since the 1990s[89], and has been named the pyramid of evidence. According to the pyramid of evidence, the randomised trial is superior to the observational studies, such as natural experiments. However, in the case of paucity of evidence others have argued that this hierarchy might be too rigid and simplistic[89]. Instead, a wavy version of the pyramid of evidence have been proposed (Figure 18). Thus, making room for study-specific arguments for why, for example, a natural experiment could be equal to/hold priority over randomised trials in certain cases.

This would certainly be relevant for the evaluation of NSEs of OPV and IPV, as routine vaccinations or as introduction of vaccines, for which randomised trials are unattainable due to research ethics. Thus, a wavy version of the pyramid of evidence, could contribute to a demand for exploiting the unplanned opportunities for research to strengthen the evidence base.



Figure 18. The proposed new and wavy evidence-based pyramid[89]. The wavy pyramid being less hierarchical and instead of systematic reviews and meta-analysis being in the top it should be the lens for viewing the evidence base.

08.03 NSEs of OPV vs IPV: what do we know now?

The results of the studies conducted underlines the importance of using real-life data to be able to identify and consider different health outcomes as well as the complexity of the setting, such as potential effect modifiers, time period and confounding factors, when evaluating the NSEs of OPV and IPV. The current evidence does not allow for conclusions on whether a difference in the effects of OPV and IPV is due to beneficial NSEs of OPV and/or negative NSEs of IPV. However, the evidence of NSEs of OPV and IPV is growing.

08.03.01 OPV vs IPV or OPV vs OPV+IPV

As previously mentioned, only two studies have compared the effect of IPV vs OPV (Section 04.06.03). Both studies found OPV to be associated with better health outcomes; lower duration of diarrhoea[46] and lower incidence of physician-diagnosed otitis media[43]. The study described in Paper 3, comparing penta3+OPV3+IPV vs penta3+OPV3 is most comparable to those studies. Furthermore, their outcomes are most comparable to our outcome consultation. However, we did not find a higher risk of consultation for children vaccinated

with penta3+OPV3+IPV compared to penta3+OPV3. The discrepancy between the previous findings and our results may be due to the disease-specific outcome definition as our outcome was not disease-specific. Supporting this, a Danish study assessing the rate of hospital admission by cause after OPV vs DTaP-IPV-Hib found, like the Finish study, a marked reduction in only respiratory events[42]. It might also be that the studies are not comparable as we compared penta3+OPV3+IPV vs penta3+OPV3 whereas the previous studies compared OPV vs IPV in different ages and in different vaccination schedules. Thus, the potential negative effect of IPV may be diluted when IPV is co-administered with other vaccines, such as penta and OPV.

08.03.02 OPV vs no-OPV

The effects of OPV on both mortality and morbidity have been investigated extensively, and they have all indicated a beneficial effect of C-OPV on mortality[26-32]. We found no statistically significant effect of C-OPV on the composite outcome, and a potential effect seemed strongest for hospital admissions, not mortality (Section 07.01; Paper 1 - Section 12.01). Possible explanations for the discrepancy between previous studies and our results could be related to a change in disease pattern over time as well as the narrow age group considered in our trial[90]. The previous studies of C-OPV were mainly based on data from 2000-2014 whereas RECAMP-OPV was conducted between 2017-2020. Previous studies[76, 91] as well as the United Nations – Populations Division[92, 93] have shown that child mortality declined in Guinea-Bissau between these periods. Furthermore, the Institute for Health Metrics and Evaluation Global Burden of Disease study have estimated a decline in the infectious diseases death rate including the respiratory diseases and the diarrhoeal diseases death rate during the period from 2014 to 2018[94-96]. The introduction of PCV and Rota in 2015 could have facilitated such a decline as these vaccines target diseases in these cause categories. We categorised events into pre-defined main cause categories as previous studies have found OPV to be protective against respiratory infections[42, 43] and diarrhoea[47, 48]. However, relatively few events were categorised into our cause categories respiratory infections (rate per 1,000 PYRS: 3.5 intervention/3.8 control) and gastrointestinal infections (rate per 1,000 PYRS: 8.1 intervention/10.7 control) (Paper 1). Thus, it might be that the proportion of respiratory and gastrointestinal cases have declined generally in Guinea-Bissau, and therefore we could also expect smaller beneficial NSEs of OPV if OPV provides better protection against these causes of disease.

As we hypothesised in Paper 2, other natural experiments have also investigated whether co-administration of OPV with DTP have implications for overall survival and morbidity[19, 37, 38, 41]. These all found indications that co-administration of OPV attenuated possible negative effects of DTP. However, our results did not support this. Despite the methodological limitations discussed (Section 08.01.02), our findings raise questions. The previous studies assessed either mortality[19, 37, 38] or in-hospital case fatality[41]. We assessed severe morbidity (hospital admission/at-hospital deaths), driven primarily by admissions, among a population attending outpatient consultations at HNSM. Compared with previous studies, our outcome might be a proxy for less severe disease, disease at an earlier stage, or disease with more pronounced symptoms. A possible explanation might therefore be that DTP-only makes the child less symptomatic when severely ill and thus likely to die without any hospital contact, but the susceptibility to less severe disease (admission) might not be affected. Two other studies might help explain why we see no difference in the risk of severe morbidity; DTP has been found to induce tolerance to unrelated antigens[97], and despite the beneficial effects of OPV, OPV in adults appeared to increase the number reporting symptoms, but not consultations, admissions and mortality[60]. Hence, co-administration of OPV with DTP may have implications for survival, but less so for hospital admissions.

08.03.03 IPV vs no-IPV

The NSEs of IPV have not yet gained much interest from the research community for evaluation. IPV was, like most other vaccines, introduced with no assessment of its effect on general child health. Although we assessed the risks associated with co-administration of IPV, and not IPV-only, Paper 3 indicates that such assessments are relevant, and that the effect of IPV may differ depending on the outcome measured. The indication of potential negative NSEs of IPV was initially based on a study from urban Bissau, showing higher F/M MRRs among IPV-vaccinated than among MV-vaccinated[55]. Our results supported a higher F/M MRR among IPV-vaccinated (Paper 3 - Section 12.03). However, differences in risk of consultation and hospital admission were less pronounced (Section 07.03). IPV was co-administered with penta3+OPV3, so the results indicating a higher mortality for girls than boys who have received penta3+OPV3±IPV may not be surprising given that higher F/M MRRs have also been found after vaccination with penta[98-100]. However, as the F/M MRR has, in prior studies, been observed to grow with increasing number of doses of DTP[101], which is part of penta (Section 04.04), an additional negative effect of IPV in addition to penta3 for girls compared to boys may not be

expected. Hence, assessing the risk of morbidity and mortality after co-administration of IPV with penta3+OPV3 vs penta3+OPV3 have most likely diminished the potential to observe the true effect of IPV. On the other hand, the study provides relevant information for the use of IPV in the routine vaccination programme, where IPV is co-administered with penta3+OPV3.

08.04 Effect modification

If we were to interpret the NSEs of OPV and IPV based only on the results presented above, important information relevant for their NSEs in the real world might be missed. Therefore, in all three studies conducted, we investigated potential effect modifiers, which could give insights into whether OPV and IPV have differential effects in different circumstances.

08.04.01 Sex

While sex-differential effects were small in most of the analyses, we did observe tendencies of larger beneficial effect of C-OPV on consultation and mortality/hospital admission in boys (Paper 1 – Section 12.01), and a lower risk of consultation, however a higher risk of mortality, after penta3+OPV3+IPV in girls (Paper 3 – Section 12.03).

Noteworthy, of the many studies which have indicated a beneficial effect of OPV on mortality in after- vs before-C-OPV studies[27-32, 35, 65-67], the majority also found a better effect in males[27, 29, 35, 65, 67]. Few morbidity studies have analysed OPVs sex-specific effects. However, a sex-differential effect in the benefit of boys have also been found for duration of diarrhoea in Bangladesh[46], and for hospital admissions due to lower respiratory infections in Denmark[42]. In contrast, a trial of BCG+OPV vs BCG-only at birth in Guinea-Bissau found no sex-differential effects on consultations within the first month of life[35]. The underlying sex-differences in mortality might help explain why we see these sex-differential effects. More boys than girls die in early life[102]. In RECAMP-OPV, the control-group event rates/risks were higher for males across the outcomes; illness, consultation, mortality, and hospital admission (Paper 1 – Section 12.01). Hence, boys might have the most to gain, and this is why OPV seem to be most beneficial for boys.

In Paper 3, sex was also identified as an effect modifier. As described in sections 07.03, the risk of mortality was higher for girls compared to boys after penta3+OPV3+IPV. However, compared with girls who received penta3+OPV3,

girls who received penta3+OPV3+IPV appeared to have a lower risk of consultation whereas there was no difference in risk of consultation for boys. A possible explanation could relate to the discussion of Paper 2 in section 08.03.02; the tolerance and thus fewer symptoms induced by DTP (here included in penta) might be more pronounced for girls when co-administered with IPV. Thus, the fewer symptoms could result in fewer consultations, but might not necessarily mean that the children are healthier. Hence, IPV may, like DTP, induce tolerance, whereas OPV induces a stronger immune response to subsequent illness. However, to investigate this hypothesis, further studies measuring the effects on different levels of disease severity are needed.

08.04.02 Timing of OPV at birth

In RECAMP-OPV, timing of OPV0 significantly modified the effect of C-OPV. As an explanation of the immunological mechanism, it could be that early timing of OPV0 induce a beneficial reaction to subsequent OPV doses later in life, potentially mediated through effects on the microbiome[53]. However, the fact that we measured morbidity in various levels allowed for extending the interpretation of timing of OPV0. In RECAMP-OPV, we found higher rates of admission and consultation among children primed with OPV0 early, however, not a higher absolute risk of self-reported illness. If children have the same risk of illness, but those who received OPV0 early have higher rates of consultation than those who received OPV0 later, timing of OPV0 might also describe the access to healthcare. Provided that this is true, we did find a beneficial effect of C-OPV among those with better access to healthcare (those who received OPV0 early). On the other hand, the rates of mortality/hospital admission were similar in the other three groups; those who received C-OPV and received OPV0 early, and those who received OPV0 later. This might indicate that the effect of C-OPV is blurred by a lack of access to healthcare. Thus, to measure an effect of C-OPV we might need a certain level of healthcare access. However, the above hypothesis is not supported by data, and will have to be investigated further in studies looking more specifically into the timing of OPV0 and its potential interaction with C-OPV.

08.04.03 Time since vaccination

As presented in the results, the potential beneficial NSE of C-OPV might change over time (Section 07.01). We did not find a beneficial effect of C-OPV on non-accidental mortality/hospital admission within the first months of enrolment, but after 3-4 months of enrolment a beneficial effect appeared. Although

the differences were very small, penta3+OPV3+IPV vs penta3+OPV3 might also provide a potential protective effect on the rate consultation and hospital admission only during the first months after vaccination (Paper 3, Supplementary Material - Section 12.03). Within the RECAMP-OPV sub-study, we also observed particularly strong effects for subgroups visited within a short interval and those not likely to receive vaccines during follow-up (Figure 14; Paper 1, Supplementary Material - Section 12.01). Thus, despite being based on explorative analyses and small numbers, it may indicate that time since vaccination influence the effect of OPV and IPV. This might be explained by routine and/or campaign vaccinations during follow-up changing the sequence of vaccine and thus the most recent vaccine received. Hence, any exposures during follow-up are important to identify and account for when investigating the NSEs of OPV and IPV. However, given that we censored for OPV campaigns in both analyses, it might also have to do with not yet defined immunological changes over time.

08.05 Choice of outcome

For both OPV and IPV, the NSEs appeared to depend on the outcome measured. The outcomes assessed in this dissertation express different levels of disease severity. Comparing these outcomes between urban and rural settings might be complex since access to health services is more limited in rural areas than urban areas, due to barriers such as difficulty in retaining health personnel, out-of-pocket payments and cost of transportation[103]. Given that health care is more accessible in urban settings than rural settings, we could also expect to observe a potentially different effect in urban settings than in rural settings.

In the context of OPV and IPV, the level of disease severity defined by the outcome, could also express different manifestations of disease, or as it has been proposed; manifestation of health[104]. In the context of vaccines, and OPV, this would mean that the manifestation of a disease in relation to induced innate immune training, can be understood as a healthy response and thus merely a manifestation of health. In contrast, the lack of manifestation of disease, as discussed for IPV in section 08.04.01, might express a lack of immune training for fighting off unrelated infections. Thereby, having more negative implications for more severe outcomes, such as death.

08.06 The ideal study

Based on the pyramid of evidence and the paucity of evidence, the NSEs of OPV vs IPV should be evaluated in a cluster-randomised trial randomising

children to OPV, IPV or no vaccine. Furthermore, outcomes that measure the effect on different levels of disease severity combined with immunological data; from illness to death, should be defined, to facilitate interpretation of the NSEs as manifestation of disease or health. Based on our observations, the overall effect as well as the time since vaccination should also be investigated in contexts with limited interference from other vaccines including vaccination campaigns. In this way it may be possible to compare the effects of OPV vs IPV vs no vaccine in a more homogenous population, on more comparable outcomes, and during the same period of time. Such a study was not possible within the timeframe of this PhD. Furthermore, when planning the RECAMP-OPV, IPV was still not introduced in Guinea-Bissau. With no studies but one, indicating potential negative NSEs of IPV[43], before the initiation of RECAMP-OPV, it would have also been unethical to conduct a study, which could potentially be harmful.

09 CONCLUSION

This thesis has attempted to provide insights in to the NSEs of OPV and IPV. By combining different study designs and analytical approaches, this thesis has demonstrated both the possibilities for strengthening the evidence base on NSEs of OPV and IPV, and the limitations for causally linking the risk of an outcome with vaccination with IPV and/or OPV.

The results of this thesis provide important findings and insights into the NSEs of OPV and IPV. The overall estimated effect of C-OPV was less strong than anticipated and did not reduce mortality and morbidity by 25%. Not co-administering OPV with DTP did not increase hospital admissions and in-hospital mortality among children attending outpatient consultations. Co-administering IPV with penta3+OPV3 did not increase consultation, hospital admission, and mortality rates. However, potential interactions, such as timing of OPV0, sex, and time since vaccination were identified, and underline the need to explore these interactions further. Furthermore, the varying effects and associations observed dependent on the level of disease severity assessed facilitated interpretation and raised questions for further investigation. Thus, underlining a need for considering different outcomes when assessing the NSEs of OPV and IPV in real-life settings.

Despite the paucity of evidence on the underlying immunological mechanism of the NSEs of OPV and IPV, the results of this dissertation partly support that it is not enough to evaluate OPV and IPV for only their polio-specific effect. The reality of a polio free world should not compromise the possibility of better health and survival for future generations of children. Therefore, the NSEs of OPV and IPV need to be clarified further to ensure that both the scheduled vaccinations as well as the unscheduled vaccinations (campaigns) with OPV and IPV optimise the impact of vaccinations on general child health.

10 PERSPECTIVES

Despite a global plan for polio eradication and replacement of OPV with IPV, the future use of OPV and IPV use is still affected by many uncertain factors, such as vaccine hesitancy/uptake, emergence of epidemics, polio outbreaks and re-emergence, and vaccine infrastructure. NSEs of OPV and IPV, as evidenced by previous evidence and partly by the results presented and discussed in this dissertation indicate that emergence of new infections/epidemics or eradication of a disease should not necessarily imply introduction or cessation of a vaccine, unless both perspectives are based on solid scientific foundations.

Although the cessation of OPV has been justified by the need to stop the spread and risk of cVDPV and VAPP, this thesis challenges the paucity of evidence in the scientific foundation for replacing OPV with IPV, the methodological challenges for assessing their NSEs, and the need for further research into potential effect modifiers. Here, HDSS sites, in particular, should exploit the unique opportunities for research into the NSEs of OPV and IPV as natural experiments. Furthermore, research into the immunological mechanisms underlying the NSEs should be prioritised to enhance the understanding and interpretation of the potential of vaccination.

11 REFERENCES

1. WHO. History of polio vaccine. <https://www.who.int/news-room/spotlight/history-of-vaccination/history-of-polio-vaccination>, **2023**.
2. Global Polio Eradication Initiative. OPV: Oral poliovirus vaccine. <https://polioeradication.org/polio-today/polio-prevention/the-vaccines/opv/>, **2023**.
3. Global Polio Eradication Initiative. Polio + Prevention. <https://polioeradication.org/polio-today/polio-prevention/>, **2023**.
4. Bernier RH. Some observations on poliomyelitis lameness surveys. *Reviews of infectious diseases* **1984**; 6 Suppl 2: S371-5.
5. World Health Organisation. Polio vaccines: WHO position paper - March, 2016. *Wkly Epidemiol Rec* **2016**; 91(12): 145-68.
6. WHO. Coronavirus disease (COVID-19): Herd immunity, lockdowns and COVID-19. <https://www.who.int/news-room/questions-and-answers/item/herd-immunity-lockdowns-and-covid-19>, **2023**.
7. UNICEF. Oral polio vaccine (OPV) price data. <https://www.unicef.org/supply/media/15226/file/OPV-vaccine-prices-13122022.pdf>, **2022**.
8. Global Polio Eradication Initiative. IPV - Inactivated poliovirus vaccine. <https://polioeradication.org/polio-today/polio-prevention/the-vaccines/ipv/>, **2023**.
9. UNICEF. Inactivated Polio Vaccine (IPV) price data. <https://www.unicef.org/supply/media/16266/file/IPV-vaccine-prices-14032023.pdf>, **2022**.
10. Estivariz CF, Kovacs SD, Mach O. Review of use of inactivated poliovirus vaccine in campaigns to control type 2 circulating vaccine derived poliovirus (cVDPV) outbreaks. *Vaccine* **2023**; 41: A113-A21.

11. World Health A. Global eradication of poliomyelitis by the year 2000. Geneva: World Health Organization, **1988**.
12. Global Polio Eradication Initiative. Polio eradication and endgame strategic plan 2013-2018. . Geneva, Switzerland: Global Polio Eradication Initiative, **2013**.
13. WHO. Update on vaccine-derived polioviruses worldwide, January 2015-May 2016. Wkly Epidemiol Rec **2016**; 91(31): 365-75.
14. Hampton LM, Farrell M, Ramirez-Gonzalez A, et al. Cessation of use of trivalent oral polio vaccine and introduction of inactivated poliovirus vaccine worldwide, 2016/Abandon du vaccin antipoliomyélitique oral trivalent et introduction du vaccin antipoliomyélitique inactivé à l'échelle mondiale, 2016. Weekly Epidemiological Record **2016**; 91(36/37): 421-7.
15. Meeting of the Strategic Advisory Group of Experts on immunization, October 2016 – conclusions and recommendations. Wkly Epidemiol Rec **2016**; 91(48): 561-82.
16. WHO. Global polio eradication initiative applauds WHO African region for wild polio-free certification. <https://www.who.int/news/item/25-08-2020-global-polio-eradication-initiative-applauds-who-african-region-for-wild-polio-free-certification>, **2023**.
17. Global Polio Eradication Initiative. Circulating vaccine-derived poliovirus. <https://polioeradication.org/wp-content/uploads/2023/06/weekly-polio-analyses-cVDPV-20230530.pdf>, **2023**.
18. WHO. Polio Eradication Strategy 2022–2026: Delivering on a promise. World Health Organization; Geneva, **2021**.
19. Mogensen SW, Andersen A, Rodrigues A, Benn CS, Aaby P. The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natural Experiment. EBioMedicine **2017**; 17: 192-8.
20. WHO. Vaccine introduction in Guinea-Bissau. Available at: <https://immunizationdata.who.int/pages/vaccine-intro-by-country/gnb.html?YEAR=>. Accessed 25-04-2023.

21. Fisker AB, Nebie E, Schoeps A, et al. A Two-Center Randomized Trial of an Additional Early Dose of Measles Vaccine: Effects on Mortality and Measles Antibody Levels. *Clin Infect Dis* **2018**; 66(10): 1573-80.
22. Aaby P, Hedegaard K, Sodemann M, et al. Childhood mortality after oral polio immunisation campaign in Guinea-Bissau. *Vaccine* **2005**; 23(14): 1746-51.
23. Benn CS, Fisker AB, Rieckmann A, Sørup S, Aaby P. Vaccinology: time to change the paradigm? *Lancet Infect Dis* **2020**; Oct;20(10):e274-e283.
24. Higgins JP, Soares-Weiser K, Lopez-Lopez JA, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* **2016**; 355: i5170.
25. Aaby P, Nielsen S, Fisker AB, et al. Stopping Oral Polio Vaccine (OPV) After Defeating Poliomyelitis in Low- and Middle-Income Countries: Harmful Unintended Consequences? Review of the Nonspecific Effects of OPV. *Open Forum Infectious Diseases* **2022**; 9(8).
26. Andersen A, Fisker AB, Rodrigues A, et al. National Immunization Campaigns with Oral Polio Vaccine Reduce All-Cause Mortality: A Natural Experiment within Seven Randomized Trials. *Frontiers in public health* **2018**; Feb 2;6:13.
27. Andersen A, Fisker AB, Nielsen S, Rodrigues A, Benn CS, Aaby P. National Immunization Campaigns With Oral Polio Vaccine May Reduce All-cause Mortality: An Analysis of 13 Years of Demographic Surveillance Data From an Urban African Area. *Clin Infect Dis* **2021**; 72(10): e596-e603.
28. Aaby P, Andersen A, Martins CL, et al. Does oral polio vaccine have non-specific effects on all-cause mortality? Natural experiments within a randomised controlled trial of early measles vaccine. *BMJ open* **2016**; 6(12): e013335.
29. Benn CS, Jacobsen LH, Fisker AB, et al. Campaigns with oral polio vaccine may lower mortality and create unexpected results. *Vaccine* **2017**; 35(8): 1113-6.
30. Nielsen S, Khalek MA, Benn CS, Aaby P, Hanifi SMA. National immunisation campaigns with oral polio vaccine may reduce all-cause

- mortality: Analysis of 2004-2019 demographic surveillance data in rural Bangladesh. *EClinicalMedicine* **2021**; 36: 100886.
31. Clipet-Jensen C, Andersen A, Jensen AKG, Aaby P, Zaman K. Out-of-Sequence Vaccinations With Measles Vaccine and Diphtheria-Tetanus-Pertussis Vaccine: A Reanalysis of Demographic Surveillance Data From Rural Bangladesh. *Clin Infect Dis* **2021**; 72(8): 1429-36.
 32. Schoeps A, Nebie E, Fisker AB, et al. No effect of an additional early dose of measles vaccine on hospitalization or mortality in children: A randomized controlled trial. *Vaccine* **2018**; 36(15): 1965-71.
 33. Welaga P, Oduro A, Debpuur C, et al. Fewer out-of-sequence vaccinations and reduction of child mortality in Northern Ghana. *Vaccine* **2017**; 35(18): 2496-503.
 34. United Nations Inter-agency Group for Child Mortality Estimation (UN IGME). Levels & Trends in Child Mortality: Report 2021, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation. United Nations Children's Fund. New York, **2021**.
 35. Lund N, Andersen A, Hansen AS, et al. The Effect of Oral Polio Vaccine at Birth on Infant Mortality: A Randomized Trial. *Clin Infect Dis* **2015**; 61(10): 1504-11.
 36. Lund N, Andersen A, Monteiro I, Aaby P, Benn CS. No effect of oral polio vaccine administered at birth on mortality and immune response to BCG. A natural experiment. *Vaccine* **2012**; 30(47): 6694-9.
 37. Aaby P, Jensen H, Gomes J, Fernandes M, Lisse IM. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. *Int J Epidemiol* **2004**; 33(2): 374-80.
 38. Øland CB, Mogensen SW, Rodrigues A, Benn CS, Aaby P. Reduced Mortality After Oral Polio Vaccination and Increased Mortality After Diphtheria-tetanus-pertussis Vaccination in Children in a Low-income Setting. *Clin Ther* **2020**; 43(1): 172-84.e7.
 39. Aaby P, Mogensen SW, Rodrigues A, Benn CS. Evidence of Increase in Mortality After the Introduction of Diphtheria–Tetanus–Pertussis Vaccine to Children Aged 6–35 Months in Guinea-Bissau: A Time for Reflection? *Front Public Health* **2018**; 6: 79.

40. Aaby P, Ravn H, Benn CS. The WHO Review of the Possible Non-Specific Effects of Diphtheria-Tetanus-Pertussis Vaccine. *Pediatr Infect Dis J* **2016**; 35(11): 1247–57.
41. Aaby P, Rodrigues A, Biai S, et al. Oral polio vaccination and low case fatality at the paediatric ward in Bissau, Guinea-Bissau. *Vaccine* **2004**; 22(23-24): 3014-7.
42. Sorup S, Stensballe LG, Krause TG, Aaby P, Benn CS, Ravn H. Oral Polio Vaccination and Hospital Admissions With Non-Polio Infections in Denmark: Nationwide Retrospective Cohort Study. *Open Forum Infect Dis* **2016**; 3(1): ofv204.
43. Seppala E, Viskari H, Hoppu S, et al. Viral interference induced by live attenuated virus vaccine (OPV) can prevent otitis media. *Vaccine* **2011**; 29(47): 8615-8.
44. Nielsen S, Sujan HM, Benn CS, Aaby P, Hanifi SMA. Oral Polio Vaccine Campaigns May Reduce the Risk of Death from Respiratory Infections. *Vaccines* **2021**; 9(10): 1133.
45. Nielsen S, Sujan HM, Benn CS, Aaby P, Hanifi SMA. Oral Polio Vaccine Campaigns May Reduce the Risk of Death from Respiratory Infections. *Vaccines (Basel)* **2021**; 9(10).
46. Upfill-Brown A, Taniuchi M, Platts-Mills JA, et al. Nonspecific Effects of Oral Polio Vaccine on Diarrheal Burden and Etiology Among Bangladeshi Infants. *Clin Infect Dis* **2017**; 65(3): 414-9.
47. Voroshilova MK. Potential use of nonpathogenic enteroviruses for control of human disease. *Prog Med Virol* **1989**; 36: 191-202.
48. Contreras G. Sabin's vaccine used for nonspecific prevention of infant diarrhea of viral etiology. *Bull Pan Am Health Organ* **1974**; 8(2): 123-32.
49. Benn CS, Netea MG, Selin LK, Aaby P. A small jab - a big effect: nonspecific immunomodulation by vaccines. *Trends Immunol* **2013**; 34(9): 431-9.
50. Aaby P, Benn CS. Saving lives by training innate immunity with bacille Calmette-Guerin vaccine. *Proc Natl Acad Sci U S A* **2012**; 109(43): 17317-8.

51. Arts RJW, Moorlag SJCFM, Novakovic B, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell host & microbe* **2018**; 23(1): 89-100.e5.
52. Kleinnijenhuis J, Quintin J, Preijers F, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci U S A* **2012**; 109(43): 17537-42.
53. Alam MJ, Rashid MM, Kabir Y, Raqib R, Ahmad SM. On birth single dose live attenuated OPV and BCG vaccination induces gut cathelicidin LL37 responses at 6 week of age: a natural experiment. *Vaccine* **2015**; 33(1): 18-21.
54. Medeiros MM, Ingham AC, Nanque LM, et al. Oral polio revaccination is associated with changes in gut and upper respiratory microbiomes of infants. *Frontiers in Microbiology* **2022**; 13.
55. Aaby P, Garly ML, Nielsen J, et al. Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines: Observations from vaccination trials in Guinea-Bissau. *Pediatr Infect Dis J* **2007**; 26(3): 247-52.
56. Aaby P, Jensen H, Samb B, et al. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. *Lancet* **2003**; 361(9376): 2183-8.
57. Blok BA, Arts RJW, van Crevel R, Benn CS, Netea MG. Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines. **2015**; 98(3): 347-56.
58. Byberg S, Ostergaard MD, Rodrigues A, et al. Analysis of risk factors for infant mortality in the 1992-3 and 2002-3 birth cohorts in rural Guinea-Bissau. *PloS one* **2017**; 12(5): e0177984.
59. Byberg S, Aaby P, Rodrigues A, Stabell Benn C, Fisker AB. The mortality effects of disregarding the strategy to save doses of measles vaccine: a cluster-randomised trial in Guinea-Bissau. *BMJ Glob Health* **2021**; 6(5).

60. Fisker AB, Martins JSD, Nanque LM, et al. Oral Polio Vaccine to Mitigate the Risk of Illness and Mortality During the Coronavirus Disease 2019 Pandemic: A Cluster-Randomized Trial in Guinea-Bissau. *Open Forum Infectious Diseases* **2022**; 9(9).
61. Hansen JS, Thyssen SM, Rodrigues A, Martins C, Fisker AB. Is early measles vaccination associated with stronger survival benefits than later measles vaccination? *BMC Public Health* **2018**; 18(1): 984.
62. de Bree LCJ, Koeken VACM, Joosten LAB, et al. Non-specific effects of vaccines: Current evidence and potential implications. *Seminars in Immunology* **2018**; 39: 35-43.
63. Jensen H, Benn CS, Lisse IM, Rodrigues A, Andersen PK, Aaby P. Survival bias in observational studies of the impact of routine immunizations on childhood survival. *Trop Med Int Health* **2007**; 12(1): 5-14.
64. WHO. First ever vaccine listed under WHO emergency use. Accessed November 20.
65. Andersen A, Bjerregaard-Andersen M, Rodrigues A, Umbasse P, Fisker AB. Sex-differential effects of diphtheria-tetanus-pertussis vaccine for the outcome of paediatric admissions? A hospital based observational study from Guinea-Bissau. *Vaccine* **2017**; 35(50): 7018-25.
66. Welaga P, Hodgson A, Debpuur C, et al. Measles Vaccination Supports Millennium Development Goal 4: Increasing Coverage and Increasing Child Survival in Northern Ghana, 1996-2012. *Front Public Health* **2018**; 6: 28.
67. Andersen A, Fisker AB, Rodrigues A, et al. National Immunization Campaigns with Oral Polio Vaccine Reduce All-Cause Mortality: A Natural Experiment within Seven Randomized Trials. *Front Public Health* **2018**; 6: 13.
68. Thyssen SM, Fernandes M, Benn CS, Aaby P, Fisker AB. Cohort profile : Bandim Health Project's (BHP) rural Health and Demographic Surveillance System (HDSS)—a nationally representative HDSS in Guinea-Bissau. *BMJ open* **2019**; 9(6): e028775.

69. INDEPTH network. Indepth Verbal Autopsy. Available at: http://www.indepth-network.org/index.php?option=com_content&task=view&id=96&Itemid=184. Accessed 04-04-2017.
70. Varma A, Jensen AKG, Thysen SM, Pedersen LM, Aaby P, Fisker AB. Research protocol of two concurrent cluster-randomized trials: Real-life Effect of a CAMPAign with Measles Vaccination (RECAMP-MV) and Real-life Effect of a CAMPAign with Oral Polio Vaccination (RECAMP-OPV) on mortality and morbidity among children in rural Guinea-Bissau. *BMC Public Health* **2019**; 19(1): 1506.
71. Craig P, Katikireddi SV, Leyland A, Popham F. Natural Experiments: An Overview of Methods, Approaches, and Contributions to Public Health Intervention Research. *Annual Review of Public Health* **2017**; 38(1): 39-56.
72. Rodrigues A, Schellenberg JA, Kofoed PE, Aaby P, Greenwood B. Changing pattern of malaria in Bissau, Guinea Bissau. *TropMedIntHealth* **2008**; 13(3): 410-7.
73. Bale C, Garly ML, Martins C, Nielsen J, Whittle H, Aaby P. Risk factors for measles in young infants in an urban African area with high measles vaccination coverage. *Pediatr Infect Dis J* **2011**; 30(8): 689-93.
74. Singh R, Mukhopadhyay K. Survival analysis in clinical trials: Basics and must know areas. *Perspect Clin Res* **2011**; 2(4): 145-8.
75. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organization, **2006**.
76. Nielsen BU, Byberg S, Aaby P, Rodrigues A, Benn CS, Fisker AB. Seasonal variation in child mortality in rural Guinea-Bissau. *Trop Med Int Health* **2017**; 22(7): 846-56.
77. McNutt L-A, Wu C, Xue X, Hafner JP. Estimating the Relative Risk in Cohort Studies and Clinical Trials of Common Outcomes. *American Journal of Epidemiology* **2003**; 157(10): 940-3.

78. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate behavioral research* **2011**; 46(3): 399-424.
79. Nguyen T-L, Collins GS, Spence J, et al. Double-adjustment in propensity score matching analysis: choosing a threshold for considering residual imbalance. *BMC medical research methodology* **2017**; 17(1): 78.
80. Leuven E, Sianesi B. PSMATCH2: Stata module to perform full Mahalanobis and propensity score matching, common support graphing, and covariate imbalance testing. <http://ideas.repec.org/c/boc/bocode/s432001.html> Version 4.0.12 30jan2016, **2003**.
81. Donner A, Klar N. Design and analysis of cluster randomization trials in health research. London: Arnold, **2000**.
82. Kish L. Survey sampling. New York, Wiley, **1965**.
83. Peter C, Cyrus C, David G, et al. Using natural experiments to evaluate population health interventions: new Medical Research Council guidance. *Journal of epidemiology and community health* **2012**; 66(12): 1182.
84. Shiba K, Kawahara T. Using Propensity Scores for Causal Inference: Pitfalls and Tips. *Journal of epidemiology / Japan Epidemiological Association* **2021**; 31(8): 457-63.
85. Biai S, Rodrigues A, Gomes M, et al. Reduced in-hospital mortality after improved management of children under 5 years admitted to hospital with malaria: randomised trial. *BMJ* **2007**; 335(7625): 862.
86. Aaby P, Ravn H, Roth A, et al. Early diphtheria-tetanus-pertussis vaccination associated with higher female mortality and no difference in male mortality in a cohort of low birthweight children: an observational study within a randomised trial. *Arch Dis Child* **2012**; 97(8): 685-91.
87. Sørensen MK, Schaltz-Buchholzer F, Jensen AM, et al. Retesting the hypothesis that early Diphtheria-Tetanus-Pertussis vaccination increases female mortality: An observational study within a randomised trial. *Vaccine* **2022**; 40(11): 1606-16.

88. Ranganathan P, Pramesh CS. Censoring in survival analysis: Potential for bias. *Perspect Clin Res* **2012**; 3(1): 40.
89. Murad MH, Asi N, Alsawas M, Alahdab F. New evidence pyramid. *Evidence-based medicine* **2016**; 21(4): 125-7.
90. Welaga P, Mutua MK, Hanifi SMA, Ansah P, Aaby P, Nielsen S. National immunisation campaigns with oral polio vaccine may reduce all-cause mortality for older but not younger infants: 20 years of demographic surveillance cohort data from rural Northern Ghana. Submitted to *EClinicalMedicine*.
91. Rieckmann A, Fisker AB, Øland CB, et al. Understanding the child mortality decline in Guinea-Bissau: the role of population-level nutritional status measured by mid-upper arm circumference. **2022**.
92. UN Inter-agency Group for Child Mortality Estimation (UN-IGME). Under-5 mortality rate, 1985 to 2020. <https://ourworldindata.org/grapher/under-5-mortality-rate-sdgs?tab=chart&country=GNB>.
93. UN Inter-agency Group for Child Mortality Estimation (UN-IGME). Infant mortality rate. <https://ourworldindata.org/grapher/infant-mortality?tab=chart&country=GNB>.
94. IHME Global Burden of Disease. Death rate from infectious diseases, 1990 to 2019. <https://ourworldindata.org/grapher/infectious-disease-death-rates?tab=chart&country=GNB>.
95. IHME Global burden of Disease. Diarrheal diseases death rate, 1990 to 2019. <https://ourworldindata.org/grapher/diarrheal-disease-death-rates?tab=chart&country=GNB>.
96. IHME Global burden of Disease. Death rate from chronic respiratory diseases, 1990 to 2019. <https://ourworldindata.org/grapher/respiratory-disease-death-rate?tab=chart&country=GNB>.
97. Blok BA, de Bree LCJ, Diavatopoulos DA, et al. Interacting, Nonspecific, Immunological Effects of Bacille Calmette-Guérin and Tetanus-diphtheria-pertussis Inactivated Polio Vaccinations: An Explorative, Randomized Trial. *Clinical Infectious Diseases* **2019**; 70(3): 455-63.

98. Fisker AB, Biering-Sorensen S, Lund N, et al. Contrasting female-male mortality ratios after routine vaccinations with pentavalent vaccine versus measles and yellow fever vaccine. A cohort study from urban Guinea-Bissau. *Vaccine* **2016**; 34(38): 4551-7.
99. Pfeiffer G, Fisker AB, Nebie E, et al. Non-specific effects of childhood vaccinations - A case control study nested into a Health and Demographic Surveillance System in rural Burkina Faso. *Vaccine* **2017**; 35(51): 7114-20.
100. Hanifi SMA, Biering-Sørensen S, Jensen AKG, Aaby P, Bhuiya A. Penta is associated with an increased female-male mortality ratio: cohort study from Bangladesh. *Human vaccines & immunotherapeutics* **2020**: 1-8.
101. Hanifi SMA, Fisker AB, Welaga P, et al. Diphtheria-Tetanus-Pertussis (DTP) Vaccine Is Associated With Increased female-Male Mortality. Studies of DTP administered before and after measles vaccine. *J Infect Dis* **2021**; 223(11): 1984-91.
102. Sawyer CC. Child mortality estimation: estimating sex differences in childhood mortality since the 1970s. *PLoS medicine* **2012**; 9(8): e1001287.
103. World Bank. Guinea Bissau : Qualitative Assessment of Demand Side Constraints to Access Maternal and Child Health Services (English). <http://documents.worldbank.org/curated/en/985561561653405142/Guinea-Bissau-Qualitative-Assessment-of-Demand-Side-Constraints-to-Access-Maternal-and-Child-Health-Services>: Washington, D.C. : World Bank Group,, **2019**.
104. Jobst KA, Shostak D, Whitehouse PJ. Diseases of meaning, manifestations of health, and metaphor. *J Altern Complement Med* **1999**; 5(6): 495-502.

12 APPENDICES

Paper 1

Nanque LM, Varma A, Thyssen SM, Benn CS, JSD Martins, Jensen AKG, Correia C, Möller S, AVDB Biggelaar, Aaby P, Fisker AB. Effect of a campaign with oral polio vaccine on mortality and hospital admission: a cluster-randomised trial among children aged 0-8 months in rural Guinea-Bissau. Submitted to *Journal of Pediatric Infectious Diseases*.

Paper 2

Nanque LM, Rodrigues AM, Möller S, Umbasse P, Fisker AB. OPV shortage in 2004: Effect of not giving OPV with DTP on admission and at-hospital mortality among children attending outpatient consultations. Manuscript.

Paper 3

Nanque LM, Careme M, Djana Q, Vedel JO, Aaby P, Fisker AB. IPV introduction in the routine vaccination programme in 2016 and subsequent reintroduction: a natural experiment. Manuscript.

12.01 Paper 1

Effect of a campaign with oral polio vaccine on mortality and hospital admission: a cluster randomised trial among children aged 0-8 months in rural Guinea-Bissau

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12.02 Paper 2

OPV shortage in 2004: Effect of not giving OPV with DTP on admission and at-hospital mortality among children attending outpatient consultations

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12.03 Paper 3

IPV introduction in the routine vaccination programme in 2016 and subsequent shortage: a natural experiment

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