BANDIM HEALTH PROJECT

1978 - 2018

Forty years of contradicting conventional wisdom

Projecto de Saúde

BANDIM

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The Bandim Health Project, 2018

Editors: Christine Stabell Benn, Ane Bærent Fisker and Peter Aaby

Photos from Frank Shann, Jeanet Ravn, and many other people who visited the project and donated their photos during the years

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Contact: bandim@ssi.dk

Layout: Ida Rye Gribsvad

Thanks

To the more than 500,000 children and adults, who have come into contact with our work both in urban and rural areas of Guinea-Bissau over the 40 years.

To the mothers of Bandim, who were used to 50% of their children dying before five years of age, but understood that something extraordinary had happened when measles vaccine was introduced in 1979 (see Chapter 2). Child survival could be improved! The mothers have supported the project ever since. We pursued the implications of this observation, but sometimes failed in our subsequent attempts to improve child survival. However, the mothers have continued to collaborate with the project, appreciating the interest in the health of their children. Though child mortality has declined more than anyone thought possible (Chapter 2), the work is not finished; child mortality may go up again as has happened at earlier occasions. Hopefully, we can continue to get the resources to demonstrate this concern.

To the many individuals who have worked for the project over the 40 years: more than 600 Guinean assistants, drivers, guards, accountants, lab technicians, nurses, and physicians, and more than 250 expatriate students, volunteers and experts.

To the many colleagues who have shared their experiences with us as members of Bandim's advisory board – Ib Christian Bygbjerg, Kaare Mølbak, Jørn Olsen, Nils Strandberg Pedersen – or as members of the many data safety and monitoring boards – Per Kragh Andersen, Jørn Attermann,Robin Bailey, Katie Flanagan, Morten Frydenberg, Rana Hajjeh, Andreas Kryger Jensen, Jukka Jokkinen, Poul-Erik Kofoed, Kim Mulholland, Jørn Olsen, Erik Parner, Anja Poulsen, Frank Shann, and Torben Sigsgaard.

To Jette Bukh, Ida M. Lisse, Ove Jessen, Henning Andersen, and Anja Vollmer, who helped so much and were passionate about our results, but did not survive to see the process through.

To the foundations and institutions, which have taken an interest in and supported our work, including in alphabetic order: Aage og Johanne Louis-Hansens Fond, Aase og Ejnar Danielsens Fond, Abbott A/S, Agnethe

Løvgreens Legat (Danske Kvindelige Lægers Forening), Allan Shapira, A.P.Møller og Hustru Christine Mc-Kinney Møllers fond til almene Formaal, AIDS Fondet, Augustinus Fonden, Axel Muusfeldts fond, Beckett-Fonden, Bodil Pedersen Fonden, Brødrene Hartmanns Fond, Carl P Petersens Fond, Codan Fonden, COWI, Dagmar Marshalls Fond, DANIDA, Danish Research Network for International Health, Dansk Selskab for Pædiatrisk Allergologi og Pulmonologi, Den Danske Forskningsfond, Den danske lungeforening, Danske Lægers Pensionsforening, Det Danske Pasteur-Selskab, Direktør Jacob Madsen og hustru Olga Madsens Fond, Direktør M.C. Holst's legat til fordel for Aarhus Universitet, Dronning Louises Børnehospitals Forskningsfond, EDTCP, Effective Interventions, Eleanor Fish, Else og Mogens Wedell-Wedellsborg Fond, Elvira og Rasmus Riisforts almenvelgørende fond, Endokrinologisk Afd. M og Infektionsmedicinsk Afd. Q, Odense Universitets Hospital, EU research programmes (INCO, FP6, FP7), EU Humanitarian Aid (ECHO), EQIPO, European Research Council (ERC), Fabrikant Vilhelm Pedersen og Hustrus mindelegat, Familien Hede Nielsens Fond, Fhv. direktør Leo Nielsen og Hustrus Fond, Figo Fonden, Frimodt-Heineke Fonden, Folkekirkens Nødhjælp, Fonden af 1870, Fonden af 17-12-1981, Fonden til lægevidenskabens Fremme A.P. Møller, Forskerakademiet, Forsknings- og Innovations Styrelsen, Forskerskolen i International Sundhed, Forskerskolen i Folkesundhedsvidenskab, Forskningsfagligt udvalg under DANIDA, Forskningsrådet for Sundhed og Sygdom, Forskningsrådet Skejby Sygehus, Frank Shann, Friedrich Wilhelm Frank og hustru Angelina Franks mindelegat, Gates Malaria Partnership, Generalkonsul Friedrich Bøhm og datter Else Bøhms Fond, Gerhard Brøndsteds rejselegat, GlaxoSmithKline, Global Fund, Guldsmed A.L.&D. Rasmussens Mindefond, Grosserer L. F. Foghts Fond, Grosserer Ludvig Berlin og Frk. Marie Poulsens Fond, Grundforskningsfonden, Habitat, Handelsgartner Ove William Buhl Olesen og ægtefælle fru Edith Buhl Olesens Mindelegat, Hans og Nora Buchards Fond, Helga og Peter Korning Fond, Helsefonden, Hermann Nielsens Fond, IANPHI, Illum Fondet, INDEPTH, Infektionsmedicinsk afdeling, Aarhus Universitetshospital, International Epidemiologic Databases to Evaluate AIDS (IEDEA), Instituto Valenciano de Infertilidad, Julie von Müllens fond, Jydsk Medicinsk Selskab, Karen Elise Jensens Fond, Klinisk Institut, Aarhus Universitet, Kong Christian IX og Dronning Louises Jubilæumslegat Kong Christian den X's Fond, Konsul Johannes Fogh-Nielsens og fru Ella Fogh-Nielsens Legat, Krista og Viggo Petersens Fond, Købmand i Odense Johann og Hanne Weimann, f. Seedorffs Legat, Kræftens Bekæmpelse, L. Meltzers Høyskolefond, Laerdals Foundation, Legat til yderligere oplæring indenfor forskning,

Lions Club, London School of Hygiene and Tropical Medicine, Lundbeckfonden, Lunds Universitet, Lægeforeningens forskningsfond, Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis Mindelegat, March of Dimes, Max og Anna Friedmanns Fond, Medical Research Council, The Gambia, Michaelsen Fonden, MINSAP Guinea-Bissau, Mogens Svarre Mogensens Fond, Murermester Jacob Johansen og hustru Maren Johansens legat, Mærsk, Nelly Amenyogbe, NordForsk, Norway's Research council, Novo Nordisk Fonden, Odense University Hospital, Oticon Fonden, Overlæge Ekkert Petersens Mindelegat, Overlæge Johan Boserup og Lise Boserups Legat, PIER – American College of Physicians, Region Midts Sundhedsvidenskabelige Forskningsfond, Reinholdt W. Jorck og hustrus fond, Rosalie Petersens Fond, Rådet for Udviklingsforskning (RUF), SAREC, Scandinavian Society of Antimicrobial Chemotherapy, SIDA, Skejby Sygehus, Skejby Sygehus' Forskningsinitiativ, Smittskyddsinstitutet, Stockholm, Statens Sundhedsvidenskabelige Forskningsråd, Statens Serum Institut, Steenbecks legat, Swedish Embassy, Bissau, Svenska Läkaresällskapet, Søster og Verner Lipperts Fond, Task Force Sight and Life Foundation, The Anthony Cerami and Anne Dunn Foundation for World Health, The Global Fund to Fight AIDS, TB, and Malaria (Global Fund), Th. Maigaards Eftf. Fru Lily Benthine Lunds Fond af d. 1. juni 1978, Thrasher Research Foundation, Tobias Kollmann, Tode legatet, UNICEF-Bissau, University of Aarhus, University of Bergen, University of Copenhagen, University of Southern Denmark (SDU), UNDP, UNFPA, Ville Heises Legat, Virogates, Vissing fonden, WAPHIR, W. Buhl Olsens Mindelegat, Welcome, World Health Organization (WHO), WHO/GPA, WHO/TDR, World Bank, Aarhus Amt, Aarhus Universitetshospital, Aarhus Universitets Forskningsfond, Aarhus Universitetshospitals Forskningsinitiativ, Aarhus Universitetshospitals Forskningsråd. Hopefully, they will continue to find it worthwhile to support BHP.

To those who supported our work. BUT first and foremost thanks to those who claimed our results were impossible, unplanned, and biologically implausible and thereby obliged us to document even better that most of our unexpected observations are reproducible. New knowledge does not start by planning to see the expected and biologically plausible - it would not be new. New knowledge starts by pursuing the contradictions in our common assumptions and seeing the same pattern again and again.

Thanks to all those, who contributed by observing the same thing again and again, identifying the consistency and contradictions, and made us able to see something new.

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1. All the light we cannot see: 40 years of being an anthropologist within the field of medical culture

In the acknowledgements, we have thanked those who fought against us. They obliged us to dig deeper and to repeat the observations to assure that the controversial epidemiological observations were indeed reproducible, that we were on the right track, or needed to find a better explanation. If the initial reception from the medical researchers had been "Interesting! – we will pursue this", I had probably left it to them and I might still have been practicing anthropology. I am grateful that did not happen!

I wish you luck in your own profession

The initial reception was not interest. To many members of the medical profession it was appalling that an anthropologist could raise questions about fundamental assumptions in medical science, e.g. the assumption that malnutrition was the main explanation of the higher mortality in low-income countries (Chapter 4). Stories circulated about the crazy anthropologist, who was said to think that he could teach medicine something new. Telephones calls were cut short, letters were not answers, and collaborators were warned against collaborating with me. The feelings of many physicians was expressed elegantly by a well-known UK professor in a review of our first paper on the beneficial non-specific effects (NSEs) of measles vaccine (MV): "*It would be laughable if it was not deplorable that a renowned institution like University of Copenhagen could employ a person who wrote such a paper*". However, the strongest statements came from the secretary of the Nobel committee, a virologist who had been asked by SAREC to evaluate the report from Guinea-Bissau: "*This is the worst crap I have ever seen*". The following discussion of how then to account for the controversial observations made in Bissau was ended by the statement: "*I wish you luck in your own profession. I will do anything I can to stop you*". Swedish funding for the project in Guinea-Bissau was discontinued.

There was obviously a need to continue working to understand the determinants of high child mortality in countries like Guinea-Bissau. Fortunately, there were also physicians like Sven Britton, David Morley, and Ove Jessen, who supported the scientific questioning, and encouraged us to continue.

With financial difficulties, we managed to keep the data collection alive in Guinea-Bissau through collaboration with a medical students' organization, International Medical Cooperation Committee (IMCC).

Post-hoc insight and triangulation

Out of this encouragement has come a process of continuously contradicting conventional wisdom and providing unexpected explanations. The data collection in Guinea-Bissau has not stopped contradicting conventional wisdom, presumably because medical culture and practice so often is built on conventions rather than data consistency. When we found a new explanation, we tried to take it to other low-income countries and even to high-income countries like Denmark, to be certain that the explanation was general and that we dared suggest a solution based on this explanation. This has been a process built on data: could we find the same thing again if we tested it in a different context? Could we change mortality if we tested the new answer?

Following the internal rule that unexpected results should be observed at least twice before being published, the answers have nearly always turned out to be consistent: Nutritional status was not the explanation for high measles mortality. The intensity of exposure to measles infection turned out to be the major determinant of measles mortality both in current low-income countries, but also in historical data from UK, Denmark and Germany. Subsequently, it was shown to apply not only to measles infection but also to chickenpox, polio and whooping cough (Chapter 5). MV turned out to have very strong beneficial NSEs in numerous low-income and high countries (Chapter 6). However, if DTP was given after MV the effect was negative (Chapter 7). Very often, these unexpected effects were different for girls and boys (Chapter 16). The effect of Vitamin A supplementation (VAS) could not be explained by prevention of vitamin A deficiency (Chapter 23). All the live vaccines turned out to have beneficial effects, etc. In most cases, these unexpected results have been triangulated, e.g. by showing parallel effects for another infection or a similar vaccine.

The consistency of totally unexpected observations in the medical field has been surprising to an anthropologist. However, more surprising has been the extent to which mainstream global health permits itself to dismiss controversial observations by re-asserting methodological purity, without actually showing that the initial observations were wrong or that they had better

explanations for the data (Chapter 25). The observations on high-titre measles vaccine (HTMV) was dismissed as biologically implausible and unplanned. The negative effects of DTP were claimed (undocumented) to be due to uncontrolled confounding in the data set from Guinea-Bissau. The NSEs of MV have not been used and it has been suggested (not documented) that it could be due to bias. For none of these or the other examples described in the following chapters has there been any attempt to refute our observations in independent studies conducted in the same way. Apparently, global health satisfy itself with the fact that some have suggested that it could be due to bias. Even though randomised trials (RCTs) could not be made of most issues, it is often enough to dismiss the observation by the claim that it is observational and not grounded in RCTs. It appears that the purity of methods have become more important than the health issue being studied.

WHO is the brain in the system?

WHO has apparently become the brain in global health. WHO has to decide new policies and if someone questions current practices, WHO alone has to decide whether such claims are right or wrong (Chapter 25). Given that WHO sometimes appear more bureaucratic than brainy, this may not be the optimal solution. We may need independent health science professionals, who can evaluate the potential fruitfulness and health implications of new observations and intervene when WHO is ignoring controversial but fruitful issues.

The health issues and lives at stake are enormous: WHO is about to stop oral polio vaccine (OPV) even though OPV campaigns have been associated with major beneficial effects, e.g. 20% reduction in the mortality rate after OPV campaigns (Chapter 11). WHO is testing the RTS,S malaria vaccine in 720,000 African children even though it was associated with two-fold higher female mortality in the first two RCTs (Chapter 19). WHO is planning to introduce more non-live vaccines in the second year of life even though we have shown that non-live vaccines after the live MV will reduce or remove the beneficial NSEs of MV (Chapter 21). WHO is using, and thereby promoting, the 3rd dose of DTP (DTP3) as the main performance indicator to monitor the vaccination programme even though DTP3 is associated with increased female mortality (Chapter 20).

Though the self-perception is that “research” in global health is getting better and better with more and more rigorous rules and methods, with more

and more ***a priori*** hypotheses and analyses plans, we may have built a system, which can only see what was planned. Increasingly, unexpected observations will be dismissed as invalid because they were observational, unplanned and potentially due to bias. Since there is little continuous data collection, these inconsistencies will not be followed.

The only recipe for better global health is: Follow all contradictions and inconsistencies and continue real-life data collection until we have consistent patterns. This is why the following brief descriptions of the Bandim Health Project's research are emphasizing the times we have contradicted conventional wisdom.

2. Mission accomplished? Child mortality declined 7-fold in 40 years

Introduction

When SAREC/SIDA from Sweden in 1978 provided support to a research project on the nutritional situation in the newly formed post-colonial state Guinea-Bissau (1974), the objective was clear. The research should contribute to reducing the very high child mortality in Guinea-Bissau. A Swedish mission to the Oio region in 1975 had suggested that under-five child mortality was as high as 500/1000. We did in fact observe that under-five mortality was nearly 500/1000 in the first year of the project (Figure 1).

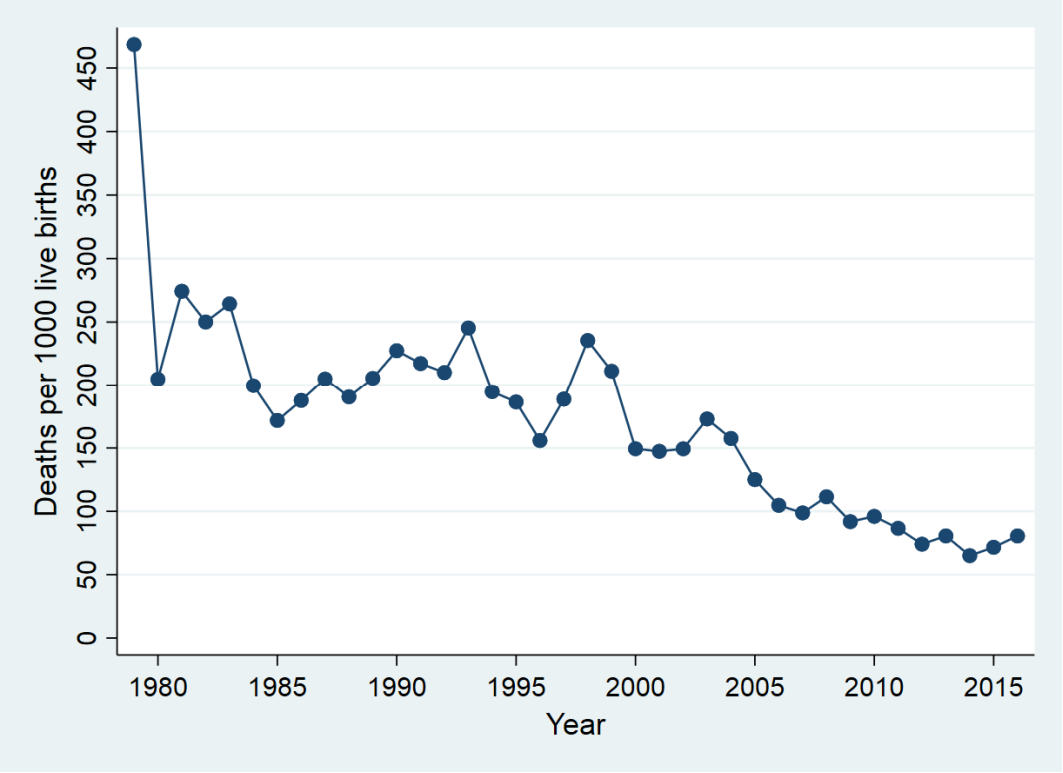

Figure 1. Accumulated under-five mortality in the urban study area in Bissau. 1978 to 2017.

The deputy minister of health did not have much confidence in the possibility of changing the ways of the older generations of Guineans for a healthier

lifestyle. However, he wanted the SAREC-project to find out how the nutritional situation of the children could be improved so that better practices could be taught in the schools. In the school gardens, which were an essential part of the new school curriculum, the next generations should also learn to grow the fruits, legumes and crops, which could secure a healthier life. That was a daunting job description.

Now, 40 years later under-five mortality has declined to around 70/1000 in the urban study area, Bandim (Figure 1). We have only data from a shorter period from the rural areas but the process has been similar with a major decline from around 315/1000 in the mid-1990s to around 95/1000 in 2015 (Figure 2).

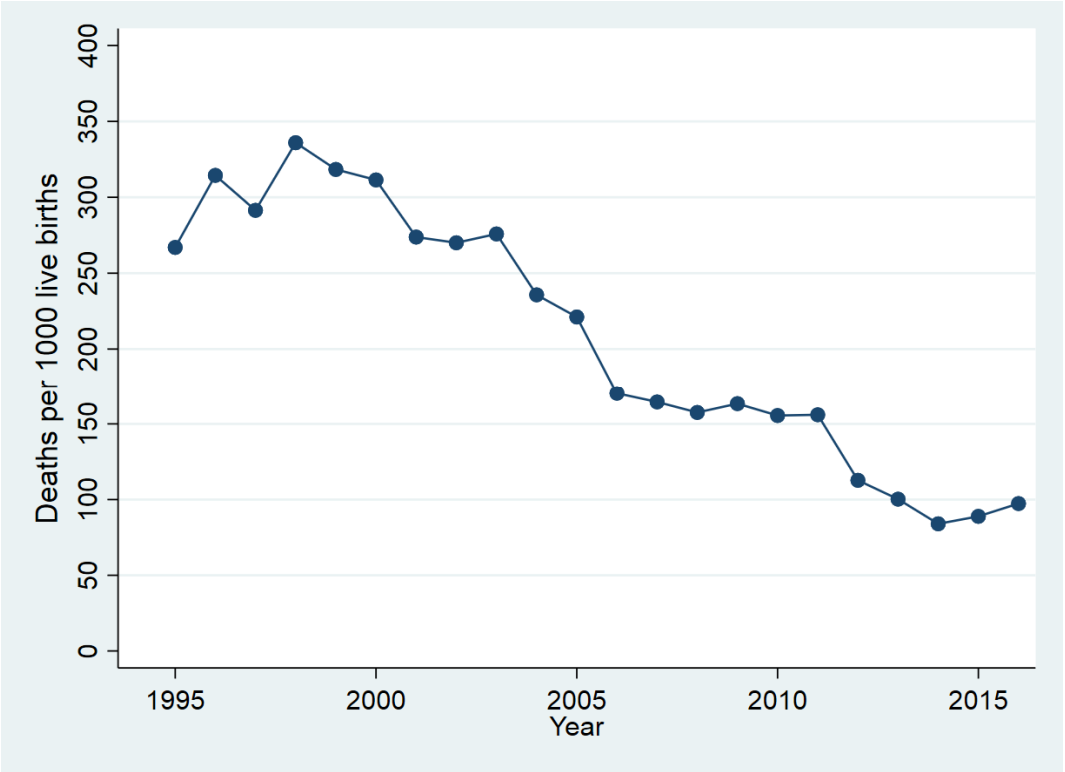

Figure 2. Accumulated under-five mortality in the rural areas of Guinea-Bissau. 1995 to 2017.

Nonetheless, the mission is not accomplished. If public health was a well-grounded science, one should expect that the mortality rate declined with each new evidence-based intervention to improve child survival. The annual mortality estimates should show a steady decline in mortality rate. That is

not what the mortality curves for Guinea-Bissau show. There are numerous declines, but also many increases in mortality; the increases should not be there if we knew all the processes, which affect child mortality.

Through the 1980s and 1990s with the introduction of many programmes to improve child care and child survival, mortality changed little. Surprisingly mortality started declining after the 1998-war, even though many physicians and nurses had fled during the war and the public health system had not become fully operational again. The many vaccination campaigns and decline in malaria morbidity may have contributed to this result (1). The challenge is to determine the relative importance of these interventions, i.e. what are the major determinants of child survival.

Though the decline in the last 20 years - since the civil war in 1998-1999 - looks promising, we cannot be certain that mortality will not increase again.

In the 1970s when we started, everybody believed that malnutrition was ***the*** major determinant of high child mortality. This paradigm turned out to be completely wrong (Chapter 4) (2). At the time, this was a major contradiction of conventional wisdom. Hence, we had to pursue many different lines of research to understand what determines child mortality and what improves child survival. This approach has continued to produce numerous contradictions of conventional wisdom (3). The chapters in this book report on the major lines of research that we have undertaken. We have pursued the contradictions we encountered. These were evidently not in the original research plan, but resolving the contradictions have turned out to be necessary to understand our data.

Public health implications

Most public health interventions implemented in low-income countries were never fully tested for their overall impact on child survival. Instead, in the name of urgency to reduce child mortality, the global health community has generated intervention policies based on common beliefs, limited tests of efficacy and no assessment of possible interactions with other interventions. Once the intervention is recommended by WHO, it is unethical to test it by withholding the (presumed) beneficial intervention from some study participants. Instead of evidence-based policy, global health has gotten policy-based evidence. This is a recipe for numerous contradictions, but to see the contradictions we need the reliable data. The huge advantage of a HDSS

is that it may force you to see the contradictions between policy-based expectations and real-life. That is the basis for improving preventive child care and further reducing child mortality.

Recommended reading

1. 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.
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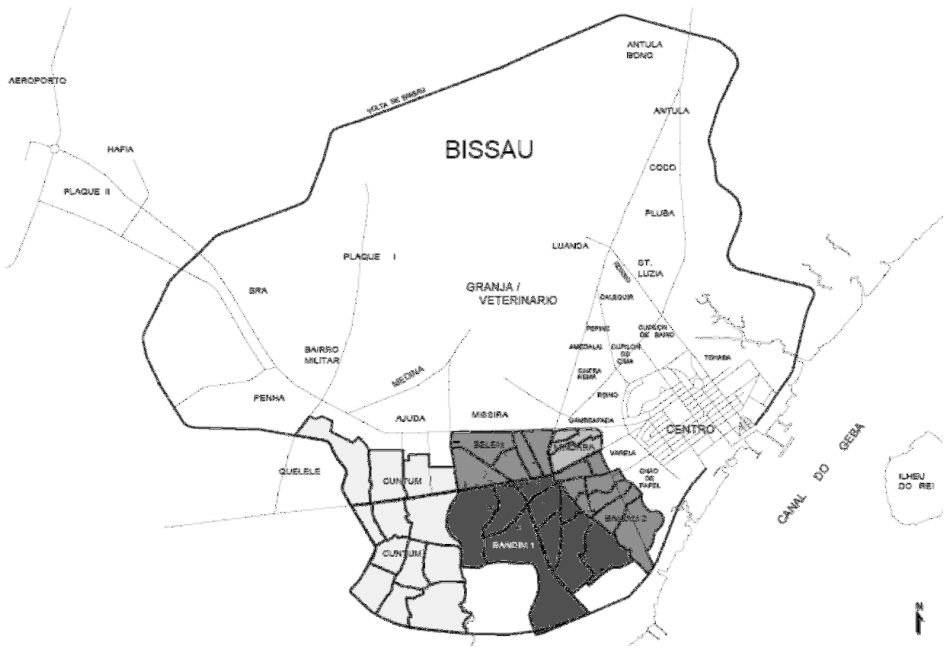
3. Fisker AB, Hornshøj L, Rodrigues A, et al. Effects of the introduction of new vaccines in Guinea-Bissau on vaccine coverage, vaccine timeliness, and child survival: an observational study. Lancet Glob Health 2014;2:e478-87.

3. Bandim Health Project's Health and Demographic Surveillance System: Collecting the real-life data

The basis for BHP's work on assessing health, diseases, and interventions are the health and demographic surveillance systems (HDSS) we have established in six districts in the capital (Map 1) and in 182 randomly selected village clusters in all health regions of Guinea-Bissau (Map 2).

The urban study area: Bandim

BHP has followed the population in the district Bandim 1 in the capital Bissau since December 1978, when the first census and general child anthropometric examination was made. At the time, the population was 6,200 (1). The population followed has gradually increased to more than 100,000. The population concentration has increased dramatically with many more houses being built – Bandim 1 has now more than 35,000 inhabitants - and we have gradually included the neighbouring districts, Bandim 2 and Belem in 1984, Mindara in 1993, and Cuntum 1 and Cuntum 2 in 2002 (Map 1).

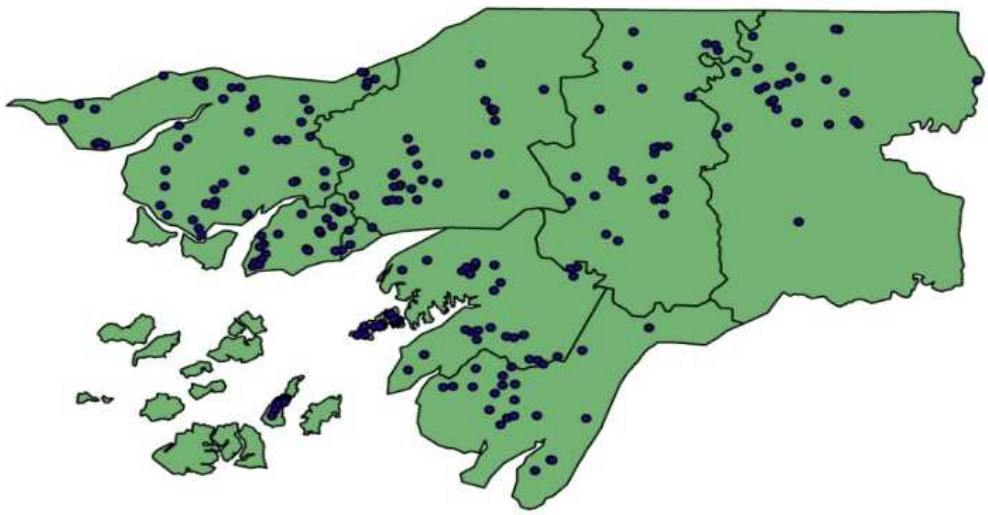


Map 1: Bissau city. The different phases of expansion have been illustrated. The project started in Bandim 1 in 1978; Bandim 2 and Belem were included in 1984; Mindara in 1993 and Cuntum 1 and Cuntum 2 in 2002.

The rural study areas: A national, representative cluster sample for Guinea-Bissau

Initially in the early 1980s, BHP followed 4-6 villages in major regions including Biombo, Cacheu, Farim, and Tombali. These villages had been selected in the initial nutrition studies to represent the different ethnic groups and eco-systems; the selection was partly based on accessibility in 1979 and partly on size as it was more cost-effective to examine large villages with many children.

In 1989-1990, we changed strategy and randomly selected 20 village clusters in each of the five most populous regions, i.e. Biombo, Cacheu, Oio, Bafata and Gabu (2). These regions represented 83% of the rural population. This change was partly in response to UNICEF's need for an assessment of the prevalence of neonatal tetanus, and partly to measure the mortality impact of the DANIDA-supported Danchurch Aid project on primary health care in Biombo and Oio. In 2006, the study was extended with selection of village clusters in the remaining health regions Tombali, Quinara, Bolama and the Bijagos Islands and more village clusters in Cacheu/Sao Domingos were added (3). Hence, the 182 clusters now cover the whole country (Map 2).

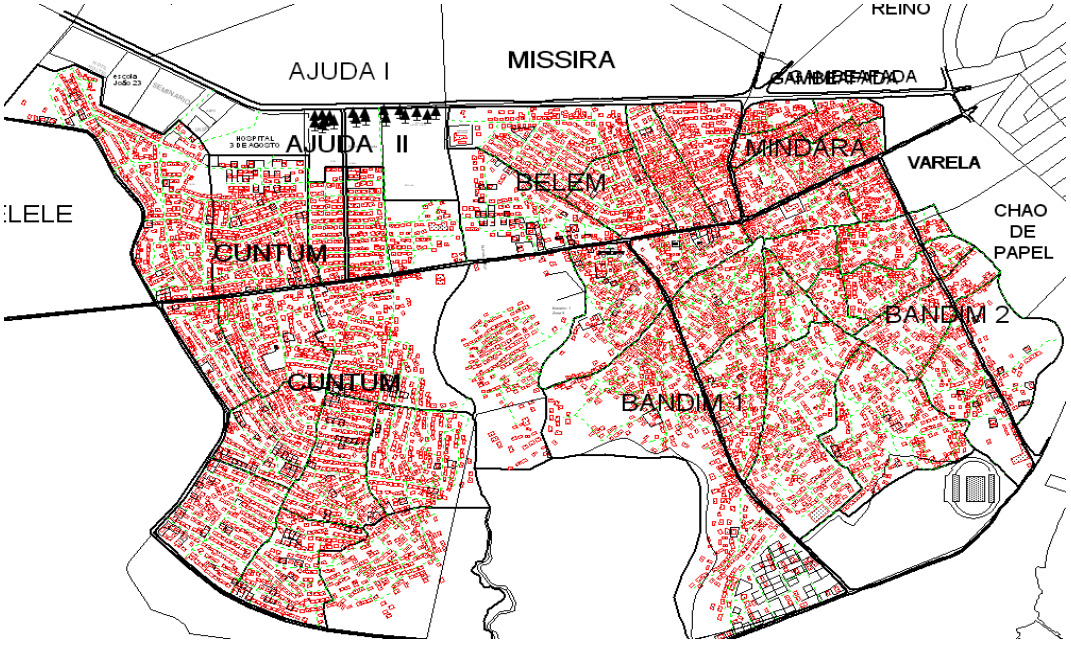


Map 2: National sample of 182 village clusters.

The data collection methodology – core HDSS data

All houses in the urban study areas are numbered and mapped using GPS (Map 3). Here all residents in the six districts are followed, and censuses conducted every 2-4 years (dependent on funding) to update the population list. In the rural areas, since the key objective is assessment of child mortality, we emphasise the identification of all compounds and all women of fertile age in the selected clusters; we then follow the children under 5 years of age of the identified women. However, we do not conduct a general census for all age groups as done in Bissau.

Since the start, BHP has had an emphasis on registering pregnancies, encouraging participation in antenatal care at the local health centres, and on regular home visits to all children under 3 (urban) or 5 years of age (rural). The frequency of home visits varies depending on area; in the urban area the child visits are performed every 3 months, while the rural areas have been every 6 months with more frequent visits in some periods/regions. At all visits to children, follow-up information on growth, morbidity, participation in health programmes and survival is collected.



Map 3: Mapped houses in the BHP urban study area.

Due to the emphasis on pregnancy registration, BHP has reported higher perinatal mortality than essentially all other HDSS sites in Africa. In most HDSS systems mothers are asked retrospectively about deliveries since the last visit by the HDSS staff. It is assumed that this captures all births in the population. However, women are less likely to report pregnancies, which resulted in a stillbirth or neonatal death and therefore this method leads to underestimation of perinatal and early childhood mortality. In the BHP system, we calculate rates of stillbirths and neonatal deaths among the registered pregnancies and therefore avoid including a biased sample.

The data collection methodology: Point of care

In Bissau, BHP is also monitoring all deliveries at the maternity ward (since 2002) and admissions at the paediatric ward (since 1990) of the national hospital. The maternity ward registration has provided the opportunity to implement early interventions: BCG-vaccination to low-birth weight children (Chapter 9), neonatal vitamin A supplementation (Chapter 24), and comparisons of different strains of BCG vaccine (Chapter 9). The hospital

admission data is important for monitoring the most important infections and we have increasingly used hospital admissions as an outcome to measure the effect of different health interventions for the overall health status.

In addition to the national hospital, BHP has staff present at the three health centres in the study area. Here we have registered vaccinations and consultations for children < 5 years of age and tuberculosis cases are registered since 1996 (Chapter 35, 36).

Public health implications

The longitudinal data of BHP allows us to ask and answer questions, which may improve health policies and their implementation. With 40 years of demographic surveillance, we have a unique opportunity to study trans-generational effects, e.g. how early childhood measles vaccination of girls affect the transfer of maternal measles antibodies to the next generation. It also allows us to go back and test new hypotheses in past data. For example, when we detected that vaccines had non-specific effects we were able many years later to go back to examine what happened to child mortality when diphtheria-tetanus-pertussis (DTP) vaccine and oral polio vaccine (OPV) were introduced in 1981 (1,4) (Chapter 17).

Recommended reading

1. Mogensen SW, Andersen A, Rodrigues A, et al. The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natural Experiment. EBioMedicine 2017;17:192-198.
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3. Fisker AB, Hornshøj L, Rodrigues A, et al. Effects of the introduction of new vaccines in Guinea-Bissau on vaccine coverage, vaccine timeliness, and child survival: an observational study. Lancet Glob Health 2014;2:e478-87.
4. Aaby P, Mogensen SW, Andersen A, et al. The introduction of diphtheria-tetanus-pertussis vaccine to older children in an urban African community: A natural experiment. Front Public Health 2018;6:79.

4. Malnutrition and measles mortality: The first contradiction

Background and assumptions

When the original SAREC-sponsored project was planned and initiated in 1977-78, under-5 mortality was known to be very high probably around 500/1000. At the time, child mortality had become low in high-income countries and no European or American public was in doubt why mortality continued to be very high in developing countries: Malnutrition! Anyone looking at the photo would see a “malnourished” child and assume that this would aggravate common infectious diseases like measles, diarrhoea and pneumonia, which then would become fatal.

Therefore, when the work started in the fall of 1978, the SAREC team, consisting of a paediatrician, a nutritionist and an anthropologist, the aim was

to conduct anthropometric surveys of children under 5-6 years of age to examine the prevalence of and the determinants of malnutrition. Through 1978 and 1979, the team conducted surveys in Bandim 1, a district in Bissau, the capital of Guinea-Bissau, and in clusters of villages in five rural areas covering the major ethnic groups and ecological zones in the country.

Real-life data

Contradiction the assumptions about high mortality, severe malnutrition was uncommon when we examined the 1200 children under 6 years of age in Bandim 1. We found no kwashiorkor and only two marasmic children, who had both lost their mother, and their malnutrition was therefore not difficult to explain. All children were breastfed to 18-24 months of age or even longer in some ethnic groups. The American nutritionist left the project since she did not find that she could contribute to improving the situation.

Measles was the largest killer of children in the 1970s and there was no disease where the belief in the importance of malnutrition was more pronounced. In the words of the leading professors from Johns Hopkins University: "*Children whose death might be prevented by measles vaccine are ...on the "road to death", and their nutritional status is so poor that they are more likely to die of any infectious disease. Thus preventing a death with vaccine among these children may not necessarily save a life, but only change the cause of death*".

During the dry season of 1979 from February to May, an epidemic of measles swept through Bandim 1. With the relative good nutritional status, according to the assumptions at that time, the epidemic should have had a limited effect. However, more than 60 children died of measles and the case fatality was 21% for children less than 5 years of age. Surprisingly, there was no association between likelihood of dying of measles infection and the nutritional status for weight and height that we had measured just a few months before the epidemic (Figure 1) (1).

The SAREC team also observed outbreaks of measles during 1979 and 1980 in the villages followed in Quinhamel, the region just outside the capital Bissau. Measles was again extremely severe with a case fatality of 34% for children under 5 years of age. There was again no association between the state of nutrition and the likelihood of dying of measles (2).

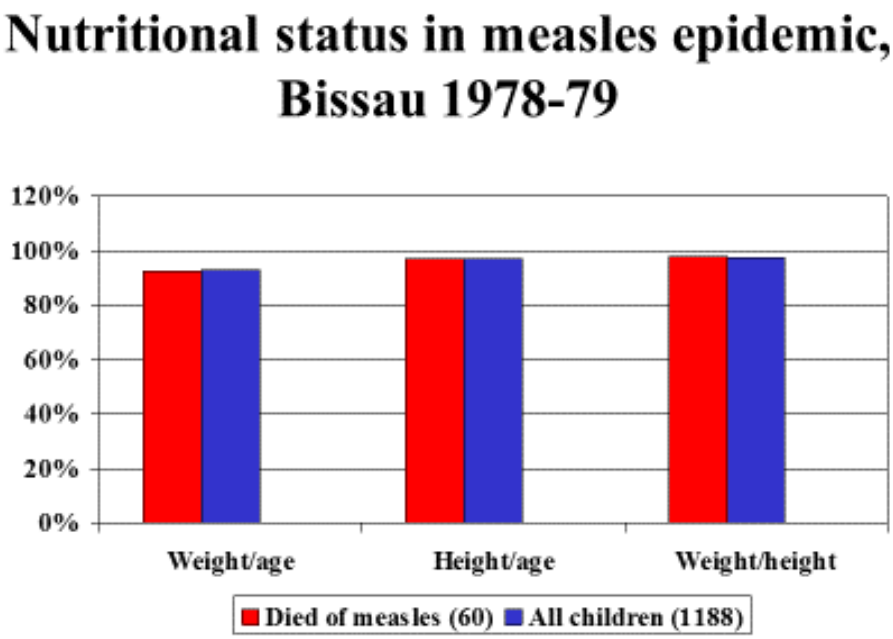

Figure 1. Nutritional status measured by weight/age, height/age and weight/height for children who died of measles infection and all other children in the community. Bandim 1979.

Following the introduction of measles vaccine in December 1979, the case fatality declined but measles continued to occur and there was still no association between measles mortality and malnutrition.

In retrospect, the data previously used to argue that nutritional status or nutritional intervention programmes determined measles mortality turned out to very slim (3). Most data was from hospitals and the malnourished children who died of measles might have been malnourished due to the rapid weight loss following the early phase of severe measles infection rather than having had severe measles due to pre-infection malnutrition. Community data on pre-existing nutritional status was clearly needed to clarify whether malnutrition was the cause of severe measles infection. It was such community studies that the SAREC team ended up producing (3).

A general consideration of global nutritional patterns and mortality levels in measles infection should actually have suggested that malnutrition could not be the main cause; measles mortality was high in Africa where nutritional status was relatively good and low on the Indian subcontinent where nutritional status was poor. No subsequent study has documented that it

was the nutritional status, which determined the severity of measles infection (Figure 2).

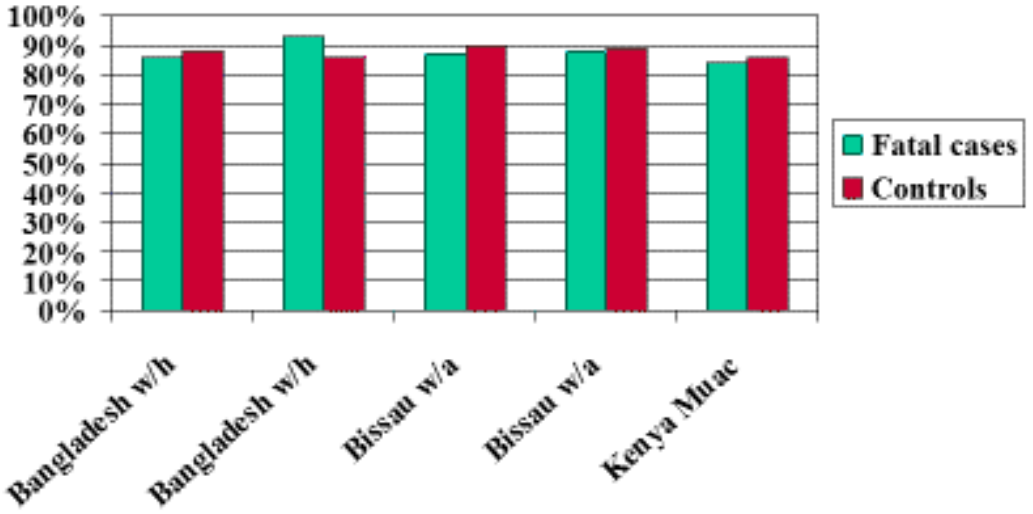

Figure 2. Nutritional status in % of Harvard standard measured by weight/height (w/h), weight/age (w/a) and mid-upper-arm-circumference (Muac) for fatal measles cases and controls.

When we introduced measles vaccine in December 1979 in connection with the first re-examination 12 months after the project had started, mortality declined in a major way (Chapter 6) so it was not true that measles vaccination just changed the cause of death among the "weak" children (4).

Public health implications

The malnutrition paradigm had many consequences; for example, at that time preventive health intervention to reduce child mortality focused on growth monitoring. The malnutrition paradigm was also linked to a popular Darwinistic model of survival of the fittest and there was therefore little belief in vaccinations, because the "weak" children likely to die from measles would probably die from something else if saved from measles by vaccination.

Given the major contradictions we had encountered it was clearly necessary to examine other possible determinants of high mortality in common infections.

Recommended reading

1. Aaby P, Bukh J, Lisse IM, et al. Measles mortality, state of nutrition, and family structure: A community study from Guinea-Bissau. J Infect Dis 1983; 147:693-701.
2. Aaby P, Bukh J, Lisse IM, et al. Determinants of measles mortality in a rural area of Guinea-Bissau: Crowding, age, and malnutrition. J Trop Pediatr 1984; 30:164-69.
3. Aaby P. Malnourished or overinfected. An analysis of the determinants of acute measles mortality. Dan Med Bull 1989; 36:93-113
4. Aaby P, Bukh J, Lisse IM, et al. Measles vaccination and reduction in child mortality: a community study from Guinea-Bissau. J Infect 1984; 8:13-21.

5. Measles: Overcrowding and intensity of exposure

Background and assumptions

The SAREC project had mainly focused on malnutrition and child mortality and initially nothing was done to set up surveillance for specific diseases or epidemics. However, observations during the first measles epidemic in Bandim in 1979 suggested that the case fatality was higher in polygamous households and when there were several cases in the same house (Figure 1). We therefore conducted retrospective interviews with all mothers in the study area about the pattern of disease transmission in both the epidemic in Bandim and in the Quinhamel rural area. Since these studies suggested that "overcrowding" was important for the case fatality in measles infection, we subsequently conducted studies in other low-income countries and of historical records to establish the general importance of overcrowding as a determinant of severe disease.

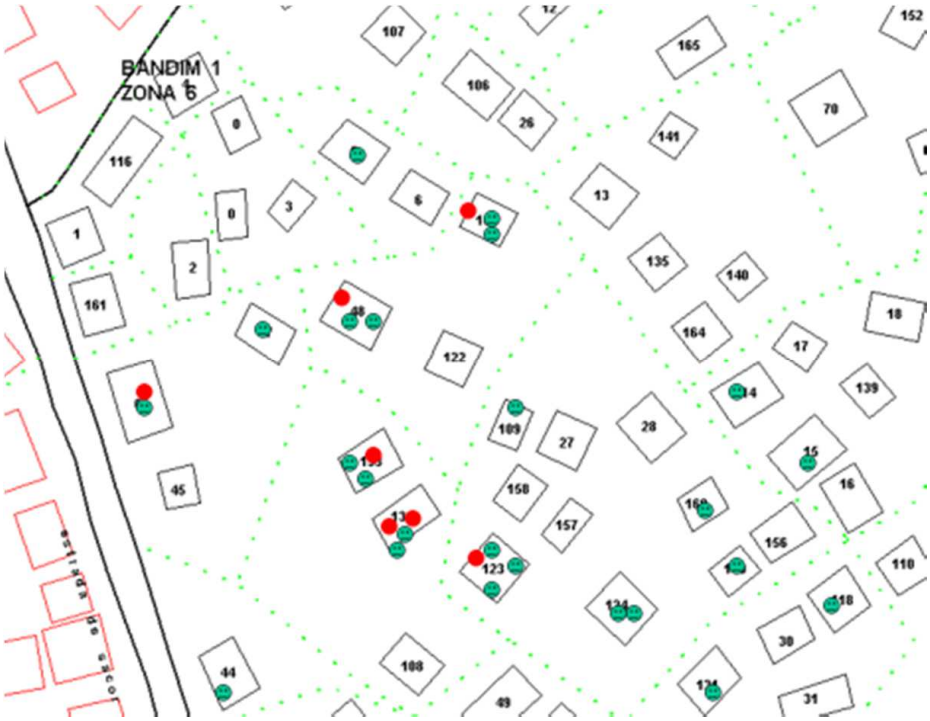

Figure 1. Plot of measles cases in zone 6 of Bandim 1. Green cases survived and red died, 1979.

Real-life data

Clustering of cases and overcrowding. The principle that clustering of measles cases increased the case fatality turned out to be systematic not only in Bissau but also in other low-income countries and in historical records (1). In all the studies, the case fatality was higher in houses with multiple measles cases compared with houses with single cases (Figure 2). In fact, most historical and geographical variation in severity could be explained by variation in the clustering of cases (1).

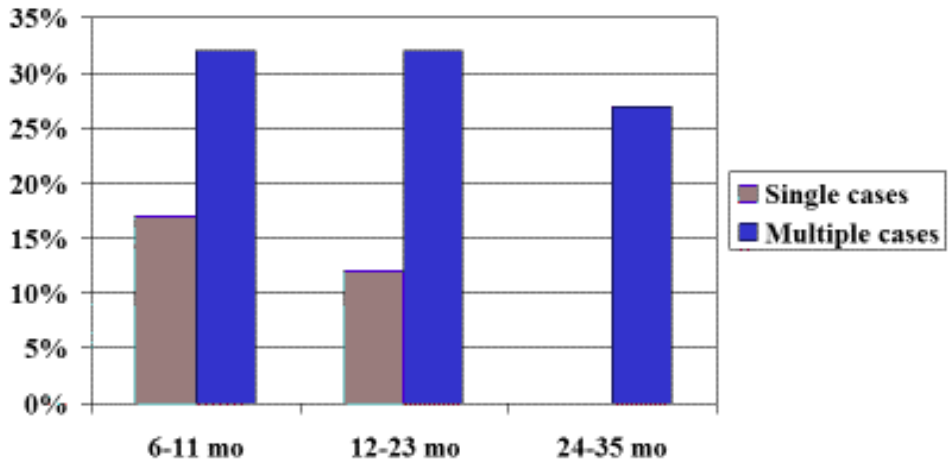

Figure 2. Case fatality in measles infection according to whether there were multiple cases or just a single case in the house. Bandim 1979 (2).

Intensity of exposure: It turned out that the intensive exposure that one would get as a secondary case within the household or house (Figure 3) increased the case fatality in measles infection; this mechanism explained why clustering and multiple cases were important for the case fatality.

In all the subsequent studies in Guinea-Bissau, other low-income countries and historical data (see Figure 4) the case fatality was much higher for secondary than for index cases of measles infection. Hence, these data clearly supported the emphasis in historical records on overcrowding and large family size as risk factors for severe measles infection (1). Subsequently we were able to show that intensity of exposure was also important for the severity of polio, whooping cough and chickenpox.

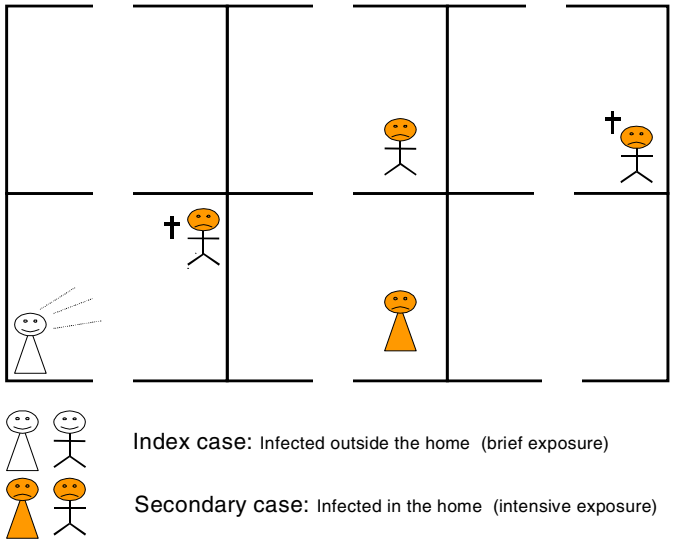

Figure 3. Intensity of exposure: index cases infected outside the house and secondary cases infected within the house by the index case.

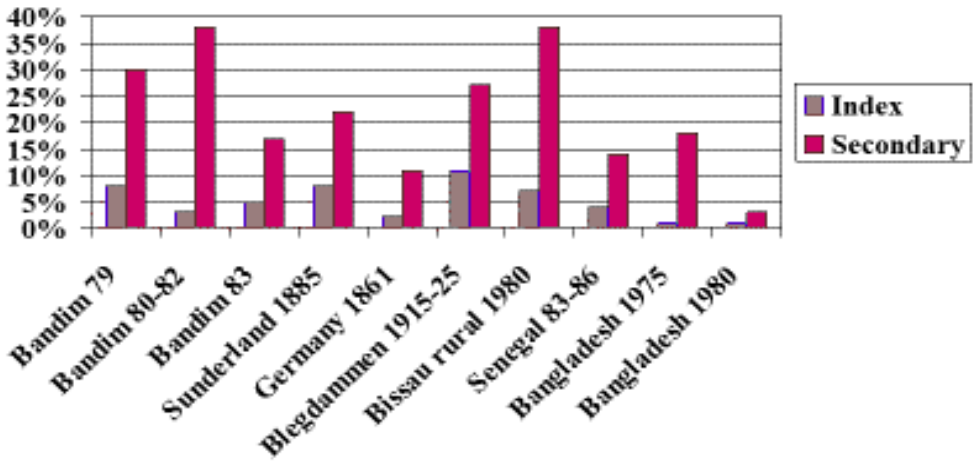

Figure 4. Case fatality in measles infection for index and secondary cases in low-income countries and historical data.

Dose of infection and length of incubation: In experimental animal studies dose of infection is an important determinant, which affects the incubation period (high dose -> short incubation) and subsequent severity. Our studies

of both measles and polio infections suggested that severity is associated with length of incubation (short incubation -> high mortality) making it likely that dose of infection determines severity also in human disease.

Amplification of severity: Severity is transmissible and secondary cases have even more severe infection, when the transmitting cases had severe infection, giving rise to a pattern in which the case fatality increases with each new generation of cases in large families (3). This is presumably the explanation of the extremely high case fatalities experienced in virgin soil outbreaks (Pacific Islands, American Indians) and closed institutions such as military barracks, immigrant ships, refugee camps, and child institutions.

Cross-sex transmission: By accident, we detected in the 1979-outbreak in Bandim that measles infection was more severe when contracted from the opposite sex (4). This has subsequently been found in many studies in other countries and historical records. Apparently, the same principle applies to polio infection, chickenpox and possibly to respiratory syncytial virus (RSV).

An accident: Cross-sex transmission*. Our paper describing severe fatal cases of measles infection (2) was being translated to Portuguese. In the English text, most fatal cases had been infected by a "sibling". As there is no word for sibling in Portuguese, the translator had written "irmão"(brother). Since it was not possible that all fatal cases had been infected by a "brother" we had to go back to the original records. Hence, it became clear that when a male died it was most likely a sister who had infected him and when a female died a brother had infected her.*

Age and sex patterns of severe disease: It was only when we emphasised intensity of exposure and secondary cases that is was possibly to see that cross-sex transmission mattered. The focus on secondary cases provided also new answers to a number of enigmas in disease epidemiology: For example, why are many infections more severe in young adults (see "Society" in Figure 5) when the young adults will react less than a child to the same dose in a vaccine (see "Nature" in Figure 5) and why are they more severe to male than females.

The pattern of transmission of infections varies by age and sex with the lowest proportion of secondary cases among children in the age group where susceptible children are brought together, usually children aged 3-10 years

depending on entry age for social institutions like kindergarten and schools. Severity will be highest in the age groups where there are many secondary cases (Figure 5), i.e. the ages before and after the susceptible are brought together. This gives rise to the characteristic U-formed curve of severity found in many transmissible infections where severity is highest among the youngest, who are likely to have been infected by an older sibling, and among the young adults, who are likely to have been infected by their own children. Hence, the U-formed curve reflects both the severity of infections but also the proportion of secondary cases in different age groups.

In a culture where there are no particular restrictions on the movements of girls and boys, girls are likely to get infected before the boys, presumably because they are more likely to have close contacts than the boys (5). If girls are more likely to pick up infections and become index case, then boys will by implication have a higher risk of becoming a secondary case. This explains at least to some extent why boys have higher mortality in common childhood infection in the Western world. The pattern can be reversed in societies with some restriction on female mobility, if for example only boys are sent to school; then boys will be the index cases and the girls will be secondary cases and have higher mortality.

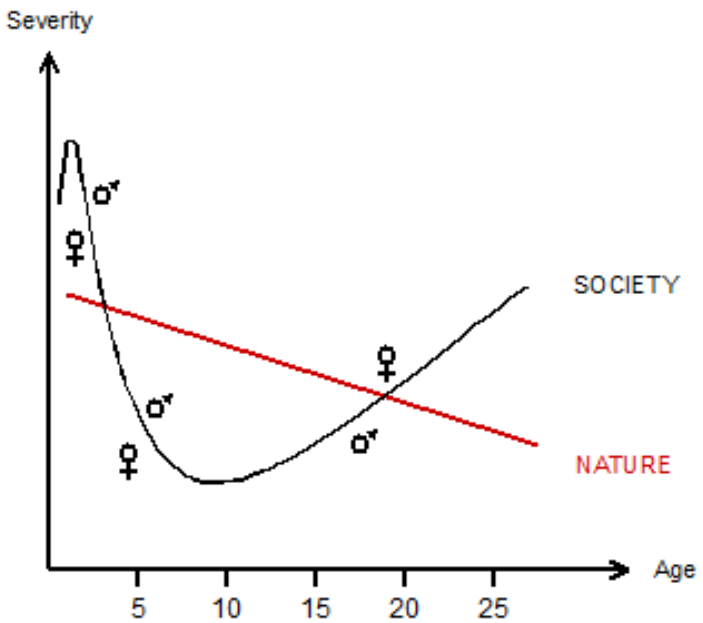

Figure 5. Curve of disease severity by age and sex.

Public health implications

The crowding-intensive exposure model was a break with the medical profession's usual model for understanding what was wrong with children who died. This model tended to emphasise that there was something wrong with the "host", be it malnutrition, genetics or the care received. The Bandim model came to emphasise that a lot of the "causality" is not host-dependent but related to the transmission process: intensity of exposure, amplification, and cross-sex transmission.

With crowding as a major determinant of high child mortality levels in both Africa and in Europe at the turn of the 20th century, much stronger emphasis should be put on improving housing and reducing family size. For example, we consistently found bed crowding to increase transmissibility and severity of many infections as well as child mortality. Much more ought to be done in this area. The importance of crowding and dose of infection would also contradict the emphasis on "weak" children as being the cause of high mortality and hence, strengthen the possible role of disease-specific prevention. We had no funding for organising family planning services or starting construction of better and less crowded house. Therefore, immunisations became the immediate and cheapest solution to substantially reduce

child mortality levels. Through the 1980s, BHP established routine immunisation services in the study area and a system of outreach services in all of Bissau city before UNICEF started supporting routine immunisation services from 1986-1987.

International health experienced a similar change in focus towards immunisations through the 1980s – not because of our observations on crowding –probably more because the rich countries had shown with the smallpox eradication campaign that final solutions were possible for specific diseases and that the same infrastructure could be used to control other infections.

Recommended reading

1. Aaby P. Malnourished or overinfected. An analysis of the determinants of acute measles mortality. Dan Med Bull 1989; 36:93-113.
2. Aaby P, Bukh J, Lisse IM, et al. Overcrowding and intensive exposure as determinants of measles mortality. Am J Epidemiol 1984; 120:49-63.
3. Garenne M, Aaby P. Pattern of exposure and measles mortality in Senegal. J Inf Dis 1990;161:1088-1094.
4. Aaby P. Influence of cross-sex transmission on measles mortality in rural Senegal. Lancet 1992;340: 388-391.
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6. Measles vaccine: Generating the concept of non-specific effects of vaccines

Background and assumptions

Due to the severe measles epidemic in 1979, we organised the first measles vaccination (MV) campaign in December 1979, when the children were anthropometrically re-examined after 12 months. At the time, there was no general vaccination programme in Guinea-Bissau. The large majority of children attended (85%); they received MV if they had not already had measles infection. The campaign was not a research project but only a service to the community. However, it soon became clear that it was necessary to follow the effect of MV much more closely.

Real-life data

MV had a marked effect on measles mortality by preventing measles cases but also by reducing crowding among the remaining cases and therefore lowering the case fatality. Furthermore, those children, who got measles infection in spite of vaccination, had much lower case fatality, and vaccinated cases transmitting milder infection contributing to less severe community outbreaks.

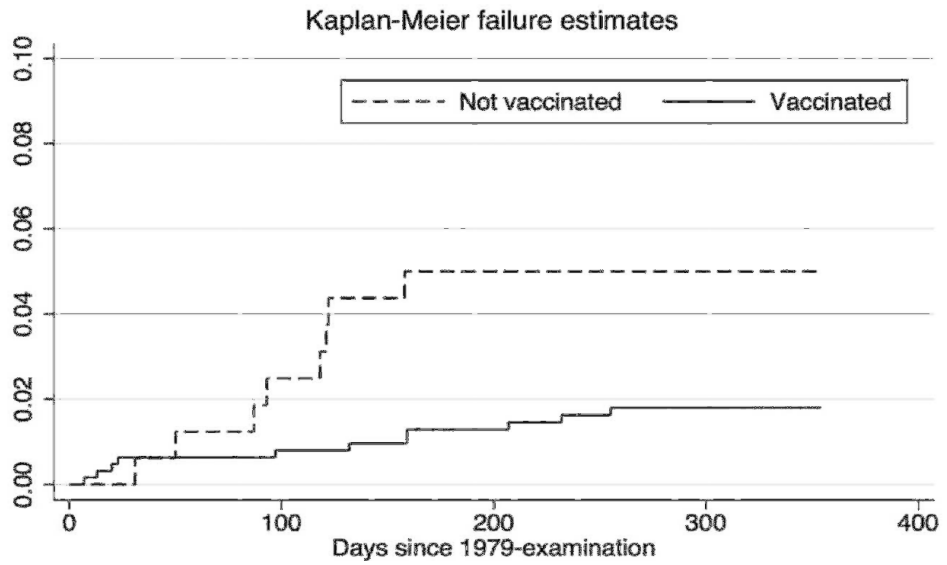


Figure 1. Accumulated mortality curves for measles vaccinated and measles-unvaccinated children. Bandim, 1979-1980 (1).

However, more important was the overall effect of MV on child mortality levels. We followed all children and had documented who had participated and should have received MV and who had been absent due to travels, and it was therefore possible to assess the difference in mortality for measles vaccinated and measles-unvaccinated children. As seen in Figure 1, the measles-vaccinated children had 70% (27-88%) lower mortality than measles-unvaccinated children. The unvaccinated children did not die of measles infection (1).

Since most children had received MV, the introduction of MV changed the community mortality level completely as illustrated in Figure 2. In the global literature, there are only four other studies, which have information on the mortality rate before and after the introduction of MV in a community (Figure 2). Interestingly, all studies suggest at least 50% reduction in mortality rate in the affected age group (2). Since WHO has estimated that 10% of deaths were due to measles infection, these results clearly created an enigma.

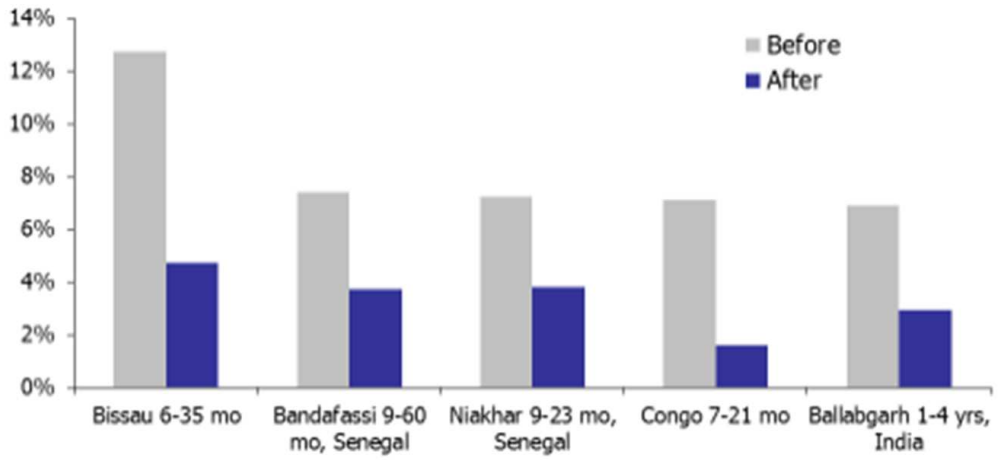


Figure 2. The community mortality rate before and after the introduction of measles vaccine.

The simplest explanation appeared to be that measles infection due to immunosuppression was associated with long-term excess mortality. MV presumably prevented this long-term excess mortality and therefore had a much larger effect on child survival than usually assumed. We conducted

many epidemiological and immunological studies to test if measles infection was associated with long-term immune suppression and excess mortality. Once again, results were contrary to expectations. Children who survived the acute phase of measles infection had subsequently lower mortality than uninfected individuals, particularly mild index cases (Table 1) (3).

To assess how much of the mortality reduction was associated with prevention of measles infection and how much was not explained by measles prevention, we estimated vaccine efficacy against death (VED) in all community studies comparing the survival of vaccinated and unvaccinated children (Figure 3). Censoring for measles infection, i.e. comparing uninfected-vaccinated and uninfected-unvaccinated children, and comparing these results with VED for all children should indicate how much of the estimated VED was explained by the specific prevention of measles infection. Surprisingly, there was virtually no difference in the VED estimates with and without censoring for measles infection (Figure 3) (4).

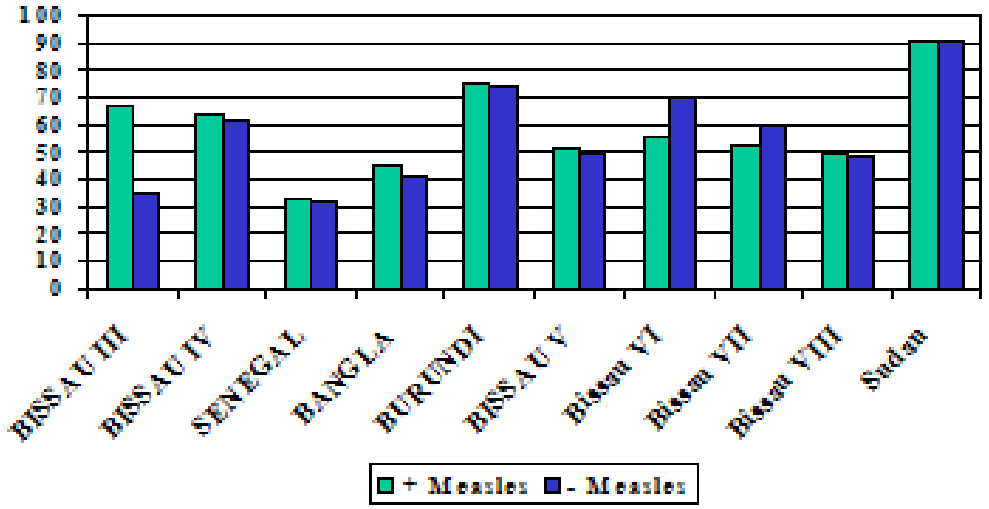


Figure 3. Vaccine efficacy against death (VED) comparing measles-vaccinated and measles unvaccinated children in analyses with measles cases (green) and without measles cases (blue).

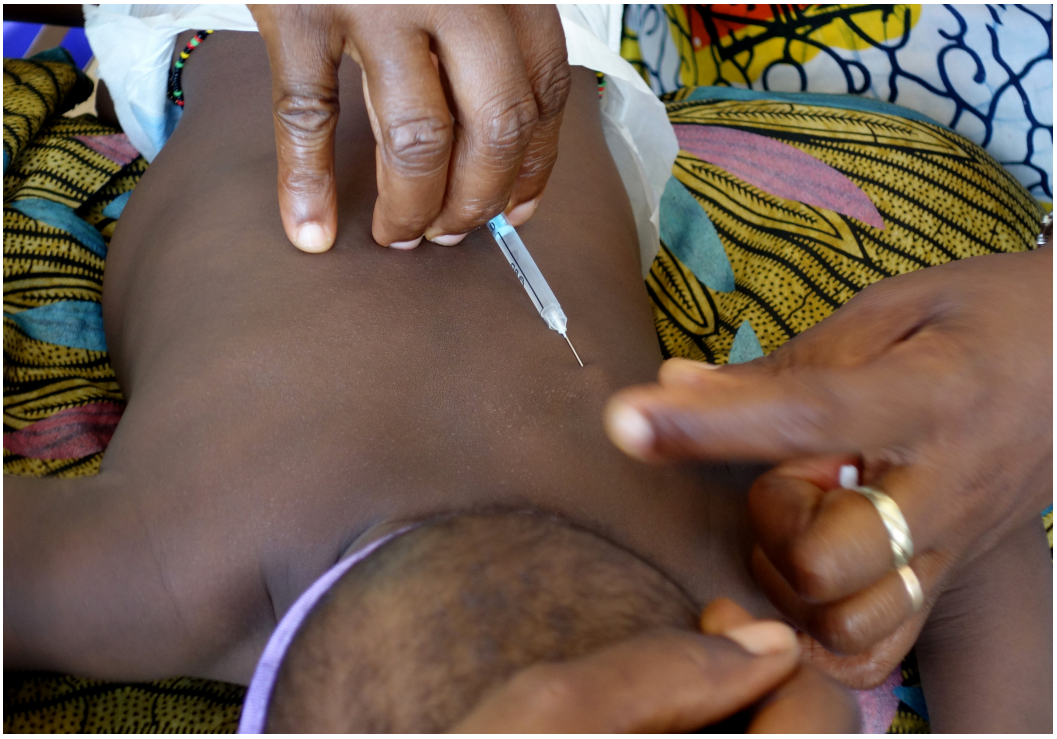
Since there was a marked difference in mortality for vaccinated and unvaccinated children in the absence of measles infection, MV apparently had an independent effect on child survival. Since MV does prevent measles deaths, the VED should have been much stronger in the analyses including

measles infection. However, since the VED did not differ (Figure 3), this pattern suggests that while some unvaccinated suffered from measles death others may indeed have had lower mortality after measles infection (Table 1) to compensate and produce the unchanged VED.

Country, time, reference	Age group	Period after follow-up after measles infections	Groups being compared	Measles infected vs. non-measles infected (95%CI)	Comments
Guinea-Bissau, 1979-80	0-6 years	6-18 months	Previous measles inf. vs not vaccinated	0.62 (0.16-2.30)	
Guinea-Bissau, 1988	Under 3 years	1 to 60 months	Measles cases vs controls	0.50 (0.22-1.16)	Adjusted age, sex, immunizations
Senegal, 1983-1986	0-9 years	1 to 48 months; censored Dec 1986	Unimmunized measles cases vs unvaccinated, uninfected controls	Index cases: 0.27 (0.09-0.85) Secondary cases: 1.10 (0.80-1.51)	
Senegal, 1992-1996	0-6 years	1-48 months, censored Dec 1996	Exposed with clinical or subclinical measles vs exposed uninfected	0.20 (0.06-0.74)	Adjustment had no effect
Bangladesh, 1982-1985	Under 5 years	3-12 months post measles	Measles cases vs uninfected controls	0.40 (0.16-0.98)	Adjusted age, sex, siblings, maternal education, area
All studies				0.49 (0.26-0.91)	

Table 1. Community studies, which had individual level data and assessed long-term mortality after measles infection.

The before-after studies (Figure 2) and the studies comparing mortality for vaccinated and unvaccinated children (Figures 1, 3) clearly suggested that MV had beneficial effects, which could not be explained merely be prevention of measles infection. At the same time, high-titre MV (HTMV) was associated with higher female mortality (Chapter 7). How could a framework be created to understand these diverse effects? Initially, we hypothesised that standard MV had beneficial non-specific immunological effects (NSEs) in addition to the preventive specific-disease effects (4). These NSEs have subsequently also been termed "heterologous effects", "non-targeted effects" or "off-target effects". Surprisingly the NSEs of standard MV were stronger for females than males. Hence, it became a simple explanation of the HTMV incident that HTMV in contrast to standard MV did not have the capacity to produce beneficial NSEs; since the girls no longer experienced the beneficial effect of standard MV, female recipients of HTMV appeared to have higher mortality. This was a poor explanation; it did not take account of the trend in all HTMV studies that female recipients not only had higher mortality than female recipients of standard MV but also had higher mortality than male recipients of HTMV. As explained in the chapter on HTMV, there was a totally different explanation for the higher female than male mortality among HTMV recipients (Chapter 7).



Public health implications and future perspectives

The conclusion that emerged from these studies was that MV contributed far more to reduction in child mortality than explained by prevention of measles. MV and possibly mild measles infection were associated with a beneficial immune training effect that we called NSEs. In hospital studies and RCTs we later showed, that NSEs had their strongest impact on respiratory infections (5).

This change of perspective had numerous implications that we are still pursuing. First, MV should be given even earlier to provide more benefit (Chapter 8). Second, stopping MV after eradication of measles infection could potentially increase child mortality in the poorest countries. Third, increasing the MV coverage would lower mortality in the poorest countries. Hence, the MV coverage, and not DTP3, should be used as the coverage indicator by international agencies. Fourth, to explain the immunological basis for the NSEs, it is a major priority to study the immune training effects of MV.

Importantly, it became clear to us that if MV had major beneficial NSEs, we needed to examine the potential NSEs of all the other vaccines, so this became the start of a whole new research agenda.

Recommended reading

1. Mogensen SW, Aaby P, Smedman L, et al. Introduction of standard measles vaccination in an urban African community in 1979 and overall child survival: a reanalysis of data from a cohort study. BMJ Open 2016;6:e011317.
2. Aaby P, Whittle HC, Benn CS. Vaccine programmes must consider their effect on general resistance. BMJ 2012;344:e3769.
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7. High-titre measles vaccine and the importance of the sequence of vaccinations

Background and assumptions

After the experience with MV when first introduced in Bandim (Chapter 6), we became very enthusiastic about testing high-titre measles vaccine (HTMV) in randomised trials (RCTs) in Bissau and Senegal when these vaccines were developed in the early 1980s. HTMV could be given from 4 months of age and had the potential to reduce child mortality much more than standard MV recommended from 9 months of age. HTMV was fully protective against measles infection in both Bissau and Senegal; it was as good as standard MV though given already at 4 months when maternal antibodies could reduce efficacy. It had the potential to help eradicate measles infection.

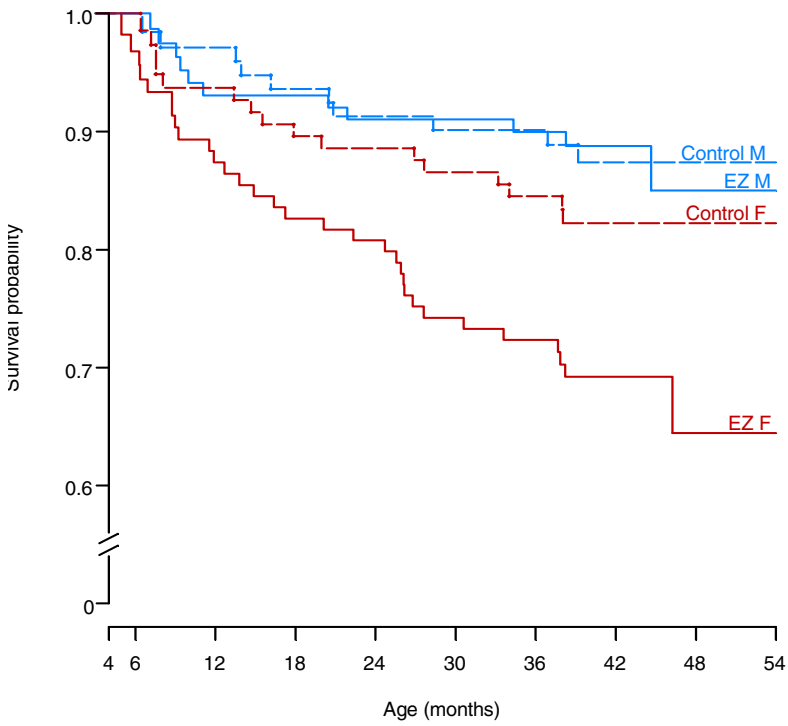

Figure 1. Survival curves for females (red) and males (blue) among recipients of HTMV(EZ) and controls.

In 1989, WHO recommended HTMV at 6 months of age. However, when we followed recipients of HTMV within the HDSS setting in Bissau, it turned out that they had higher mortality than the control children receiving standard MV at 9 months of age (Figure 1) (1). The deleterious effect was only seen after 9 months of age when the control group had also received MV (Figure 1). The deleterious effect of HTMV was seen only for females. These observations were subsequently confirmed in Senegal, Sudan and Haiti. In the African meta-analysis of HTMV studies, HTMV was associated with a 33% higher child mortality between 4 months and 5 years of age; the excess mortality was nearly two-fold higher for female recipients of HTMV. WHO withdrew the vaccine in 1992.

These trends made little sense: Why would a protective vaccine have negative effects? Why would these effects be seen only after 9-10 months of age? Why were they only seen for females?

We had no answers in 1992, but the HTMV incident showed that a protective vaccine can have deleterious NSEs and they can be very important for child survival. Hence, the HTMV sparked the research agenda for the NSEs of other vaccines. If it happens once and we have no explanation, it could happen again. When the vaccine was withdrawn in 1992, the common interpretation was that HTMV had come too close to natural measles infection inducing immune suppression; new measles vaccines to protect against measles infection before 9 months of age were needed and encouraged by both NIH and the EU research programs. However, the general interpretation made no sense because why would “immune suppression” be a problem only for girls and only after the control group had received MV? When we detected that diphtheria-tetanus-pertussis vaccine (DTP) (Chapter 16) was associated with increased female mortality, it offered a completely different explanation, which could potentially resolve the contradictions in the HTMV story.

Real-life data

High-titre measles vaccine: In the African studies, HTMV had been administered already at 4-5 months of age and most children had received DTP or inactivated polio vaccine (IPV) after measles vaccine. We therefore tested in all the African studies whether this change of sequence was the real cause of increased female mortality (2). Indeed, excess female mortality was only found among children who received DTP and/or IPV *after* measles vaccine, the F/M MRR being 1.93 in this subgroup. In the small group of children,

who did not come back to receive an inactivated vaccine at 9-10 months of age, there was no difference, the F/M MRR was 0.96 (Figure 2).

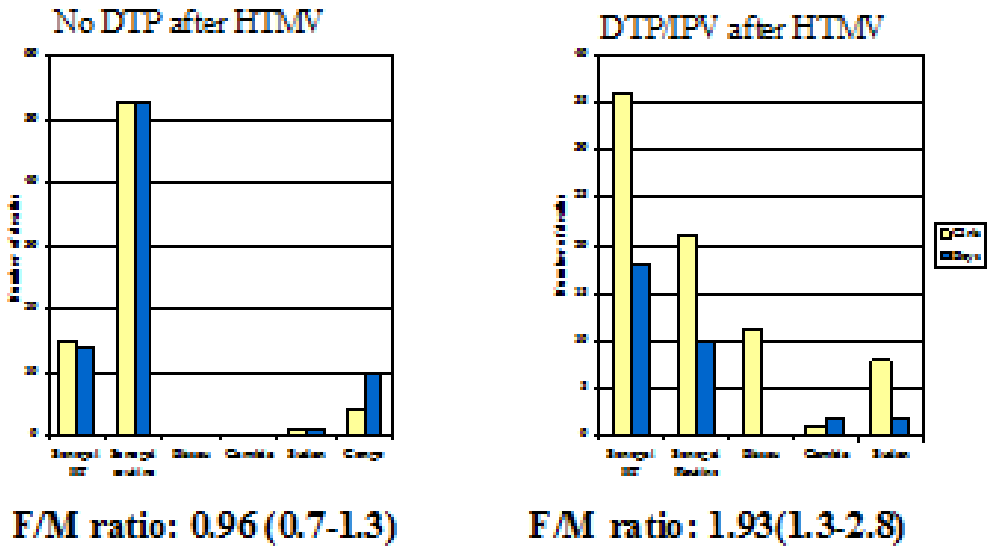

Figure 2. The number of female and male deaths in all studies of HTMV depending on whether DTP/IPV had been administered after HTMV.

Medium-titre measles vaccine (MTMV) and standard-titre MV: The official point of view was that HTMV was special and had to be withdrawn. However, if the issue was the sequence of vaccination there should be similar problems when DTP or other inactivated vaccines were given after other forms of MV. We tested this in RCTs, which had used MTMV. In total, there were seven RCTs from Sudan, Senegal, The Gambia and Guinea-Bissau in which it was possible to compare an inactivated vaccine given after HTMV/ MTMV with a standard titre MV after an inactivated vaccine (3). The RCTs had similar designs; the children were randomised at 4-5 months to early HTMV/MTMV or a control group receiving an inactivated vaccine. At 9-10 months of age, there was a switch over; the early HTMV/MTMV groups received an inactivated vaccine whereas the control group received standard-titre MV. Before the 9-10 month vaccinations, there was no excess mortality associated with HTMV/MTMV; before they received any DTP after enrolment in the trial, the early HTMV/MTMV groups had 80% (35-94%) lower mortality than the inactivated control groups in this age group (3). Hence, in contrast to the common belief that HTMV *per se* had deleterious effects,

HTMV may have been associated with low mortality.

In a meta-analysis, after 9 months, the mortality for inactivated vaccine (after HTMV/MTMV) compared with standard MV (after inactivated vaccine) was 38% (5-83%) higher. The result was similar in MTMV (41%) and HTMV trials (37%). All the excess mortality was among girls who had 89% (27-180%) higher mortality after inactivated vaccine whereas there was no difference for boys (-2%), a very significant sex-differential effect.

These studies suggested that the sequence of vaccinations are very important, the most recent vaccination determines the immunological profile and thereby the mortality risk. For girls, it was problematic if the most recent vaccination was DTP and not MV. Hence, DTP vaccination status at enrolment could determine subsequent mortality in MV trials if missing doses of DTP were administered after enrolment. As indicated in the Table, this turned out to be the case in the six trials from which we had data (4). After receiving MV, children missing one or more doses of DTP (DTP0-2) had significantly higher mortality than those who had already received DTP3, but only among girls. For boys this difference did not matter.

Public health implications and future perspectives

By combining the initial observations related to HTMV with the subsequent observation that DTP vaccine was associated with increased female mortality, it became clear that the real problem was not HTMV *per se* but DTP and the change in sequence of vaccinations. We removed a good vaccine for the wrong reason.

Previous studies of vaccines have not taken the interaction and sequence with other vaccines into consideration. Furthermore, the data of administering DTP after measles vaccine in RCTs (Table) and as out-of-sequence vaccinations in observational studies suggest that the NSEs of vaccines and the sequence and combination in which vaccines are given may be very important for child mortality levels (Chapter 21).

The reinterpretation of HTMV constitutes a strong argument for the hypothesis that DTP has deleterious effects for girls. It is implausible that one could generate a hypothesis about HTMV from unconnected studies on DTP and find that it is consistent with all existing data in the HTMV/MTMV studies unless a causal biological process is at work.

Study	Girls			Boys		
	DTP0-2	DTP3	MR (DTP0-2/DTP3)	DTP0-2	DTP3	MR (DTP0-2/DTP3)
Guinea-Bissau-A	6.0%	0%	ND	8.7%	0%	ND
Guinea-Bissau-B	17.8%	0%	ND	7.4%	0%	ND
Guinea-Bissau-C	7.5%	3.8%	1.97 (1.04-3.72)	6.4%	6.0%	1.06 (0.60-1.90)
Guinea-Bissau-D	6.1%	1.7%	3.55 (1.23-10.3)	3.2%	3.3%	0.97 (0.34-2.80)
Sudan (119)	6.0%	2.8%	2.16 (0.27-17.3)	1.4%	1.9%	0.71 (0.06-7.87)
Congo (119)	10.0%	2.8%	3.54 (0.71-17.5)	10.6%	5.1%	2.06 (0.46-9.22)
Total			2.36 (1.43-3.89)			1.11 (0.69-1.77)

Table. Mortality rate in measles vaccination trials in relation to the DTP vaccination status at enrolment.

Notes: DTP0-2=missing doses of DTP at enrolment; DTP3=had received all doses of DTP at the time of enrolment. Trials in Guinea-Bissau: A= Medium EZ-trial; B=Medium and high-titre EZ-trial; C=2-dose MV trial; D=trial of MV with vitamin A; ND=Not defined. #The test of homogeneity for the estimates for boys and girls was p=0.031.

These observations question the practice of administering missing vaccines whenever a child comes to a health centre. This may be particularly important because it is current donor policy to measure the performance of the vaccination program by the coverage for DTP3. Therefore, many children are getting DTP administered with MV or after MV. All indications are that such practices are associated with increased mortality (Chapter 21).

This may become even more important now, because WHO is advocating a 2nd-year-of-life platform for vaccinations which would imply that more non-live vaccines (booster DTP, RTS,S malaria vaccine, meningococcal vaccine) will be administered after standard MV. Based on all available data, this will lead to increased female mortality.

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8. The best measles vaccination strategy: Trials to learn by error

Background and assumptions

Since the first experiences with measles epidemics and measles vaccine (MV) campaigns in 1979-1980, it has been a priority to find the best MV strategy in terms of impact on child survival. However, our research has become a protracted story of finding interactions between MV and other health interventions. Hence, **the** best strategy may not exist because many other health interventions affect the impact of MV on child survival.

The current policy of providing MV at 9 months of age in low-income countries was defined by WHO in the late 1970s when the Expanded Programme on Immunizations (EPI) started (1). The policy was built on studies in Kenya of seroconversion after MV at different ages. To interpret the data, six explicit or implicit assumptions were made: 1) children with measles antibodies were *fully* protected; 2) children with no measurable antibodies were *fully* susceptible; 3) measles infection in vaccinated and unvaccinated children was equally severe; 4) it did not matter whether children got measles in infancy or later; 5) seeing measles in a vaccinated child, i.e. "vaccine failure", would lead to lack of confidence in EPI; and 6) it had to be a one-dose strategy.

Based on these assumptions, it was decided to vaccinate at 9 months rather than at 7 or 8 months of age, primarily because it was feared that earlier vaccination would lead to more "vaccine failures".

All assumptions were wrong. Had infant survival been assessed in an RCT, it would probably have been better to vaccinate at 6 or 7 months of age, with a second dose at 9 or 12 months of age. "Vaccine failure" cases have lower case fatality and were therefore perceived as "mild measles", and early MV moved measles infection to an older age group with lower case fatality. Contrary to expectations, "mild measles" cases strengthened the popular acceptance of MV – mothers saw that their child had measles but did not become very ill. Furthermore, MV has beneficial NSEs, which would prevent deaths of other causes, and earlier vaccination would therefore be better. However, the effect on infant survival of MV at age 9 months compared with MV at an earlier age was never tested by EPI.

Real-life data

In the late 1970s many countries vaccinated at 6-8 months of age; in the first campaigns implemented in Bissau, we vaccinated from 6 months of age. As described in Chapter 6 this gave very encouraging results for child survival. Stimulated by that experience, we embarked on several trials with medium- and high-titre MVs (MTMV/HTMV), which could be given already from 4 months of age. As described in Chapter 7 this became a disaster with two-fold higher mortality for female recipients of the new vaccines.

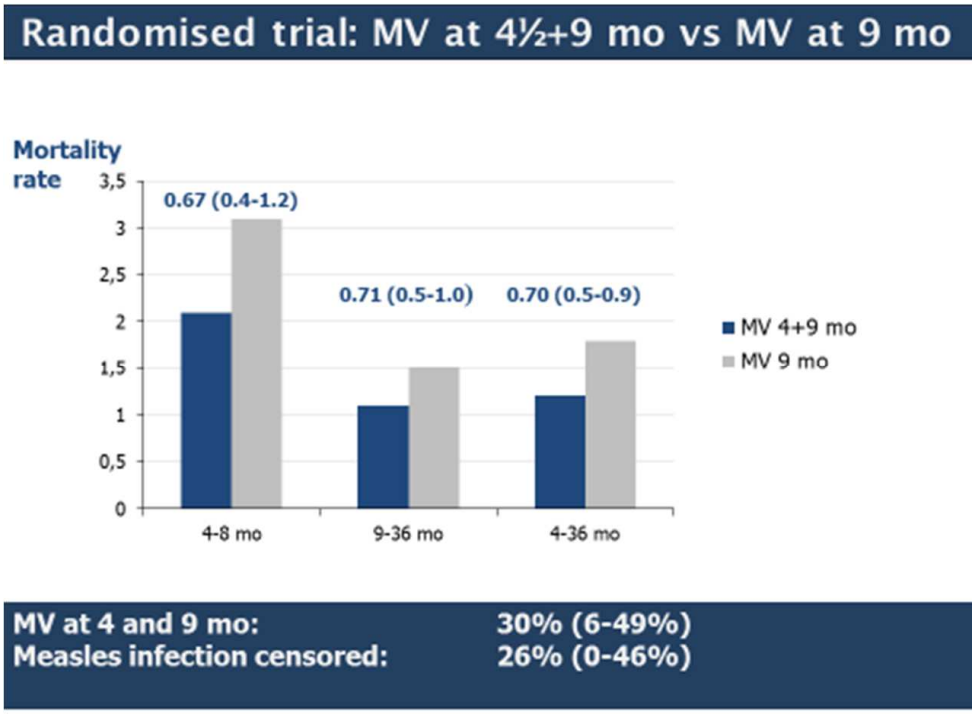

Figure 1. RCT comparing survival after two doses or one dose of MV. Bandim, 2003-2009.

We were at loss with respect to what to do when HTMV failed. However, when we used MV from 6 months of age in the early 1980s, we provided a second dose of MV to children who had received the first dose before 9 months (2). That experiment suggested a very positive effect of two doses of MV, the mortality rate being 39% (8-60%) lower for children, who had received MV between 4 and 8 months of age, compared with children, who received MV at 9-11 months as recommended by WHO.

In 1995, we therefore initiated an RCT of two-doses of standard-titre MV given at 6 and 9 months versus the official policy of MV at 9 months of age. Many things went wrong during this trial; most children had not received all doses of diphtheria-tetanus-pertussis vaccine (DTP) before entering the trial and many therefore received DTP with or after MV, which turned out to be poor combinations (Chapters 7, 21).

The RCT was also interrupted by a civil war in 1998-1999. When the war started in June 1998, 433 children had been enrolled in the RCT at 6 months and received early MV or inactivated polio vaccine (IPV), as control vaccine. Due to breakdown of the health care system during the war, these participants did not receive the 9-month MV. Hence, the war created a natural experiment in which we could measure the effect of early MV versus no MV (IPV). MV-recipients children had 70% (13-92%) lower mortality than IPV-recipients during the period with intensive war when everyone fled from Bissau. This reduction was not due to prevention of measles infection (3).

Due to the experience with DTP given after MV in the HTMV trials (Chapter 7) and the two-dose MV trial described above, we decided that in the next

two-dose trial, started in 2003, we would only include children, who had received three doses of DTP before enrolment. These children would be unlikely to receive DTP after MV. Since maternal antibodies had declined (Chapter 14), we moved the age of enrolment down to 4.5 months, 4 weeks after DTP3 (recommended at 14 weeks). Recruiting 6,648 children took 3 years and with follow-up to 3 years of age the study ended in 2009. This time, the RCT was a success as planned. There was a small outbreak of measles in 2003 and we found that MV at 4.5 months reduced the risk of early measles infection by 94% (77-99%). But more important, children who received MV at 4.5 and at 9 months had 30% (6-48%) lower mortality between enrolment and 3 years (Figure 1) (4). This was not a comparison of MV versus no MV but of an early two-dose MV strategy compared with the current WHO strategy of MV at 9 months. When measles cases were censored in the analysis, the reduction in mortality was 26% (0-45%), the beneficial effect being stronger for girls (39%) than for boys (10%). Hence, most of the mortality reduction was non-specific.

Though this trial was a success from the point of showing in an RCT that MV had beneficial NSEs and that these could be of major importance for child

survival, it also showed that other interventions could affect the results in major ways. Most participants in the 2003-2009 RCT had participated in RCTs of neonatal vitamin A supplementation (NVAS). The reduction in mortality between enrolment and 3 years of age was 50% (22-68%) among children who had not received NVAS at birth but only 2% (-50-36%) among those who received NVAS. Hence, it seemed that NVAS could effectively remove the benefit of early two-dose MV (4).

	Received no cOPV before enrolment			Received cOPV before enrolment		
	Mortality rate (deaths/person-years)		MRR (95%CI)	Mortality rate (deaths/person-years)		MRR (95% CI)
	Early 2-dose	1-dose MV		Early 2-dose	1-dose MV	
No or not yet cOPV after enrolment	1.2 % (20/1736)	2.2 % (74/3383)	0.53 (0.32-0.87)	2.0% (11/563)	2.2% (24/1077)	0.87 (0.43-1.73)
cOPV after enrolment	1.1 % (21/1974)	1.6% (57/3583)	0.68 (0.41-1.12)	1.4% (6/446)	0.5% (4/855)	2.90 (0.82-10.3)

Table. Annual mortality rates and mortality rate ratios (MRR) for children randomised to early 2-dose MV group or to 1-dose MV at 9 months depending on when campaign OPV (cOPV) had been received. Mortality between 4.5 and 36 months of age. Bandim 2003-2009.

To further explore the two-dose MV strategy, we initiated RCTs in Guinea-Bissau and in rural Burkina Faso. The trials had as enrolment conditions that children had received DTP3 before enrolment and that they had not received NVAS. Surprisingly, the RCT indicated no benefit of early two-dose MV. This was a period with numerous annual campaigns with OPV. These campaigns are associate with major declines in mortality (Chapter 11,13), making it more difficult to show a survival effect of an intervention. However, the OPV campaigns apparently also affected the relative mortality ratio between the one and the two-dose groups. If the children had received several doses of campaign OPV, it was more advantageous to have received only one dose of MV (5). As seen in the Table, in the 2003-2009 trial the MRR for 2-dose/1-dose was a 47% (13-68%) reduction in mortality for children who had received no campaign OPV before or after enrolment. However, the MRR was 190% (-18-903%) higher if they had received campaign

OPV both before and after enrolment. The pattern has been the same in the subsequent RCT. There is clearly a need to find out what is the cause of this interaction. Given the uncertain situation with respect to when OPV campaigns are implemented, we have not initiated new trials of early MV.

Public health implications and future perspectives

Our RCTs of early MV and two-dose MV have shown that MV can have major beneficial NSEs but is has also become clear that there are interactions that may neutralize these beneficial effects. DTP given with or after MV, NVAS, and several OPV campaigns may neutralise the beneficial effect of an early MV strategy. It should be possible to control the sequence of DTP3 and MV. There is no sign that NVAS will become general policy (Chapter 24). However, the OPV campaigns in West Africa have been unpredictable, among others depending on the emergence of polio cases in Nigeria.

Global health is planning to stop OPV and replace it by IPV in 2022. When the change happens, we will consider early two-dose MV strategies again. If a lot of new non-live vaccines, e.g. booster DTP and RTS,S malaria vaccine, are being introduced to be given after routine MV, this may cause new interactions and problems for any attempt to use MV to reduce child survival.

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9. Live vaccines have beneficial non-specific effects: BCG

Background and assumptions

Bacille Calmette-Guérin, BCG, is a live-vaccine that was originally isolated in 1908 from *Lait Nocard*, the milk from a cow that suffered from tuberculous mastitis. It was subsequently cultured in a beef-bile-potato medium throughout the difficulties of the World War I. By 1921, BCG was deemed safe to administer to humans as a measure against one of the biggest killers of its time – tuberculosis (TB).

Generally, BCG is widely regarded as a mediocre TB vaccine since the protective efficacy against pulmonary TB in studies from around the globe ranges from 0 to 80%; protection is better against severe forms of TB. Due to the modest efficacy towards the targeted disease, many (unsuccessful) attempts have been made to produce a novel TB vaccine.

Real-life data

Interestingly, when the vaccine was introduced, researchers noted protection against non-targeted diseases(1,2). Those observations were forgotten until many years later, when similar non-targeted protection of measles vaccination became apparent and the BHP started examining the so-called *non-specific effects* (NSEs) of BCG.

A series of BHP studies suggested that having a characteristic BCG scar or a positive Mantoux skin reaction (indicating a vaccine response) was associated with markedly lower mortality. A landmark observational study from 2000 reported a 45% (15%-64%) reduction in infant mortality among BCG-vaccinated infants (3).

Studies from Bissau also demonstrated that BCG reduced the risk of atopy and anergy. These results greatly challenged the traditional dogmas of immunology; vaccinologists did not expect such effects and could not explain them. The scientific community was broadly reluctant with the reported data, collected by researchers in one of the world's poorest countries. This sure could not be true, or could it?

Luckily, there was a group of infants in Bissau that did not routinely receive BCG at birth, infants with a low weight (LBW; <2,500g). With ethical permissions to conduct randomised controlled trials (RCTs) of BCG-at-birth vs. the usual delayed BCG, our studies of this vulnerable group of infants have greatly enhanced the understanding and acceptance of NSEs of BCG. Three consecutive RCTs demonstrated a reduction in neonatal mortality (first 28 days of life) among BCG vaccinated infants of 38% (17%-54%) (4) (Figure 1).

Across the studies, there were 73 neonatal deaths in the BCG group and 116 deaths in the control group; early BCG given to 3,298 vulnerable infants had thus saved 43 lives. This means that for each 77 neonates vaccinated with BCG, one was spared from dying in the neonatal period. TB is very rare in the neonatal period and the effects were thus entirely non-specific.

The importance of early BCG vaccination was also stressed in relation to BCG scar; among children vaccinated in the first week of life, those who developed a BCG scar had 60% (20%-80%) lower mortality. Furthermore, our hospital studies have shown that BCG may have a limited effect in preventing infection but strong beneficial effects in reducing the case-fatality among those admitted. The hospital study reported 7 cases of fatal sepsis among BCG vaccinated neonates and 20 among controls (5).

The studies were recognised by the national ethics committee in Guinea-Bissau, which now accepts that all newborns are BCG-vaccinated at birth regardless of weight. In the international scientific community, however, there are still some who find these results biologically implausible and who demand a biological explanation before accepting the findings. Luckily, the landmark LBW studies led to an array of new studies into BCG with collaborators around the world focusing on the immunological pathways. A Dutch group discovered that BCG induces an immune response in adults by epigenetic reprogramming in monocytes that lead to protection against unrelated infections; we went back and confirmed this finding in Bissau in the low-birth-weight cohort. This fundamental research provided a plausible biological mechanism.

Further evidence of the generalisability of NSEs came from studies in high-income countries. In Danish data, we found that BCG vaccination among Danish schoolchildren vaccinated in the 1970s was associated with a 42% reduction in deaths from natural causes up to 2010. It was also possible to conduct an RCT in Denmark where our group could reproduce the findings of protection against atopy and, among the group of infants whose mother

had been BCG vaccinated (just like most mothers would be in Guinea-Bissau), BCG was associated with protection against hospital admission for infectious diseases.

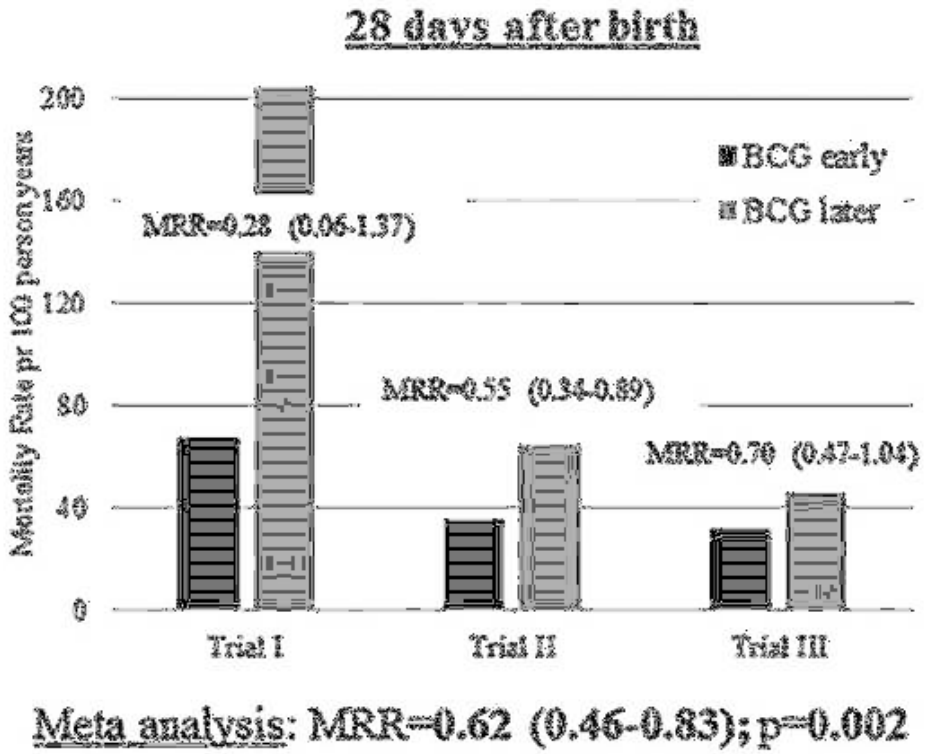

Figure 1. Mortality Rate Ratio (MRR) at 28 days after birth for early BCG vs. delayed BCG.

Our current research priorities are the characteristics of local BCG skin reactions, effects of different BCG strains, whether co-administration of BCG with diphtheria-tetanus-pertussis vaccine (DTP) can be used to reduce the negative effects of DTP (Chapter 16), and whether boosting with BCG can be used to improve health among both children and elderly people. Many strains of BCG are being used worldwide, with very limited data on differences for both protective efficacy against TB and for NSEs. Between 2014-2017, the BHP performed the first study to compare the major BCG strains. Differences in mortality and morbidity associated with different BCG strains add another element to BCG's NSEs as do substantial differences in mortality for the different BCG skin reactions.

Public health implications and future perspectives

Despite of the public health importance of early BCG vaccination, several identified constraints to universal early BCG vaccination remain due to logistical challenges and restrictive vial policies. These constraints urgently need to be resolved. A public health approach of 1) identifying the most efficient BCG strain, 2) vaccinating as early and broadly as possible, and 3) ensuring that all infants produce a BCG scar by revaccinating neonates with no scar, are low-hanging fruits with wide implications that have been ready to harvest for a long time. Approaching its 100th birthday, BCG is administered more than 100 million times each year and a total of 3 billion infants have been BCG vaccinated in the Expanded Programme on Immunization. Providing protection against atopy, anergy, TB, leprosy, Buruli ulcer, sepsis and even long-term mortality, BCG remains a treasure to humanity; a gift that keeps giving.

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10. Optimising the benefit of BCG

Background and assumptions

WHO recommends BCG at birth in countries with high tuberculosis (TB) burden to prevent TB infection. BCG coverage at 12 months of age exceeds 90% in most countries, and is thus among the highest of the vaccines given in infancy. However, currently coverage is only assessed at age 12 months, and that means that there is little emphasis on delivering the vaccine at birth.

BCG is administered at the left upper arm. Following a correctly administered BCG vaccine, children usually develop a BCG scar. The BCG vaccine is hard to administer, and not all children develop a BCG scar following vaccination. Currently, revaccination with BCG is not recommended, and BCG scars are not assessed as part of the evaluation of the vaccination programme.

Real-life data

BCG coverage: Official BCG coverage estimates are influenced by much uncertainty. Due to imprecisely estimated target population size and repeated vaccination of some individuals coverage estimates sometimes exceed 100% (Figure 1). Exploiting the BHP setup, we have been able to estimate BCG coverage more precisely, by only including children with assessed vaccination status. This revealed that while 12 months coverage is indeed very high, most children are not timely vaccinated. Few children thus experience a benefit from BCG during the neonatal period and by one month of age, only 38% of children were BCG vaccinated (1).

There are several obstacles to early BCG vaccination. BCG is produced as 20-dose vials and once reconstituted the vial has to be used within 6 hours. Vaccine wastage rates is one of the indicators, vaccine donors use to assess the performance of the vaccination programmes. These conditions have led to local policies in Guinea-Bissau and other countries of not opening a vial of BCG unless 10-12 children are present for vaccination. Thus, although WHO recommends BCG at birth or at the first encounter with the health system, there is sometimes far from policy recommendation to policy implementation and as we observed, the majority of children do still not receive BCG at birth.

Figure 1. Official regional vaccination coverage estimates from the Quinara Health Region in Guinea-Bissau in 2006. The BCG coverage is 117%.

In rural Guinea-Bissau, many unvaccinated children had either been born at a health facility or been in contact with the health system, thus there were many missed vaccination opportunities. We calculated that if all children were vaccinated at the first contact with the health facilities, 54% of children would have been BCG vaccinated within the first month of life (1).

BCG scarring: Among BCG-vaccinated children, having a BCG scar is associated with lower mortality. However, scarring rates vary between cohorts: In urban Bissau the scarring rates were 80-95%, but in rural Guinea-Bissau, we found that only 52% of BCG-vaccinated children had developed a BCG scar when assessed between 2009 and 2011 (2). We have found consistently in six studies covering both high- and low-scar prevalence cohorts that having a BCG scar is associated with lower mortality among BCG-vaccinated children (Figure 2).

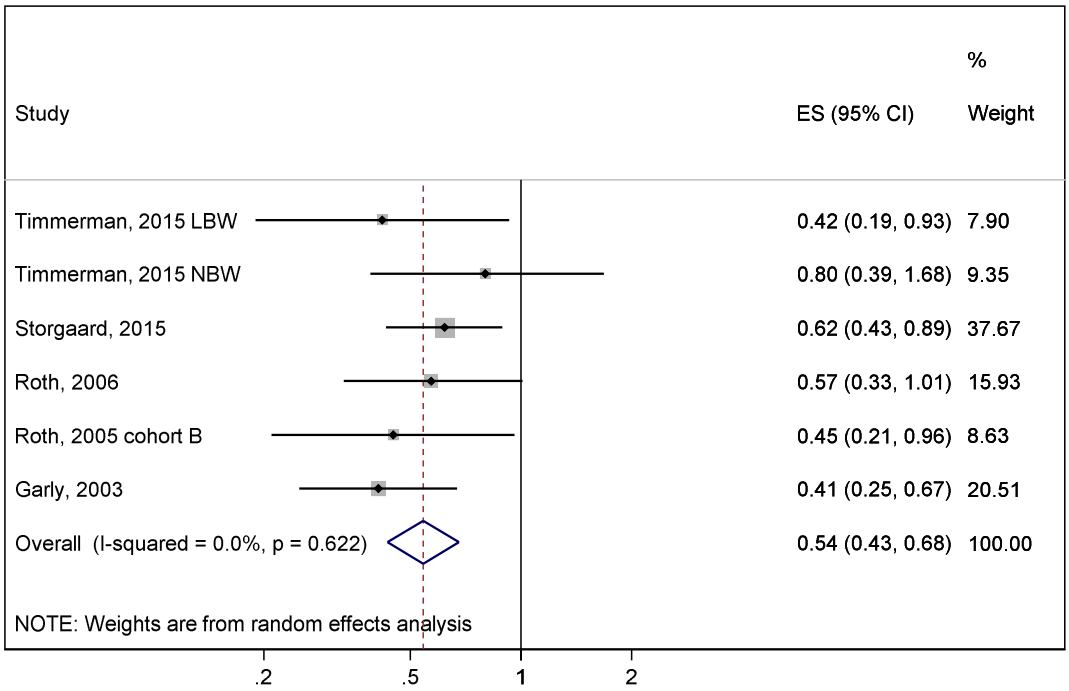

Figure 2. Meta-analysis of studies assessing the effect on mortality of having a BCG scar among BCG-vaccinated children.

Thus, it may not only be important to receive the BCG vaccine, but it might also be important that BCG is correctly administered to develop a scar. We have identified vaccination technique and vaccine strain as main determinants for developing a BCG scar, whereas markers of underlying health status were not associated with scar development (3-5). This is further supported by the fact that among children vaccinated by trained BHP nurses, 98% developed a BCG scar, whereas only 79% of children vaccinated elsewhere developed a BCG scar (5).

Public health implications and future perspectives

Recently, WHO updated their policy paper on BCG. It was emphasised that BCG should be given at birth in countries with high TB burden. However, this will not ensure that children are timely vaccinated as long as the indicators do not reveal delays. We recommend that in addition to the already reported 12-months BCG coverage, BCG coverage at 1 week and 1 month should also be reported to provide countries with an incentive to ensure timely BCG vaccination by opening a vial of BCG even if there is only one child present for vaccination. Furthermore, it may be useful to monitor the

rate of BCG-scar development to ensure that most children develop a BCG scar. According to our research, proper training and vaccination technique may be important to increase the BCG scarring rate. As elaborated in Chapter 9, BCG may have important beneficial effects on child health. We should strive to exploit these beneficial effects by ensuring that all children are vaccinated as early as possible and that all children receive a correctly administered BCG vaccine.

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11. Live vaccines have beneficial non-specific effects: Oral polio vaccine (OPV)

Background and assumption

Poliomyelitis is nearly eradicated. This has only happened due to the very liberal use of oral polio vaccine (OPV) in the routine vaccination program in low-income countries and in campaigns to eradicate polio infection. The world has now decided that OPV has to be stopped and replaced by inactivated polio vaccine (IPV) in 2022 because OPV can cause paralysis in very rare cases, i.e. vaccine derived polio virus (VDPV), or revert to a more pathogenic transmissible virus.

When OPV was first introduced in South America in the 1960s, reports suggested that OPV was associated with fewer diarrhea deaths. Researchers from the Soviet Union reported that vaccination with OPV and other non-pathogenic enteroviruses reduced the risk of influenza and respiratory morbidity 2 to 4-fold among healthy adults. However, apart from these suggestions that OPV had beneficial non-specific effects (NSEs), there are no study of what OPV might have contributed to child survival in low-income countries. OPV was not included in the recent WHO review of the NSEs of routine vaccinations.

OPV has been used in three different ways in public health programmes: First, OPV has been co-administered with the three routine doses of diphtheria-tetanus-pertussis (DTP) vaccine. Second, OPV is given as a priming dose at birth (OPV0). Third, OPV is used in campaigns to eradicate polio infection. BHP has had the possibility of assessing OPV in all of these contexts.

Real-life data

Routine vaccinations with OPV and DTP: There have been situations where *DTP was missing* and children received OPV-only (Table 1). In two studies from the early 1980s in Bandim, children with OPV-only had significantly lower mortality than children who had received DTP (+/-OPV) as their most recent vaccine, mortality being 78% (5-95%) lower for the OPV-only children (1). Twenty years later, when DTP was missing for several months in Bissau, the case fatality at the paediatric ward was 71% (23-89%) lower for children who had received OPV-only compared with children who had received

DTP1+OPV as recommended (2). Conversely, when *OPV was missing*, compared with DTP-unvaccinated children, DTP-only-vaccinated children tended to have higher mortality than DTP+OPV-vaccinated children (1). Hence, OPV may have reduced the deleterious effect of DTP.

Study	**Study design**	**Mortality rate per 100 person-years (deaths/person-years)**		**Relative risk**
		Most recent vaccine: OPV-only	Most recent vaccine: DTP (+/-OPV)	
Urban Bissau, introduction of DTP and OPV 1981-1984	Natural experiment; children aged 3-5 months	0 (0/8.6)	15.8 (12/76.0)	0.0 (0.00-3.81)
Urban Bissau, introduction of DTP and OPV 1981-1984	Children aged 6-35 months	1.7 (2/119.2)	6.2 (28/451.1)	0.27 (0.06-1.12)
Combined estimate				*0.22 (0.05-0.95)*

Table 1. DTP missing. Relative risk of mortality of OPV-only vaccinated compared with DTP+OPV-vaccinated children

OPV at birth: We conducted the first RCTs of OPV0 with infant mortality as the main outcome. Overall, there was a 17% reduction in infant mortality. This estimate included the effect of subsequent campaigns with OPV (see below). In the analysis censoring for OPV campaigns, allocation to OPV0 was associated with a 32% (0-57%) reduction in infant mortality (3).

In some periods, OPV has not been available for administration at birth and that has provided some natural-experiments. We initially published a "natural experiment" arising from a situation in which OPV0 was not administered for 6 months. Comparing periods with and without OPV0, routine OPV0 had a significant negative effect for boys. However, when we later tested the possible modifying effect of OPV campaigns, it turned out that far more children missing OPV0 had subsequently received campaign-OPV

and that this explained why we had found that OPV0 (=not receiving campaign OPV) had a negative effect for boys (4). Hence, in a combined analysis of the RCTs and the observational studies, OPV0 was associated with 27% (4-44%) lower infant mortality before OPV campaigns.

OPV campaigns: Over the last 20-25 years, there have been numerous OPV campaigns in most low-income countries. To our knowledge, only one study has compared mortality of participants and non-participants in OPV campaigns, i.e. when the first two OPV campaigns were conducted in February-March 1998 in Guinea-Bissau. Adjusting for numerous background factors, campaign-OPV was associated with 19% (-21-46%) lower mortality rate. Having received campaign-OPV was also associated with 73% (24-90%) lower risk of subsequent hospital admission.

The coverage has been very high in more recent OPV-campaigns, and we have therefore conducted subsequent analyses assuming that all eligible children received campaign OPV. We have estimated the MRR comparing the “after-campaign” mortality rate with the “before-campaign” mortality

rate. Using data from seven RCTs conducted in urban Bissau between 2002 and 2014, we analysed the effect of the 17 OPV campaigns implemented in this period. With the 1244 deaths and 33,822 person-years from all seven RCTs, it was possible to adjust for age, possible seasonal effects and changes in mortality rate over time. Campaign-OPV was associated with a 19% (95% CI=5-32%) mortality reduction (5). Each additional OPV campaign reduced mortality by 13% (4-21%). The OPV campaigns and decline in mortality over this period is illustrated in Chapter 13. Beneficial effects were not found for any of the other campaigns with vitamin A supplementation, measles vaccine and H1N1 vaccine.

A similar reduction of 22% (6-36%) was seen in rural areas of Guinea-Bissau; during an RCT in rural Burkina Faso mortality was 36% (6-56%) lower after OPV campaigns than before these campaigns. Furthermore, children, who had been eligible for an OPV campaign before admission, had lower case fatality (11% (96/855)) for any cause than similar children who had not been eligible for an OPV campaign (16% (324/2089)) i.e. 28% (10-42%) lower case fatality after the OPV campaigns.

Vaccine	Design; Age group	Overall mortality reduction	Mortality reduction before OPV campaigns #
Measles vaccine	Additional MV at 4½ months; mortality 4-36 months per-protocol analysis;	30 % (6-48%)	47% (13-68%)
OPV-at-birth	RCT of OPV0 vs no OPV0; infant mortality	17 (-13-39%)	32% (0-55%)
BCG-II	BCG at birth to LBW children; infant mortality	17% (-8-39%	20% (-6-40%)
BCG-III	BCG at birth to LBW children; neonatal mortality	30 % (-4-53%)	34% (0-56%)

Table 2. Four RCTs of early vaccination: effect of OPV campaigns on overall estimate.

Notes: # follow-up was censored when the children became eligible for campaign OPV.

In all these areas, the data suggests that OPV has had beneficial NSEs. There has been no polio infection in Bissau for 20 years, so these effects are evidently NSEs. If OPV has capacity to reduce the mortality rate non-specifically, OPV campaigns may fundamentally change the outcomes in RCTs testing other interventions. This is indeed what happened in the four RCTs, where we have been able to test the modifying effect of OPV campaigns (Table 2). Only one RCT was statistically significant for both periods before and after OPV-campaigns. However, three RCTs were statistically significant before the OPV-campaigns. After the OPV campaigns, the hypothesised effect had disappeared. Hence, OPV-campaigns have reduce child mortality rates but made it more difficult to assess the impact of other interventions on child survival.

Public health implications and future perspectives

All available data suggests that OPV has beneficial NSEs. The decline in under-five mortality over the last decades seems to be strongly associated with the numerous campaigns with OPV (4,5) (Chapter 13). Hence, OPV campaigns may have been a major reason that Guinea-Bissau unexpectedly reached the millennium development goal of reducing child mortality by 2/3 between 1990 and 2015.

As part of the endgame for polio infection, it is the plan to replace OPV with IPV in 2022. The available data suggest that IPV as other non-live vaccines have no beneficial effects and may be associated with increased female mortality. Thus, it is likely that child mortality may increase again once OPV has been removed. Eradicating polio and stopping OPV may well become a major Pyrrhic victory.

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12. Live vaccines have beneficial non-specific effects: Smallpox vaccine

Background and assumptions

The last case of smallpox occurred in 1977. In 1980 WHO stopped smallpox vaccinations. When BHP started in 1978, smallpox infection was clearly an irrelevant public health problem. Unfortunately, it took 20 years before we got interested in smallpox vaccine, and then from a rather different perspective. We speculated that if MV had beneficial non-specific effects (NSEs), there was a possibility that eradicating measles and stopping vaccination could have negative consequences by no longer providing the beneficial immune training. In the worst scenario: the overall effect could be negative if the NSEs saved more individuals than had been killed by the infection. The only example of eradication and stopping vaccination was smallpox. The live smallpox vaccination was associated with a strong immune stimulation and it was possible that it could have had important NSEs. However, the health impact of discontinuing smallpox vaccination in 1980 had not been assessed. To prepare for a later eradication of measles, we decided to study the possible effects of having stopping smallpox vaccination.

To study possible NSEs of smallpox vaccine, we needed access to information on vaccination status before 1980. In Guinea-Bissau this could only be done if we could use smallpox vaccination scars as a proxy for smallpox vaccination, which turned out to be feasible (1). In Denmark we could use the school health cards (2).

Real-life data

Adult mortality in Guinea-Bissau: From January 1998 to January 1999, field workers assessed vaccination scars for 1893 adults in Bandim 1 and Bandim 2. Only individuals above 25 years of age were eligible for inclusion, since they were the only ones who had had the chance to be smallpox vaccinated. Survival was assessed in June–July 2002. Comparing individuals with a smallpox vaccine scar but no BCG scar with individuals without any scar (n=873/1373), the mortality rate adjusted for age was 40% (9-60%) lower (Figure 1). The effect of smallpox vaccination may have been stronger for

women than men. The effect of smallpox vaccine appeared to be similar among individuals with a BCG scar, and those who had any type of scar had 39% (11-59%) lower mortality than those with no scar (1). Interestingly, the beneficial effect of smallpox scars increased with additional scars, the reduction in mortality rate was 35% after 1 scar, 46% after 2 scars, and 56% after 3 or more scars.

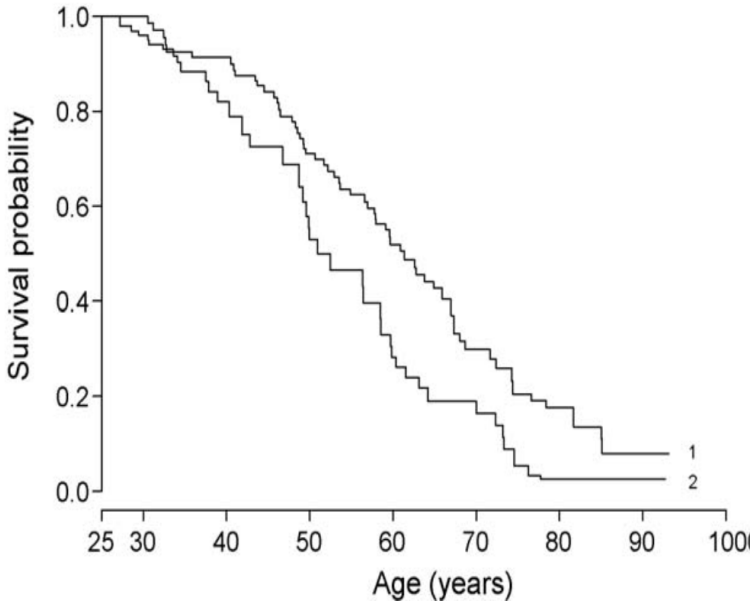
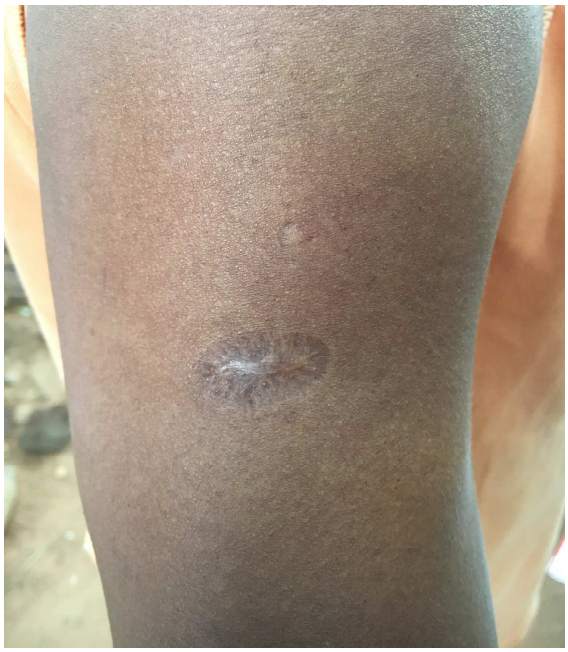


Figure 1. Kaplan–Meier survival curves for 873 people with a vaccinia scar and no BCG vaccination (1) and for 500 individuals without any scar in Bissau (2).

These findings from Bissau city were tested within a case-control study of HIV-2 infection in rural Guinea-Bissau. In 2003, 367 individuals born before 1974 and thus possibly smallpox-vaccinated were examined for vaccine scars by a physician (3). Of these, 141 were HIV-2-infected, 23 were HIV-1-infected, 29 were dually infected, and 174 were uninfected with HIV. Survival was assessed in 2006; 13% (47/367) had died. Individuals with a smallpox vaccine scar had 78% (39-92%) lower mortality than individuals without any scar, adjusting for age, sex, village and HIV status. The reduction in mortality rate was 81% (43-94%) for women and 60% (-274-96) for the small number of men in the study. Better survival was found for both HIV-negative and HIV-2 infected individuals (3).



Adult mortality in Denmark: In Denmark, smallpox vaccination was compulsory before entering school up to 1976. No Danish register of vaccinations dates back to the phase out of smallpox vaccination, but individual information has been systematically recorded for children attending Copenhagen schools since the 1930s. From such school health records, both information on vaccinations, health and social factors were digitalised and linked with national registers via personal identification numbers in order to study NSEs of the smallpox vaccine.

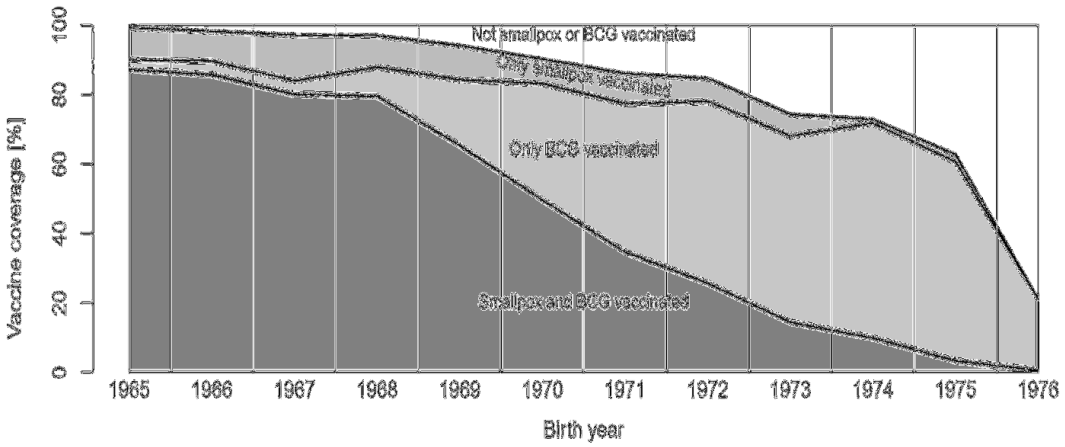


Figure 2. Smallpox and BCG vaccinations were phased out in Denmark affecting the birth cohorts born between 1965-76.

Among the 46 239 Danes born between 1965-76 and who went to school in the municipality of Copenhagen, 841 deaths could be analysed during our follow up until 2010. In a case-cohort study, we analysed how smallpox and BCG vaccinations were associated with 401 deaths due to natural causes. For both smallpox and BCG vaccination vs none of the two vaccines, the mortality rate was 46% (19-64%) lower, and for smallpox and/or BCG vaccination, the mortality rate was 43% (19-60%) lower (2) compared with those without vaccines. Interestingly, the reduction was approximately 50% for all major disease groups except cancers where no effect was observed. Vaccinations were not associated with our negative control outcomes (accidents, suicides and murders).

HIV-1-infection in Guinea-Bissau and Denmark: Unexpectedly, in both urban and rural Guinea-Bissau persons with a smallpox scar were more likely to be HIV-2-infected compared with individuals with no vaccination scar (1,3). We speculated that the smallpox vaccination campaigns could have contributed to transmission of HIV-2 due to insufficient sterilization of the scalpel or bifurcated needle used in the campaigns. Alternatively, if HIV-2 infected, smallpox-vaccinated individuals had better survival than HIV-2 infected, not-smallpox-vaccinated individuals, we would eventually get an association with HIV-2 infection being more prevalent among smallpox-vaccinated individuals than among not smallpox-vaccinated individuals.

Since HIV-1 was not present in Guinea-Bissau in the 1970s, vaccination with smallpox did not contribute to the spread of HIV-1 infection. However, HIV-infection has been an important cause of mortality among adult, so we examined whether vaccination scars offered some protection against HIV-1 infection.

In a cross-sectional study in urban Guinea-Bissau, we observed that smallpox and/or BCG vaccination scars were associated with a 38% (-7 to 64%) lower prevalence of HIV-1 compared with individuals without smallpox and BCG vaccination scars. This prevalence difference was even stronger for those with multiple smallpox vaccination scars and stronger among women than men. In Denmark, those who had been smallpox and/or BCG vaccinated had a 30% (-15 to 57%) lower chance of HIV-1 compared with peers without any of the two vaccines. The combined estimate across Guinea-Bissau and Denmark indicated that smallpox and/or BCG vaccination was associated with 34% (4-54%) fewer HIV-1 cases (4).

Public health implications and future perspectives

Given the consistency of findings between Guinea-Bissau and Denmark, which had very different vaccination contexts, it seems unlikely that confounding can explain the beneficial effects associated with being smallpox vaccinated. Hence, smallpox vaccination is likely to have had beneficial NSEs just as BCG. Preliminary studies suggest that *vaccinia* also induces innate immune training like BCG, whereas the modified *vaccinia Ankara* (MVA) does not (5). MVA has been used as a vaccine-vector for other antigens, e.g. in new TB or malaria vaccines. MVA is non-replicating and if it does not induce innate immune training, this could go some way to explain why some of the prime-boost vaccines have not worked efficiently.

The studies in Guinea-Bissau and Denmark have generated large cohorts of individuals who have been assessed for smallpox vaccine and BCG scarring. We may gain important insight into the long-term NSEs of live vaccines for both acute and chronic diseases through following these cohorts. For example, in a hospital based case-control study during a cholera epidemic in Bissau, individuals with BCG and/or vaccinia scars had reduced risk of hospital admission with cholera infection (OR=0.40; 95% CI: 0.18-0.86).

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CENTRO SAUDE DE BANDIM
PROGRAMA DIÁRIO DE SERVIÇO

13. The eradication paradigm: Defeating smallpox, polio and measles infections and their live vaccines

Ending infections - stopping vaccinations

Smallpox was eradicated in 1977. Live smallpox vaccine was stopped globally in 1980, three years after the eradication of smallpox infection. Polio infection is close to extinction, possibly in 2019. Trivalent oral polio vaccine (OPV) was stopped globally in April 2016 and live bivalent or monovalent OPV will be stopped in 2022, and replaced by the inactivated polio vaccine (IPV). Measles (and possibly rubella) will be next in line for eradication, maybe 10-15 years from now. These eradicable diseases were all controlled by ***live*** vaccines.

In current medical culture, each infection is a self-contained system of interaction between the pathogen and the immune system. Hence, once we have eradicated the disease it is believed that we can remove the vaccine without any further control. No one examined what happened to general health when smallpox vaccination was stopped in 1980. No study is planned of the consequences of stopping OPV and replacing it with IPV, even though two RCTs of OPV versus IPV in Finland and Bangladesh have found that IPV is associated with more morbidity than OPV. In the last 40 years, Denmark has stopped three live vaccines – smallpox vaccine, BCG, and OPV. No study examined possible unexpected health consequences of stopping these live vaccines.

If the underlying assumptions about a one-to-one link between one disease and one vaccine are incorrect, and the live vaccines protect against more than the targeted infection, it may have unexpected and disastrous consequences to stop these vaccines.

Live vaccines have beneficial non-specific effects for survival

The data is consistent, the three live vaccines for eradicable infections have beneficial non-specific effects (NSEs), protecting against other infections than smallpox, polio and measles. Though the beneficial NSEs of these vaccines may sometimes be reduced or removed due to interactions with other

interventions, e.g. DTP being administered after MV, there is no study to document that these live vaccines have no beneficial NSEs. Though the global health community never tested the overall effect of these vaccines in RCTs, we have conducted RCTs with OPV and MV and found strong beneficial NSEs (Chapters 6, 11). OPV-at-birth was associated with a 32% (0-55%) reduction in infant mortality and an additional dose of MV at 4.5 months of age was associated with a 30% (6-48%) reduction in mortality between 4.5 and 36 months of age (1,2).

The evidence from observational studies is also substantial for these live vaccines having beneficial NSEs. In the WHO review of the non-specific effects of vaccines, MV was associated with a reduction of more than 40% in mortality, which could not be explained by the prevention of measles infection (3).

Immunological studies have shown that both BCG and smallpox vaccine may induce innate immune training (4). Hence, it is plausible that the vaccine can protect against non-targeted diseases. It is therefore surprising that global public health is not trying to assess what might be the effects of stopping these live vaccines.

Live vaccines have beneficial NSEs long after the disease is eradicated or eliminated

In the absence of RCTs testing the long-term effects of these live vaccines, the best we can do is to examine what happens for vaccinated and non-vaccinated individuals after the disease is eradicated or eliminated.

The data for smallpox vaccine is clear. In both Guinea-Bissau and Denmark we showed that smallpox vaccinated adults had 40% lower mortality for natural causes of death than non-vaccinated individuals of similar age in the periods 20-30 years after smallpox eradication (Chapter 12). There is no strong indication that it was just the healthier individuals who were vaccinated; for example, in Guinea-Bissau the vaccination coverage was higher in the rural areas, presumably because campaigns were better organised by the colonial administration in the rural areas than in the capital.

There has been no new cases of polio infection in Bissau for the last 15-20 years. Though the first studies were done in the 1980s when there was still polio around, recent studies of routine OPV vaccinations and OPV campaigns showing strong beneficial NSEs have been done in a situation where

there has been no polio infection (1,5).

The last major measles outbreak in Bissau occurred in 2003-2004. Though a few imported cases may have occurred this will not explain why we have continued after 2004 to find very strong beneficial NSEs for measles vaccinated children both in RCTs and in campaigns (2).

Campaigns

Vaccination campaigns have been a very strong feature of the final phase of eradication. In Guinea-Bissau, there have been more than 20 OPV campaigns since 1998 and MV campaigns have been regular since 2006 but were also conducted shortly after the civil war in 1998-1999. Both OPV and MV campaigns have been associated with strong reductions in mortality rates (Figure 1). Surprisingly, we have found for both OPV and MV that the beneficial NSEs are enhanced with additional doses of these live vaccines (5); this effect cannot be explained by enhanced specific protection against measles or polio infections. In other words, boosting with live vaccine is beneficial.

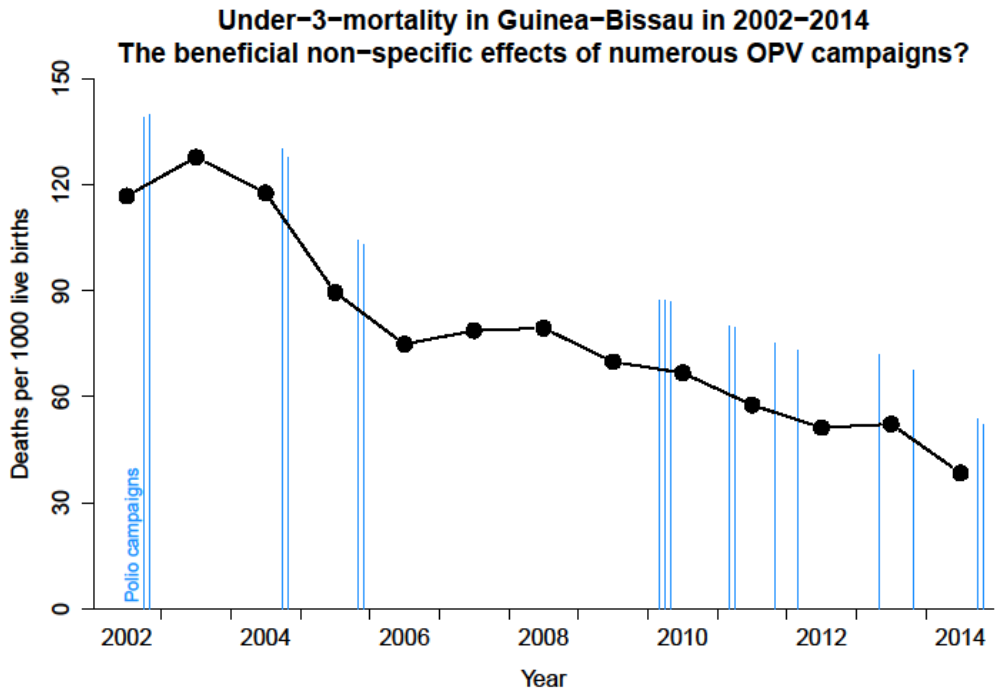

Figure 1. Decline in under-3 mortality between 2002 and 2014. OPV campaigns are marked in blue.

Predicting the public health implications of stopping OPV and MV

With what we know now, it seems likely that stopping of smallpox vaccination was associated with increased mortality among older children and adults since those were the age groups receiving the vaccine.

OPV and MV are given in the first months of life, where mortality rates are much higher, and the implications of stopping OPV and MV may therefore be much larger. OPV and MV have beneficial NSEs reducing mortality in their own right when given as single vaccines in the childhood immunization programme. However, OPV and MV have also contributed to reducing mortality when given together with other vaccines; for example, OPV given with DTP or MV given after DTP reduced the negative effects of DTP. When OPV and MV are removed, the negative effects of DTP or other non-live vaccines may become more pronounced. When the OPV and MV campaigns are going to be stopped soon, the children will no longer benefit from boosting by a live vaccine (5).

Hence, we predict that infant and child mortality will increase again in the near future, not from polio or measles but from poorer immunological con-

trol of other infections. So the mission is not accomplished (Chapter 2). Public health will have to study whether other vaccines can be used to substitute the beneficial NSEs of OPV and MV.

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14. Maternal priming enhances the beneficial non-specific effects of live vaccines for child survival

Background and assumptions

Providing vaccines in the presence of maternal immunity - typically measured as maternal antibodies - is associated with a blunted antibody response and reduced protection against the target disease. Therefore, vaccination programs strive to balance the timing of vaccines, to avoid blunting, and at the same time ensure early protection. For instance, measles vaccine (MV) is given at 9 months in low-income countries, but after 12 months in high-income countries, the latter being associated with higher antibody responses and therefore assumed superior if the risk of measles infection is low.

There is increasing evidence that live vaccines have beneficial non-specific effects (NSEs) on overall health, and therefore it may be important for overall health to provide these vaccines early. How maternal immunity affects the NSEs of vaccines has so far not been studied.

Real-life data

We investigated the overall mortality and morbidity effect of two live vaccines, MV and BCG, and specifically studied if the effect depended on maternal priming.

A) We studied the effect on mortality of providing an early MV at 4-6 months of age in the presence or absence of maternal measles antibodies in Guinea-Bissau. As we found a surprisingly beneficial effect of vaccinating in the presence of maternal antibodies, B) we hypothesised that within a randomised trial of BCG to Danish neonates, the morbidity effect of BCG would be better in children of BCG-vaccinated mothers. As this was the case, C) we looked for other opportunities to test the hypothesis and found it within a study in Guinea-Bissau, where both mothers and children had had their BCG scar assessed at enrolment and children were followed for mortality.

In a combined analysis of two MV studies from Guinea-Bissau, children who had maternal measles antibodies when they received their first dose of MV

had 78% (95% CI=36-93%) lower mortality than children with no maternal antibody between 4–6 months and 5 years (Figure 1) (1).

In Denmark, neonatal BCG-vaccination was not associated with an overall reduction in hospital admissions for infectious diseases up to 15 months. However, consistent with our hypothesis, in the subgroup of children of BCG-vaccinated mothers, BCG was associated with 35% (6-55%) lower rate of hospital admissions for infectious diseases, the effect being significantly different from that in children of BCG-unvaccinated mothers (p for interaction=0.01)(2).

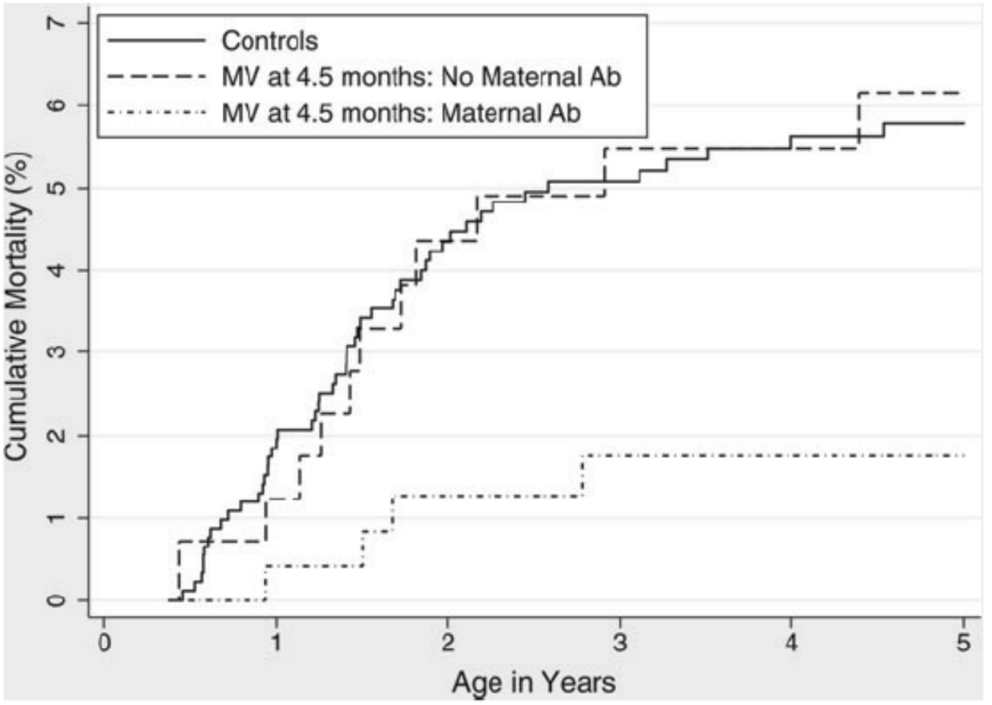

Figure 1. Cumulative mortality between 4.5 months and 5 years of age in relation to age of measles vaccination (MV) and presence of maternal antibody (trial II). Children randomized to MV at 4.5 months received also MV at 9 months of age. Controls received only MV at 9 months of age. Abbreviations: Ab, antibody; MV, measles vaccination. (1).

In Guinea-Bissau, children who had a BCG-scar had 41% (5-64%) lower child mortality between 4.5 and 36 months than children without a BCG-scar. The reduction in mortality was 66% (33-83%) if the mother had a BCG-scar but only 8% (-83-53%) if the mother did not have a BCG-scar (p for interaction=0.04)(3).

Public health implications

Three different studies in different populations, using different designs and outcomes, have uniformly supported the same overriding hypothesis: maternal priming enhances the beneficial effects of live vaccines on overall health. The underlying mechanisms are unknown. Other studies have shown that boosting with live vaccines enhance their beneficial NSEs (4, Chapter 15). Thus, it may be the presence of pre-existing immunity – be it from the mother or from previous exposure – that is important.

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15. Revaccination and campaigns with live vaccines are beneficial

Background and assumptions

Immune memory after vaccination with a live vaccine is long-lived, and normally a single dose is sufficient to protect against the targeted infection. To ensure an adequate antibody response to the vaccine, the policy is to wait with vaccination until after maternal antibodies have waned.

Live vaccines against measles, tuberculosis, polio, and smallpox reduce mortality more than explained by prevention of the target-disease. Hence, these vaccines train the immune system to protection against unrelated infections (Chapters 6-12). We recently observed that the beneficial non-specific effect (NSEs) of measles vaccine (MV) was enhanced when vaccination took place in presence of maternal antibody immunity (Chapter 14). This indicated that the vaccine could be additionally beneficial when provided in the presence of pre-existing antibody. We therefore examined whether revaccination with the live vaccines against tuberculosis, measles, polio and smallpox boosted their beneficial NSEs (1).

Real-life data

BCG: A large trial in Alger 1935-1947 included more than 40,000 children allocated to BCG or nothing at birth. Those receiving BCG got three oral doses of BCG shortly after birth; the children were revaccinated at 1, 3, 7 and 11 years of age. The reduction in overall mortality associated with BCG increased with increasing number of doses (p for same effect across doses, $p<0.001$).

More recently, we conducted a BCG revaccination trial in Guinea-Bissau. In Guinea-Bissau, nearly all children receive BCG early in life and were scheduled to receive a diphtheria-tetanus-pertussis (DTP) booster at 18 months of age. Children were randomised to BCG revaccination or no vaccination at 19 months of age. Among children, who had received DTP booster before enrolment, BCG revaccination versus no BCG revaccination at 19 months of age was associated with a 64% (1-87%) reduction in mortality between 19 months and 60 months of age (2).

Interestingly, the effects of BCG immunotherapy against bladder cancer are stronger in patients who had been BCG vaccinated prior to treatment.

Measles vaccine: In two randomised controlled trials (RCTs) in Guinea-Bissau, two doses versus one dose of MV reduced all-cause mortality by 63% (95% CI: 23–83%) from 9 to 18 months of age (1).

In the early 1980s, MV was administered in campaigns in Guinea-Bissau once or twice a year; children who received their first MV before 9 months received a second MV at the next visit. Hence, receiving one or two doses of MV was a natural experiment determined by age at the time of the first campaign. Comparing the mortality of children from the time they received either a second or a first MV after 9 months of age, those who received the second dose had 59% (25–81%) lower mortality between 9 and 59 months.

A second dose of MV is still given in campaigns in Guinea-Bissau. After recent MV campaigns in rural and urban Guinea-Bissau, we compared the effect of MV campaigns on overall mortality, stratified by whether the child had received routine MV. The mortality reductions were larger in children who *also* received routine MV (3). We also observed beneficial effects of a second dose of measles-mumps-rubella vaccine in Denmark (Chapter 22)

Oral polio vaccine: Oral polio vaccine (OPV) is recommended at birth and in three doses with DTP vaccines at 6, 10 and 14 weeks of age. Hence, OPV revaccination is normally given with DTP. It therefore becomes difficult to evaluate whether revaccination with routine OPV has an independent beneficial effect. However, OPV is also given in campaigns independent of prior vaccination status of the child. We examined how the 14 national OPV campaigns between 2002-14 in Guinea-Bissau affected the mortality rate. OPV campaigns – but not other campaigns – reduced the mortality rate significantly when controlled for age, season and time trend. Since the children were to receive campaign-OPV several times, we assessed the effect of repeated doses; each additional exposure to OPV-campaigns was associated with an additional 13% (4–21%) reduction in mortality (4).

Smallpox vaccine: For smallpox vaccine, two cohort studies from urban and rural areas of Guinea-Bissau examined the correlation between the number of smallpox vaccination scars and subsequent mortality. In the urban study, there was a significant trend for larger reduction in mortality with increasing number of smallpox vaccination scars (5). Though not statistically significant, the trend was similar in the smaller rural study.

Public health implications

Revaccination with live vaccines led to substantial reductions in overall mortality. These findings challenge the current understanding of vaccines and may explain the beneficial effects of campaigns with live vaccines. The implications are potentially major; overall mortality could be reduced significantly with increasing use of revaccination with live vaccines.

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16. Deleterious and sex-differential non-specific effects of DTP: Confronting WHO policy

Background and assumptions

Along with measles vaccine (MV), diphtheria-tetanus-pertussis (DTP) vaccine has come to play a defining role for the BHP research agenda. There are many reasons for this.

In 2000, we published the first paper, which suggested that DTP might be associated with increased child mortality (1). Based on vaccination information collected from children in the rural areas, we found that during 6 months of follow-up and controlled for age and background factors, BCG-vaccinated children had 45% (15-64%) lower mortality than BCG-unvaccinated children; however, children who had received the first does of DTP (DTP1) had 84% (10-210%) higher mortality than DTP-unvaccinated children. WHO had been advised before the publication, and we had asked whether further studies could be coordinated to find out if this was a true finding. Prior to publication, WHO sent three experts to Bissau to review data collection procedures, stored data and analyses. No critical problem was identified.

Subsequently, WHO's Global Advisory Group on Vaccine Safety (GACVS) sponsored re-analyses of data from other longitudinal field sites in Burkina Faso, Bangladesh, Papua New Guinea (PNG), and Indonesia. During the preparatory meetings planning these analyses, we emphasised that it would be very difficult to analyse the vaccination data as if the data on vaccinations was complete. There would always be children for whom data had not been collected. However, the WHO recommendation for analysis did not take that into account. The WHO-sponsored studies and a number of other studies following WHO's recommendation for analysis subsequently found marked beneficial effects of DTP.

Following these analyses, GACVS commissioned an independent "Task Force on Routine Infant Vaccination and Child Survival" which should review the evidence for a deleterious effect of DTP vaccination on child survival. The Task Force concluded, among other things, that

"The task force was unanimous that the totality of the evidence provided in the papers reviewed does not suggest a deleterious effect of DPT vaccination; on the contrary, they provide substantial evidence against such an effect. Furthermore, with the exception of the studies from Guinea-Bissau, there was little evidence of a differential effect between boys and girls. The possibility cannot be excluded that there may be an effect of DPT vaccination that is specific to Guinea Bissau but the findings presented did not convince the task force that this was likely to be the case."

Apparently, there was no reason to continue the investigation of DTP and mortality. However, the Task Force had not discussed their data or analyses with the Guinea-Bissau group, which had initiated this research.

Results

Several methodological issues should have been discussed.

First, most DTP studies were carried out in rural areas where vaccines are delayed and BCG is often given together with DTP. Giving BCG+DTP is a very different immunological exposure from having DTP after BCG as is recommended by WHO. This distinction was not considered in the WHO-sponsored analyses. For example, the largest data set from Bangladesh of nearly 38,000 children reported that DTP was associated with a 24% (12-33%) reduction in mortality. However, in a subsequent re-analysis of the same data we could show that mortality was 48% (30-62%) lower between 6 weeks (age of DTP) and 9 months (age of MV) if the children had started the vaccination schedule by receiving BCG+DTP simultaneously than if they had the WHO-recommended schedule of BCG first and then DTP (2).

Second, in contrast to the studies from Guinea-Bissau, the WHO-sponsored studies did not exclude any individual for lack of information. Since it is impossible to have complete vaccination data unless there is one institution distributing all vaccines which has a good register, many children will have been classified as "unvaccinated" - not because they were known to be "unvaccinated" but because there was no information, e.g. because the child had died. It is much easier to get vaccination information from surviving children than from dead children, whose the parents have often discarded the vaccination card. The dead children with no vaccine information will be classified as "unvaccinated" but could easily have been a misclassified vaccinated children.

Since vaccines given to children are registered at subsequent contact with a field worker, vaccination status is selectively updated for surviving children. By assigning risk-time from the date of vaccination rather than from the date the vaccination was registered, the analysis has introduced a major "survival bias". The higher the potential bias in the study, the higher will be the mortality rate among the so-called "unvaccinated" children and the stronger will be the beneficial effect of DTP in an analysis of the mortality rate ratio (MRR) for vaccinated vs unvaccinated children. Hence, we have called the MRR between "unvaccinated" and vaccinated (any vaccine) children for a ***bias index***. As can be seen in Figure 1, there is a clear correlation between the bias index and the reported MRR for DTP vs DTP-unvaccinated. Hence, the studies which "provide substantial evidence against" a deleterious effect of DTP were those which had survival bias and very high bias indexes (3).

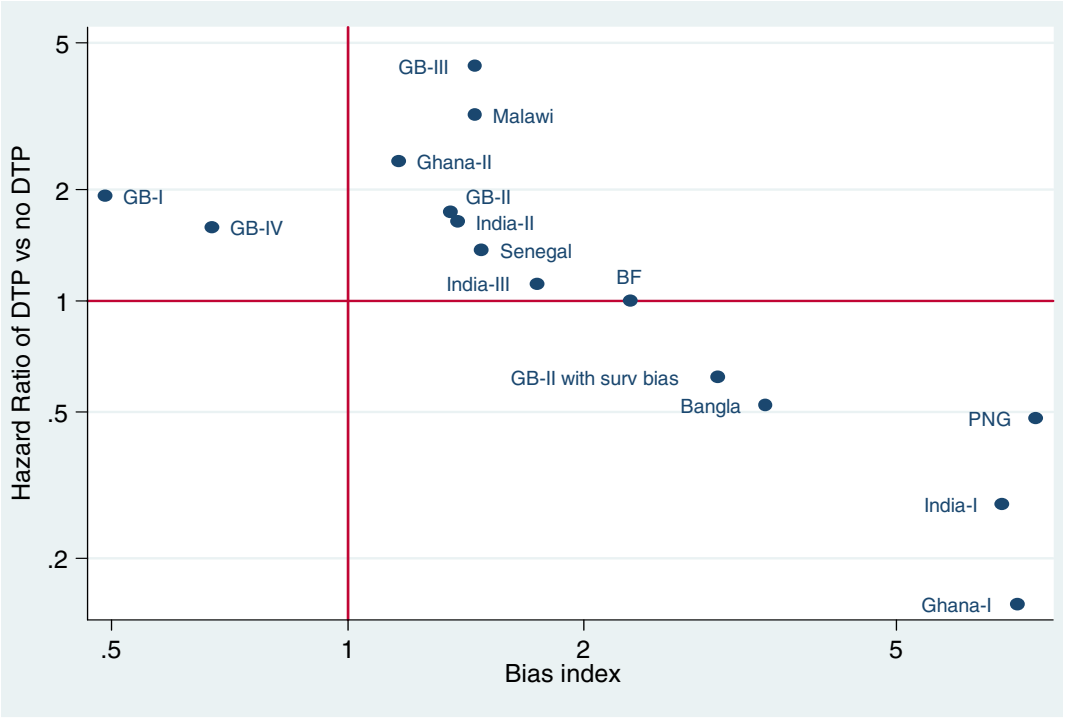

Figure 1. Scatter plot of the mortality hazard ratio (HR) for DTP-vaccinated versus DTP-unvaccinated and for the bias index (mortality HR for unvaccinated versus vaccinated (any vaccine) children). The GB-II study is represented with the originally published results (8) and with results when survival bias was introduced in the analysis.

We have therefore emphasised studies, which did not have survival bias, because they had documented the status of both vaccinated and unvaccinated children prior to follow-up and have then followed the children prospectively for survival. Eight studies fulfilled this criterion. In a combined analysis of these studies, being DTP-vaccinated was associated with 100% (50-167%) higher mortality than being DTP-unvaccinated (Figure 2) (3). The results were essentially the same in studies from Guinea-Bissau (97% (40-175%) higher) and elsewhere (111% (22-266%) higher). Even though there was no survival bias in these studies there may well have been other biases related to who were vaccinated and not vaccinated with DTP. The data clearly indicated that healthy children receive vaccination first and one should therefore have expected that the DTP-vaccinated children had lower mortality in any case. Hence, the two-fold higher mortality in Figure 2 is likely to be a conservative estimate of the "true" effect of DTP.

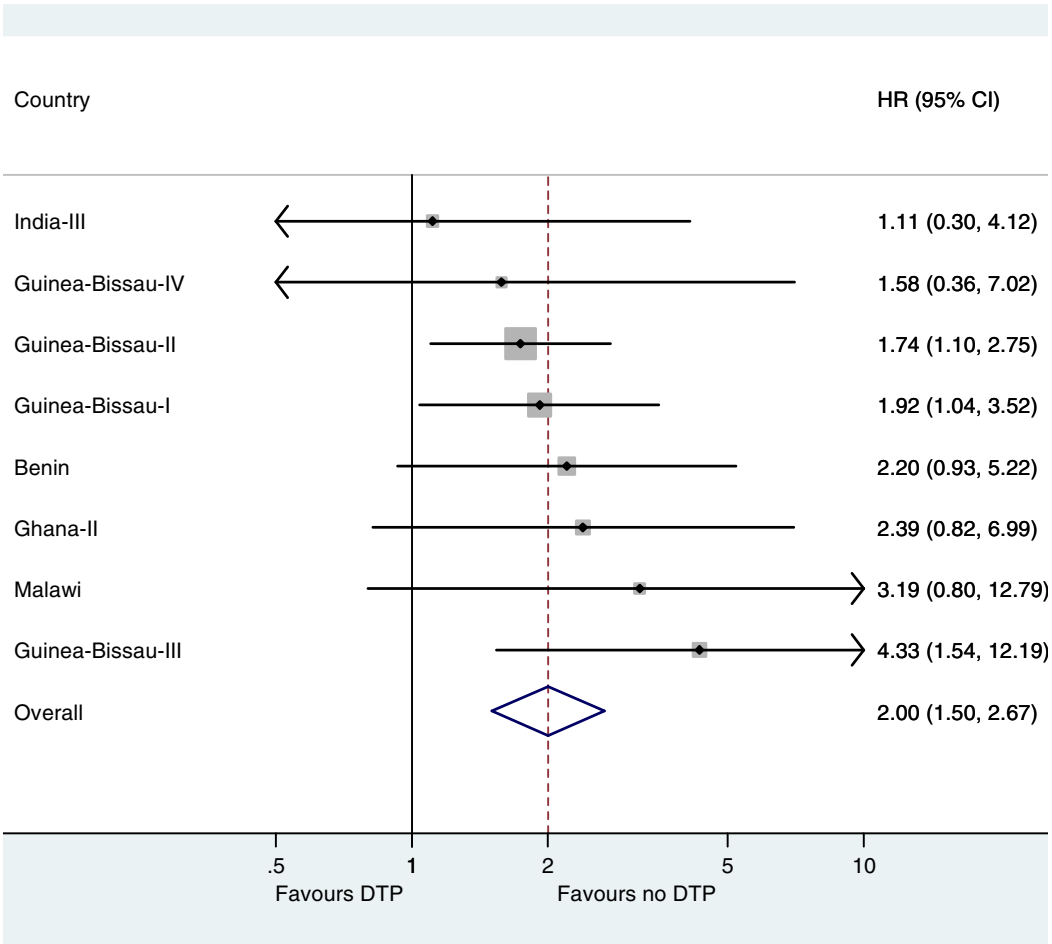

Figure 2. The mortality hazard (HR) for DTP-vaccinated versus DTP-unvaccinated children in studies with no survival bias.

To examine the female-male MRR we used all studies, which had information on sex and DTP-vaccination. As seen in Figure 3, females had 53% (21-93%) higher mortality than the males after having received DTP (4). Again the effect was the same in the seven studies from Guinea-Bissau (58% (-2-154%) higher) and the eight studies from elsewhere (56% (26-94%) higher). Hence, there is no reason to speculate that the effect of DTP is specific to Guinea-Bissau. The systematic increased female mortality is "unnatural"; in the pre-vaccination era there was no increased female mortality in the corresponding age groups.

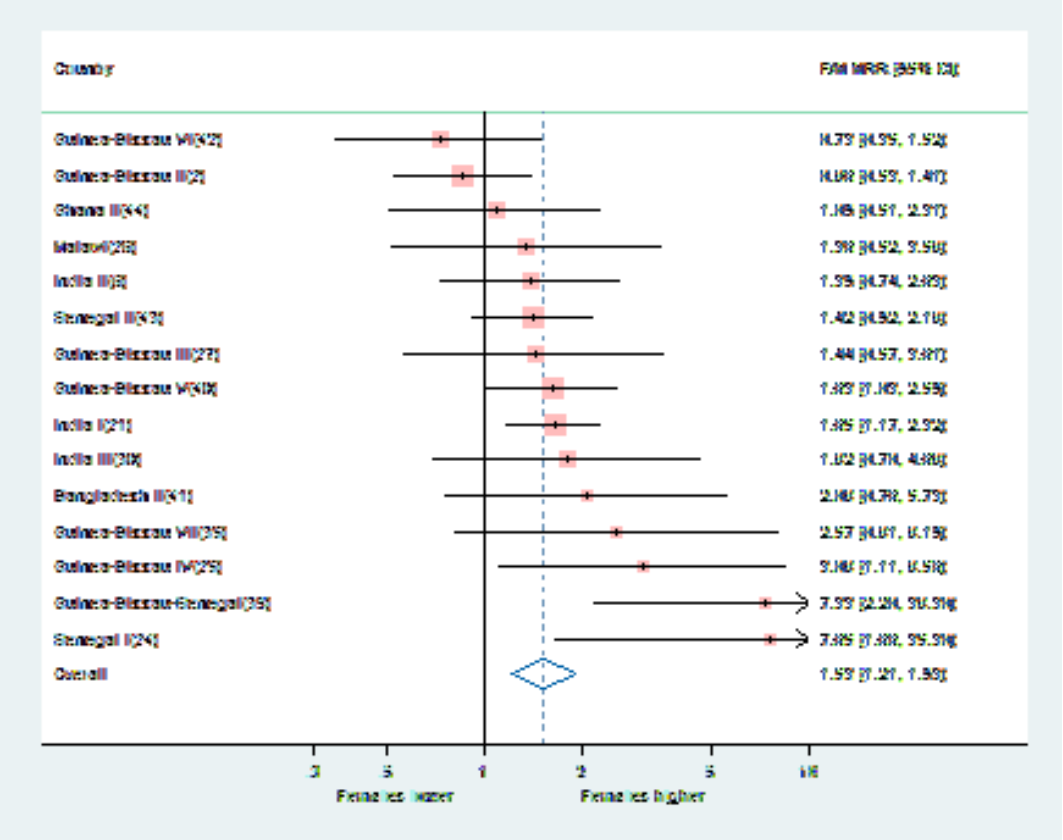

Figure 3. The female/male mortality rate ratio for female DTP-vaccinated versus male DTP-vaccinated children.

It took several years to convince key figures in global health that the WHO-sponsored analyses had in fact been wrong due to the misclassification of exposure and survival bias in the analyses. It took another 5 years before WHO's Strategic Advisory Group of Experts on Immunization (SAGE) decided to review the potential NSEs of BCG, DTP, and measles vaccine (MV).

As discussed in other chapters the data on BCG and MV suggested major beneficial NSEs and WHO recommended further studies on NSEs. For DTP, the meta-analysis found 38% (-8-108%) higher mortality for DTP-vaccinated compared with DTP-unvaccinated children (5). However, in spite of previous discussions of survival bias, the SAGE-meta-analysis included three studies from Bangladesh, PNG and Burkina Faso with a high bias index and major survival bias (Figure 1). Had these studies been excluded, the result would have been the same as the one we presented in Figure 2.

Public health implications and future perspectives

So far, all properly collected data suggest that being vaccinated with DTP-containing vaccines is associated with increased mortality compared with not being DTP-vaccinated. This deleterious effect is particularly marked for girls (4). It has occasionally been suggested, e.g. by the Task Force, that the increased female-male MRR could be due to reduced mortality among DTP-vaccinated males. There is no support for that interpretation. In the natural experiment studies of the introduction of DTP-vaccinated males had 71% (-1-193%) higher mortality than DTP-unvaccinated males (Chapter 17). Thus, the initial danger signal has been supported by many studies over the past 20 years, and testing better policies are urgently needed.

The 38% (-8-108%) increase in mortality, which was not statistically significant, has not been interpreted as sufficiently important for action. While WHO is planning large studies to further examine whether there are NSEs of BCG and MV, no study has been planned for DTP. However, a 38% mortality increase after DTP is large and significantly different from what should be expected for a vaccine, which protects against diphtheria, tetanus and pertussis.

The evidence now is: First, that DTP-vaccination contrary to expectation is associated with higher mortality than not having received DTP; no bias can explain this counter-intuitive trend. Second, the natural experiment studies document at least a two-fold increase in mortality after DTP-vaccination (Chapter 17). Third, DTP is associated with an “unnatural” higher female than male mortality. Fourth, DTP explained why high-titre MV was associated with higher female mortality and had to be withdrawn by WHO (Chapter 7).

Triangulation of these data would dictate that we should do far more to minimize the negative effects of DTP. For example, we have examined

whether co-administration of DTP with BCG or possibly other live vaccines and whether vaccination with a live vaccine shortly after the last dose of DTP can remove or reduce the deleterious effects of DTP (2).

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17. Natural experiments: the introduction of DTP and OPV in Guinea-Bissau in the 1980s

Background: Catch-22

The scientific rigor in much of medical culture has become so strict that it will virtually only accept evidence, which comes from randomised clinical trials (RCTs). Observational studies are dismissed because they could be biased. That creates lots of problems for research into the non-specific effects (NSEs) of vaccines.

Before being introduced, vaccines were not tested in RCTs, with overall health as the outcome. Once introduced and recommended by WHO, it becomes unethical to test them in RCTs as this would imply that some participants did not get the assumed beneficial intervention. Hence, this has created the unfortunate situation that the NSEs of some vaccines cannot be tested in RCTs and if observational studies suggest potential problems with vaccines, they are likely to be dismissed as biases.

For example, following the WHO review of the NSEs of DTP which had found that DTP was associated with 38% (-8-108) higher mortality, WHO's research implementation committee (IVIR-AC) decided that DTP could not be studied further, so nothing has been done to get more certain knowledge about the effect of DTP, the most commonly used vaccine in the Expanded Programme on Immunizations (EPI).

There are some alternatives. First, in some situations vaccinations are allocated without the usual biases. Such "**natural experiments**" may provide estimates of effect, which are as good as RCTs. Second, if an intervention consistently, using different analytical approaches, is associated with negative effects in spite of beneficial effects being expected, **triangulation** of the data will predict that the result will also be negative the next time. Hence, the best interpretation will be that the vaccine has negative effects.

Diphtheria-tetanus-pertussis vaccine (DTP) and oral polio vaccine (OPV) were introduced in Bandim in June 1981 (1,2) and in the different rural areas followed by the mobile team in 1984(3).

Results

Bandim: In the 1980s, we conducted three-monthly nutritional surveys for children under 3 years of age to identify malnourished children. We invited mothers in each of the eight subdistricts to come to the subdistrict's meeting place with their child on a specific day. The indicated morning, the children were weighed and the weight was noted on the growth card of the child (held by the mother) and on the individual registration card held by the BHP (Figure 1).

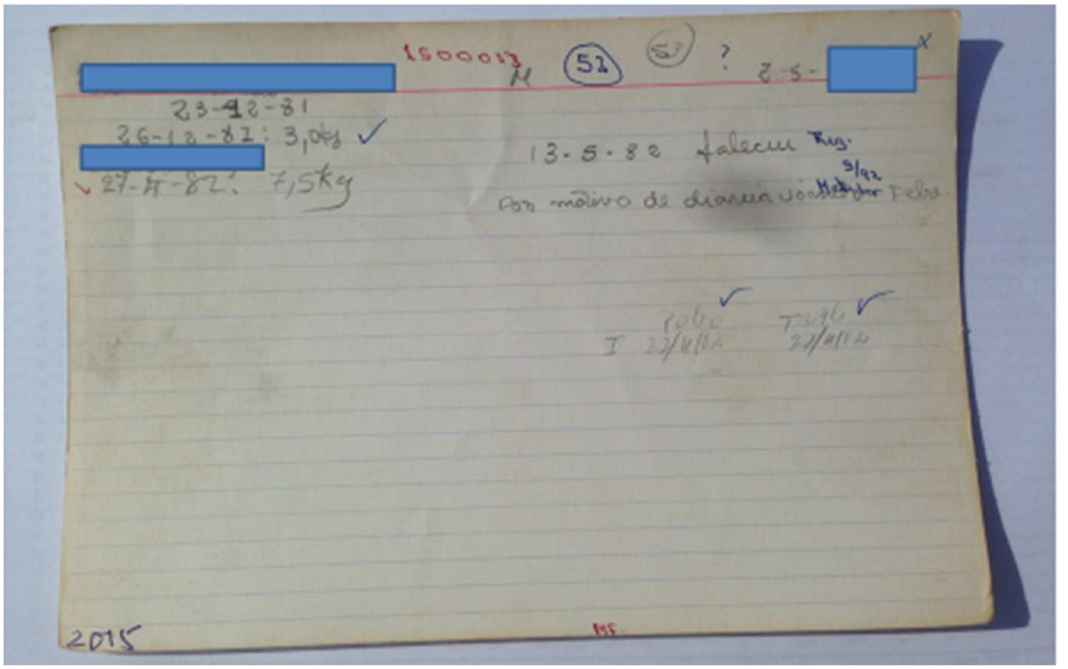

Figure 1. Vaccination card of child from Bandim.

When vaccines became available, a nurse from the local health centre administered the vaccines, which were also noted on both the growth card and the registration card. The community weighing sessions were held every third months and the age limit for DTP at the time was 3 months of age. Hence, depending on their birthday and the timing of weighing sessions, some children came just after 3 months of age and received DTP and OPV and others came at 2.5 months and were not vaccinated. The latter children would only come back at 5.5 months of age and be vaccinated at that age. So within the age interval from 3 to 6 months of age, this was a natural experiment where some children were vaccinated early with DTP

and OPV and others remained unvaccinated up to nearly 6 months of age. This made it possible to compare the mortality of DTP+OPV vaccinated children with not yet vaccinated children between 3 and 6 months of age. For example, the child in Figure 1 was unvaccinated from 3 months until 4 months of age when it received both DTP and OPV and then it died 2 weeks later of diarrhoea.

In this natural experiment, we found that having received DTP+OPV was associated with 5-fold higher mortality than not being yet vaccinated (Table 1). In some periods, there had been no OPV and for children who had received DTP-only, mortality was 10-fold increased (1).

Mortality rate (deaths/person-years)		Relative mortality (95 CI)
Unvaccinated	DTP vaccinated	
4,5% (5/111.4)	17.4 (11/63.1)	5.00 (1.53-16.3)
	DTP-only 35.2 (5/14.2)	10.0 (2.61-38.6)
	DTP+OPV 12.3 (6/48.9)	3.52 (0.96-12.9)

Table 1. Mortality rate and relative mortality (RR) from 3 to 6 months of age in relation to vaccination status. Bandim 1981-1984.

When DTP and OPV were introduced in June 1981, there were 702 children aged 6-35 months of age to whom we also offered vaccination if they attended. However, if they were malnourished or sick they were not vaccinated; the children who were not vaccinated had significantly poorer nutritional status than those who were vaccinated. Those who attended and were not vaccinated should therefore have had higher mortality than those vaccinated. Nonetheless, the vaccinated children had two-fold higher mortality than the children who attended but were not vaccinated (2).

Rural areas: BHP mobile team visited 20 villages, in four regions in the interior every six months. Since the beginning in 1980, we provided MV to older children but DTP and OPV vaccines only became available from 1984 to children aged 2-8 months; if older they would have received MV. The community was only visited one day, so some children remained unvaccinated to the next visit because they had travelled. Other children had poorer nutritional status or were sick on the day of visit and therefore not vaccinated. Again, it was to be expected that the unvaccinated children had higher mortality. However, as can be seen Figure 2, the DTP vaccinated children had two-fold higher mortality over the next 6 months, until the next visit (3).

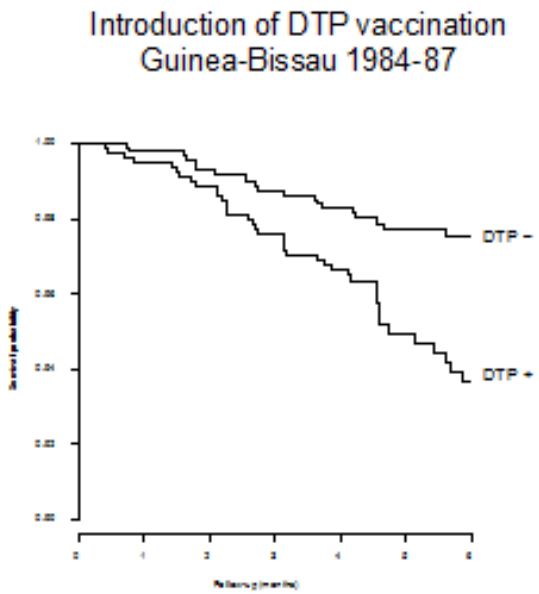

Figure 2. Survival curves for 2-8 months old DTP vaccinated children (DTP+) and children who did not get DTP (DTP-). Twenty villages in rural Guinea-Bissau.

Combining these three natural experiments (Figure 3), DTP (+/- OPV) vaccinations were associated with 114% (42-223%) higher mortality than not being vaccinated. This negative effect was strongest for girls, the increase being 160% (57-332%) for girls and 71% (-1-193%) for boys (2).

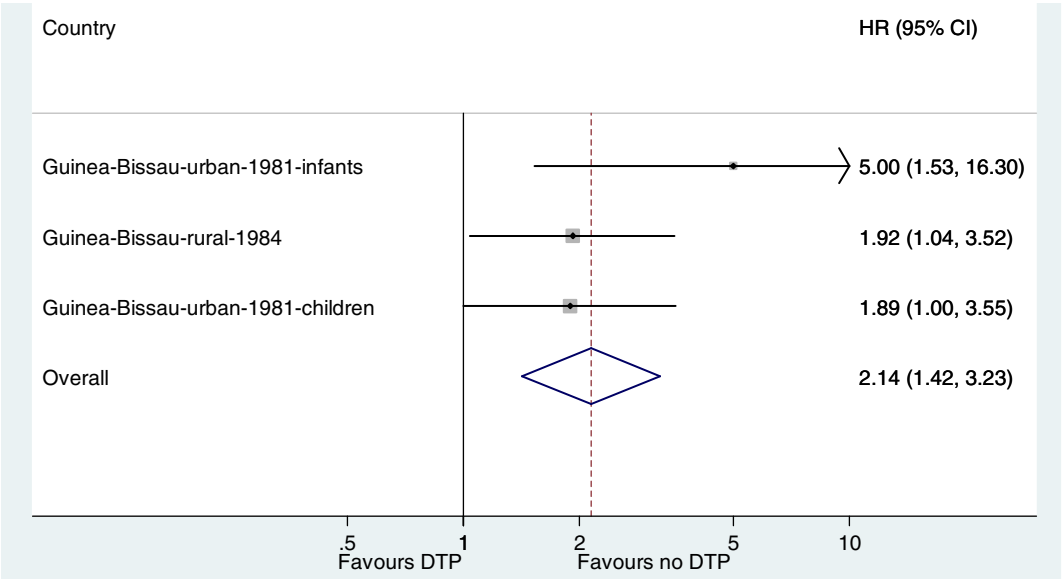

Figure 3. Meta-analysis of three studies of the introduction of DTP.

Public health implications and further perspectives

There is no bias, which can explain the unexpected results in these studies. In addition, no study with identification of vaccination status and prospective follow-up has documented that DTP vaccinated children had better survival (Chapter 16).

Since DTP is the most commonly used vaccine in the EPI and the coverage for the third dose, DTP3, is used to monitor the performance of the national EPIs, these data should clearly be a cause for concern. Are they? Apparently not. There is no attempt from WHO to find alternative vaccination strategies, which could remove or reduce the DTP-associated problems. Our data suggest that co-administration of OPV has helped reduce the negative effects of DTP (1-3) and we have found that co-administration of BCG and DTP reduce the negative effect, particularly for girls (4). Hence, it is likely that studies focusing on using other vaccines to minimise the negative effect of DTP could go a long way to reduce the problem. However, what is happening now is that global health vaccinology will remove OPV and that the booster dose of DTP is being further encouraged as part of the 2nd year of life platform for vaccinations. Hence, the situation is likely to get worse.

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18. Real-life effects of Pentavalent vaccines

Background and assumptions

Between 2001 and 2014, the GAVI alliance supported the introduction of the pentavalent vaccines in low- and middle income countries. The funding from the GAVI alliance supported purchasing the vaccine as well as grants to support the implementation of the vaccination programme. In the 73 supported countries, the trivalent diphtheria-tetanus-pertussis vaccine (DTP) has now been replaced by the pentavalent DTP-HiB-HBV vaccine (Penta). Hence, the 70 million children vaccinated through the Expanded Programme on Immunization (EPI) in these countries annually, are now not only protected against diphtheria, tetanus and pertussis but also against *H. influenzae* type B and hepatitis B. The GAVI alliance estimates that the conferred protection against these additional pathogens along with the increase in coverage caused by their investment in 2011-20 will avert 5.6 million future deaths.

Real-life data

In 2008, when the pentavalent vaccine was introduced in Guinea-Bissau, we did indeed observe an increased coverage of the DTP-containing vaccines. The year before the Penta introduction the coverage of the third dose of DTP was 73%, while the Penta-3 coverage was 81% in the year after the introduction. The increase in coverage was due to more children beginning their DTP/Penta vaccination series and to a lower dropout rate: before the Penta introduction, 94% received DTP-1 and 27% of these did not receive DTP-3, after Penta introduction, 98% received Penta-1 while only 19% did not receive Penta-3. We were also able to document that there had been improved timeliness (1). Hence, judged by the evaluation parameters commonly used to assess the performance of the vaccination programme (the coverage of Penta-3), the introduction of Penta and the increased funding for outreach was a great success.

Estimated benefits modelled based on vaccination coverage and fractions of causes of death attributable to specific pathogens, do however not reflect what happens in real life. Following the 2008-introduction, we could show that while the coverage and timeliness of the DTP-containing vaccines had improved, at the same time the coverage of measles vaccine (MV) had declined and the MV timeliness had deteriorated (1). Numerous studies have indicated that MV has beneficial effects on survival (Chapters 6, 8), while DTP-vaccine has negative effects for the survival (Chapters 16, 17). If Penta, containing DTP, had the same negative effects as DTP, the introduction of Penta could be associate with increased mortality.

In the studies where we have investigated the effects of Penta on child mortality, the effects of Penta are similar to those observed for DTP: higher female than male mortality after vaccination with Penta, and higher mortality when Penta is given with or after MV.

We have assessed the sex-differential mortality patterns in three different populations. In Guinea-Bissau, we assessed the sex-differences in mortality after routine vaccinations at the health centres in the BHP urban study area. We found that while mortality among girls was 73% (11-170%) higher than mortality among boys following Penta vaccinations, mortality in girls was 62% (-19-88%) lower after measles and yellow fever vaccination (Figure 1) (2).

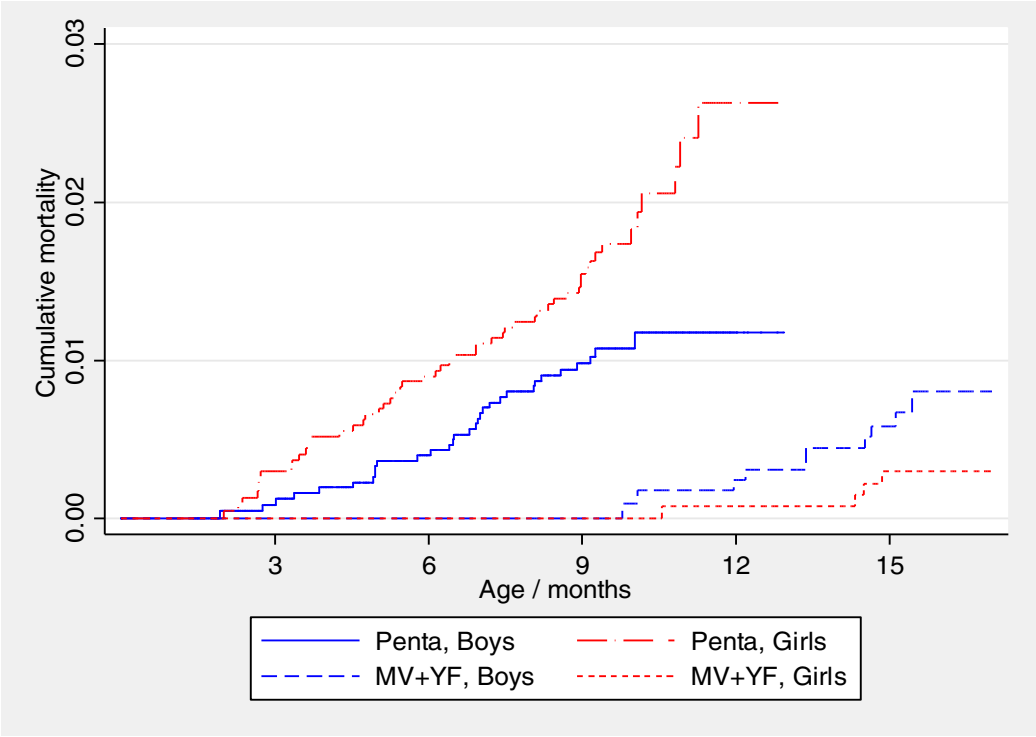

Figure 1: Sex-differential child mortality after vaccinations in Guinea-Bissau.

Similarly, in Bangladesh, at the Chakaria HDSS, among children followed between 6 weeks and 9 months of age the mortality of girls was almost 10 times higher than the mortality of boys when Penta was the most recent administered vaccination, while the mortality in girls was half the mortality of boys when BCG vaccine was the most recent vaccination (3). Finally, in Burkina-Faso, we conducted a case-control study in the Nouna HDSS and found support for sex-differential effects of Penta: Among children in the age group 2–8 months, Penta was associated with lower mortality in boys, but not in girls (4).

In Guinea-Bissau, receiving Penta with MV was associated with higher mortality than receiving MV after Penta, just as has been observed for DTP co-administered with MV (5). Penta after MV is also associated with higher mortality in both Guinea-Bissau and Ghana (Chapter 21).

Public health implications and future perspectives

The problem of increased female mortality after vaccination with DTP-containing vaccines has not been solved by substituting the trivalent DTP vaccine with the pentavalent DTP-HiB-HBV. Before the roll-out of the vaccination programme, boys and girls had similar mortality levels after the neonatal period. They no longer have that. The introduction of Penta is thus another example that we may do more harm than good by merely considering the pathogens, which we protect against, rather than the effect of the vaccine on overall health.

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19. Non-live vaccines have negative non-specific effects: RTS,S and other non-live vaccines

Background and assumptions

When new vaccines are developed and introduced, non-live vaccines are generally preferred over live vaccines, as they do not have the potential to cause disease in immunocompromised individuals. However, while the live vaccines examined so far have been associated with beneficial effects (Chapters 6-15), the non-live DTP-containing vaccines have negative NSEs, which are stronger for girls than boys (Chapters 16, 18). This made us speculate, whether the pattern observed for DTP and Penta applies to other non-live vaccines.

Real-life data

We made two types of analyses: 1) we compared the mortality of vaccinated and unvaccinated children and 2) we calculated the female/male mortality ratio among vaccinated children (Table).

Inactivated polio vaccine (IPV): Between 1985 and 1995, randomised trials in Bissau testing early measles vaccination used non-live IPV as a control vaccine. In these trials, mortality among girls was 52% (2-128%) higher for girls than for boys among recipients of IPV (analysis type 2) until they received measles vaccine (1).

Hepatitis B vaccine (HBV): In one of the trials, children recruited during one year received HBV from 7.5 months of age. In the HBV-exposed cohort, mortality was higher between 7½ and 12 months of age than between 1½ and 7½ months of age, while the mortality in the two age groups did not differ for HBV-unexposed cohorts. Compared with the HBV-unexposed cohorts, mortality in HBV-exposed cohort was almost two times higher (analysis type 1), and among HBV-vaccinated children the mortality of girls was more than twice the mortality of boys (analysis type 2) (2).

RTS,S malaria vaccine: More recently, GSK has developed and tested a new malaria vaccine, the RTS,S vaccine. When the results of a multicentre trial were published in 2015, the vaccine showed some protection against malaria, but no effect was observed on mortality. In fact, the risk of dying from

any cause was 24% (-3-58%) higher among the vaccinated children (analysis type 1) (3). The results were not reported by sex (3). When the data by sex was released, our prediction held: RTS,S was indeed associated with increased mortality in girls: girls randomised to RTS,S had 91% (30-179%) higher mortality than controls. Among boys there was no excess mortality and consequently the female mortality among children in the RTS,S group was 33% (2-74%) higher than the male mortality (analysis type 2) (4).

Vaccine	**Studies**	**Vaccinated/ unvaccinated RR**	**Female/male RR among vaccinated children**
Diphtheria-tetanus-pertussis vaccine	15 observational studies	2.00 (1.50-2.67) (Chapter 16)	1.53 (1.21-1.93) (Chapter 16)
Pentavalent vaccine	Observational study	NA	1.73 (1.11-2.70) (Chapter 18)
Inactivated polio vaccine	Three RCTs with IPV as control vaccine	NA	1.52 (1.02-2.28) (1)
Hepatitis B vaccine	Natural experiment	1.81 (1.19-2.75) (2)	2.20 (1.07-4.54) (2)
RTS,S malaria vaccine	RCT in 2 distinct age-groups	1.24 (0.97-1.58) (3)	1.33 (1.02-1.74) (4)
H1N1 influenza vaccine	Natural experiment: Campaign	1.86 (1.02-3.42) (5)	2.68 (0.44-16.4) (5)

Table. Relative risks (RR) of overall and female mortality associated with non-live vaccines.

NA: No data available on unvaccinated. Abbreviations: RCT, randomised controlled trials.

H1N1 influenza vaccine: Other non-live vaccines have been distributed in campaigns. In 2010, towards the end of the H1N1 influenza pandemic, Guinea-Bissau received and distributed H1N1-influenza vaccines to children

aged 6 months-5 years. In an ecological after versus before study, this vaccine seems to have increased mortality (analysis type 1) and to be associated with a higher female/male mortality (analysis type 2) (Table) (5).

Public health implications

The RTS,S vaccine is an example of a vaccine, which was about to be rolled out over large parts of Africa without any trials having demonstrated that the specific protective effect against malaria translated into a beneficial effect on general health. In fact, RTS,S is currently being further tested in Ghana, Kenya and Malawi, in spite of data showing that there may be increased mortality after RTS,S vaccination. The only way to ensure that our interventions have the intended effects on overall health is to ensure that the data to assess their effect on overall health is collected. Here, changes in the female to male mortality ratio may lend important clues to whether there are negative NSEs of new non-live vaccines.

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20. #MeToo-DTP3: Why are we using DTP3 to monitor the performance of the vaccination programs?

Background

The third dose of diphtheria-tetanus-pertussis (DTP3)-containing vaccine is used to monitor the performance of the national Expanded Programs of Immunization (EPI). As a result, the DTP3 coverage has increased more than the coverage for measles vaccination (MV). For example, when EPI in Guinea-Bissau received support from GAVI in 2007 for the introduction of the DTP-containing pentavalent vaccine (Penta), the Penta coverage improved considerably as expected, but the coverage for measles vaccine (MV) fell from 71% to 66% (1). Since we have previously found that MV has beneficial non-specific effects (NSEs) for child survival but DTP is associated with higher female than male mortality, the emphasis on DTP vaccinations is likely to be problematic and may contribute to increased child mortality.

We have found for all the live vaccines that revaccinations are associated with an added benefit, which cannot be explained by improved prevention against the vaccine-disease. In other words, revaccination enhances the beneficial NSEs (2). It has not been examined whether revaccination with non-live vaccines enhance the deleterious NSEs or remove them. We therefore reviewed whether DTP3 is associated with higher female-than-male mortality than DTP1 and DTP2 and what happens when DTP is administered after MV (3).

Real-life data

The WHO review of the NSEs of vaccines had 16 DTP-studies with information on mortality and we have found an additional seven new studies on DTP-containing vaccines (4). Some studies included only children with DTP1 or DTP2 or only DTP3, and many did not report data specifically by sex. In the end, we had eight studies with information for both DTP1 and DTP3. The female/male (F/M) mortality rate ratio (MRR) was 1.16 (0.89-1.53) after DTP1, 1.21 (0.91-1.60) after DTP2 and 1.66 (1.32-2.09) after DTP3 (Table 1). Hence, in a meta-analysis of the eight studies which had information on both DTP1 and DTP3, the F/M MRR was 50% (10-105%) higher after DTP3

Table 1. Female-male mortality rate ratios (MRR) by number of doses of DTP

Study	Design	F/M MRR for DTP1	F/M MRR for DTP2	F/M MRR for DTP3	Relative F/M for DTP3 vs DTP1
Guinea-Bissau, 1990-1996	Hospital case fatality; monitoring vaccination status at admission; N=461; age 1½-8 months	1.13 (0.53-2.39)	1.40 (0.61-3.22)	3.11 (1.31-8.45)	2.75 (0.83-9.12)
Guinea-Bissau, 2001-2006	Hospital case fatality; monitoring vaccination status at admission; N=2944; age 1½-8 months	1.08 (0.80-1.45)	0.96 (0.69-1.36)	1.59 (1.19-2.12)	1.47 (0.97-2.23)
Guinea-Bissau; 2008-2011	Health centre vaccinations and demographic follow-up; N=17312 vaccinations; age 1½-8 months	1.23 (0.48-3.19)	3.74 (1.04-13.41)	1.58 (0.56-4.43)	1.28 (0.32-5.22)
Senegal I, 1989-1997	Vaccine trial providing all vaccinations; N=9,683; age 3-8 months; DTP1 given with BCG	0.64 (0.38-1.05)	1.21 (0.69-2.11)	1.79 (0.91-3.52)	2.80 (1.20-6.52)
Senegal II, 1997-2000	Routine demographic surveillance; vaccination cards at home visits; N=4,102; age day 2-24 months	1.67 (0.97-2.87)	1.19 (0.62-2.29)	1.24 (0.61-2.58)	0.74 (0.30-1.83)
Malawi, 1995-1997	Routine demographic surveillance; vaccination cards at home visits and health centre records; N=751; age day 8-8 months	1.16 (0.63-2.11)	1.04 (0.36-2.97)	1.17 (0.32-4.38)	0.94 (0.19-4.58)
Bangladesh; 1995	Hospital treatment study with follow-up; DTP with vitamin A or placebo; N=200; age 1½-8 months	2.60 (1.03-6.52)	8.00 (0.91-70.04)	2.12 (0.31 – 14.69)	0.82 (0.10-6.92)
Bangladesh; 2011-2016	Routine demographic surveillance; vaccination cards at home visits; N=5,829; 1½-8 months	2.22 (0.20-24.43)	2.15 (0.20-23.76)	9.62 (1.21-76.60)	4.33 (0.18-103.5)
Combined estimate		1.16 (0.89-1.53)	1.21 (0.91-1.60)	1.66 (1.32-2.09)	1.50 (1.10-2.05)

than after DTP1 (3). Trends were similar in the two studies which used pentavalent vaccine (56% (-57-460%) higher) and the six studies using DTP (50% (9-107%) higher). DTP is not associated with reduced male mortality (Chapters 16, 17) and the increased F/M MRR is therefore reflecting a real increase in female mortality.

Among the 23 studies of DTP1-DTP3, 11 studies had no information on F/M mortality rates among measles vaccinated children, two studies had only information on DTP3 , and in one study most measles vaccinated children received MV with DTP or DTP after MV. The remaining nine studies had information on the F/M MRR for both DTP1-3 and MV vaccinated children. In all nine studies the F/M MRR declined and the MRR was 37% (4-58%) lower for females than for males after MV. Hence, this was a 3-fold reduction in the F/M MRR after MV compared with the F/M MRR after DTP1-3 vaccinations (3).

The number of studies, which had information on F/M mortality rates among children, who had received DTP after MV, is limited. In most cases, DTP after MV would be DTP3 but this was usually not reported in the papers; in a study from India, DTP after MV was the booster dose of DTP. In the meta-analysis of the seven available studies, the females had 65% (25-118%) higher mortality than males for DTP after MV (3).

Public health implications and future perspectives

All available data suggest that the negative NSEs of DTP-containing vaccines on child survival become more pronounced with each additional dose. In other words, revaccination with DTP-containing vaccines is making the deleterious effects of DTP worse for females. This strengthens the argument that DTP has sex-differential effects, which cannot be explained merely with reference to sex-differential treatment. The trend was similar for DTP and Penta so future studies should also explore whether repeated doses of other non-live vaccines are linked to enhanced deleterious effects. If non-live vaccines have increasingly negative effects with increasing number of doses, this will be very important because the vaccine community is working to introduce more non-live vaccines in the 2nd year of life, e.g. booster DTP and four doses of RTS,S malaria vaccine. Even if MV may reduce the increased F/M MRR this will be reversed again if another dose of DTP is given after MV.

In recent decades, OPV campaigns have reduced mortality rates very considerably (Chapter 11, 13) and this may also have neutralized female-male difference in mortality. It is clear in Table 1 that the increase in sex-differential effect was worse in the two older studies from Guinea-Bissau (1990-96) and Senegal (1989-97) from before the OPV campaigns were introduced. When OPV campaigns and routine use of OPV are going to be stopped in 2022, the sex-differential effects of the non-live vaccines may become more pronounced again.

Given that DTP3 is associated with increased female mortality, it is noteworthy that the international vaccine community primarily uses DTP3 to monitor the performance of the national EPIs. Using a specific vaccine as monitoring instrument will inevitably promote the coverage for that vaccine (1). Hence, using DTP3 coverage as the key monitoring index is likely to have contributed to increased female mortality. Why are we not using an index, which is positively associated with better child survival? This could be the coverage of BCG or MV, or even better both. Emphasizing a certain age of vaccination as key targets would contribute to promoting earlier vaccination with the live vaccines having the strongest beneficial NSEs.

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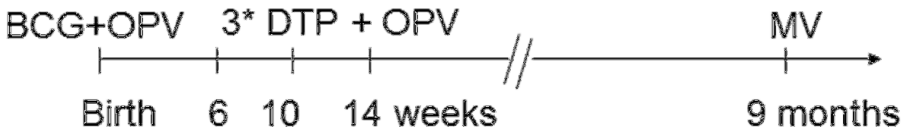
21. Out-of-sequence vaccinations: A warning for the 2nd year of life platform for vaccination

Background and assumptions

At the core of the vaccination programme are the three doses of a diphtheria-tetanus-pertussis (DTP)-containing vaccine at 6, 10 and 14 weeks and a measles vaccine (MV) at 9 months of age. The sequence in which vaccines are given receives little focus in the present implementation and evaluation of the vaccination programme (Figure 1).



BCG: Bacillus Calmette Guerin Vaccine, OPV: Oral Polio Vaccine;
DTP: Diphtheria-Tetanus-Pertussis Vaccine containing vaccine;
MV: Measles Vaccine

Figure 1. The core of the vaccination programme**.**

The recommended timing and schedule of the vaccines may however differ markedly from the way the vaccination schedule is implemented, especially in rural areas where access to vaccines is limited. In rural Guinea-Bissau, 54% of BCG-vaccinated children received BCG with or after the first dose of DTP (i.e., BCG and DTP out-of-sequence), while 28% of measles-vaccinated children received DTP and MV out-of-sequence (DTP with or after MV)(1). A contact with the health system is an opportunity to catch up on missing doses of vaccines and it is assumed that providing a missing vaccine dose will lead to better health.

Real-life data

In the WHO commissioned review published in 2016, mortality was more than 2 times higher if DTP was given after MV compared with having MV as the most recent vaccine (2). This estimate was based on a meta-analysis of

three studies from Guinea-Bissau, Senegal and India. Subsequently, three further studies have supported this finding.

We recently re-analysed the data from the 100 rural village clusters under surveillance in Guinea-Bissau between 1990-96. These data were published in the BMJ in 2000 as the first study demonstrating the contrast between the beneficial non-specific effects (NSEs) of MV and BCG and the detrimental NSEs of DTP. While the original analysis did not take into account the sequence of vaccines, the present reanalysis was based on what we had later learned. We found that among measles vaccinated children, out-of-sequence vaccinations were associated with approximately two times higher mortality than in-sequence vaccinations (3).

In 2008, DTP was replaced by the Pentavalent DTP-*H. influenzae* type b-Hepatitis B vaccine. We recently showed that providing missing doses of pentavalent vaccine to measles vaccinated children tended to increase child mortality (4). Importantly, measles-vaccinated children who did not receive missing doses of pentavalent vaccines did not have higher mortality than children who had received all three doses of Penta before receiving MV.

Finally, a study from Navrongo HDSS in Ghana, using data from 1996 to 2012 during which both DTP and Penta have been given, also indicates that both DTP-containing vaccines, given with or after MV, are associated with increased mortality. Thus, when we update the meta-analysis with the results of the new studies for Guinea-Bissau and Ghana, the conclusion is unaltered: Among children receiving DTP-containing vaccine after MV, mortality is double the mortality among children vaccinated with DTP and MV in sequence (Figure 2).

It is also worth noting that the apparent negative effect of the WHO-recommended high-titre MV (HTMV) was explained by children receiving inactivated polio vaccine/DTP after MV, rather than by HTMV *per se* (Chapter 7).

In addition to the effect on mortality, receiving DTP after MV may also increase the risk of hospital admissions. Among children with assessed vaccination status when they presented for outpatient consultation at the pediatric ward at the national hospital in Bissau, children aged 9–17 months who had received out-of-sequence DTP and MV had more than 50% higher risk of being admitted compared with children vaccinated in sequence (5). Increased admission rates has also been observed in high-income countries for children who had received an inactivated vaccine after a live MV.

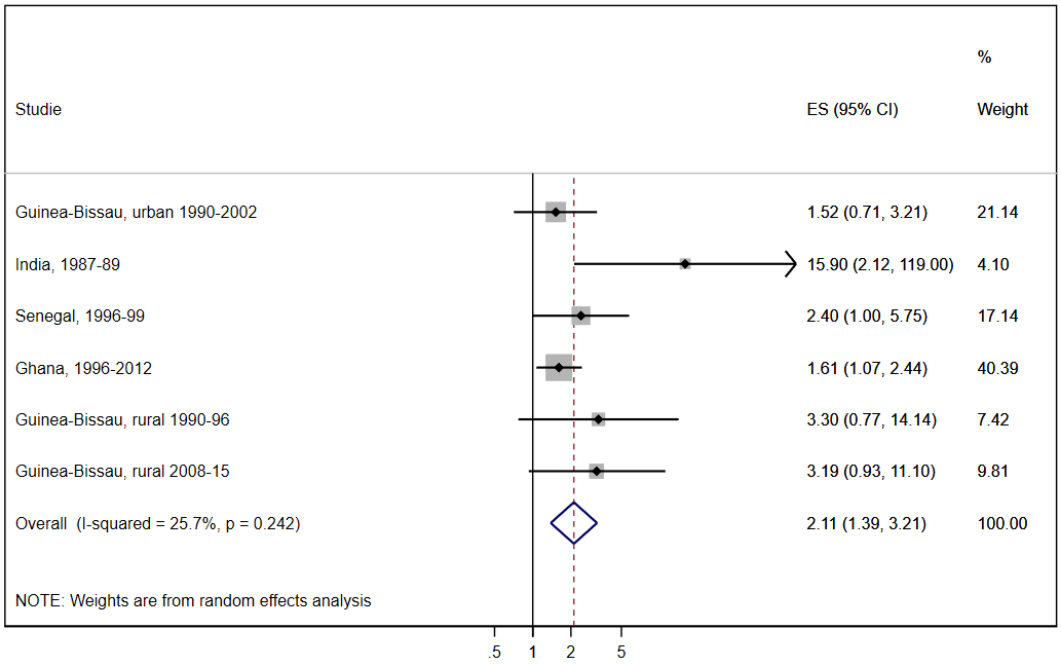


Figure 2. Meta-analysis of studies assessing the effect of giving DTP containing vaccines after measles vaccine.

While out-of-sequence DTP and MV are associated with higher mortality, out-of-sequence BCG and DTP are associated with better survival (provided that the neonate has survived to the age of receiving the first dose of DTP-containing vaccine). A meta-analysis of three studies from Bangladesh, India and Senegal comparing BCG+DTP1 versus DTP1 (after BCG) found a 48% (20-66%) reduction in mortality rate (2).

Public health implications and future perspectives

While the proportion of children receiving vaccines out-of-sequence is declining in many settings, the importance of considering the effects of providing non-live vaccines remains urgent. With the introduction of new, non-live vaccines in the vaccination programme (Meningitis A, malaria vaccine) and booster doses of the DTP-containing vaccines as well as a booster dose of MV, WHO is planning a second year of life platform, where non-live vaccines will likely be given after live vaccines. Thus, non-live vaccines given after MV may also become a concern in areas where the vaccination schedule is followed. We need to assess the effect carefully for both boys and girls to ensure that the protection conveyed by giving the extra antigen after MV is not counterbalanced by a negative effect on child health.

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22. Testing non-specific effects of vaccines in high-income countries

Background and assumptions

In low-income countries, non-live vaccines appears to have detrimental non-specific effects (NSEs) and live vaccines appears to have beneficial NSEs when the vaccine is most recently received (see the previous chapters). Therefore, a relevant question was if we could find similar associations in Denmark.

Most of the studies of NSEs from low-income countries have studied child mortality. A main cause of child mortality in low-income countries are infectious diseases and it is likely that vaccines can modify the immune system's ability to handle infections. In Denmark, child mortality is low and infections are rarely the cause of death. Therefore, hospitalisations due to infectious diseases have been the outcome in the Danish NSEs studies.

The vaccines used in Denmark are not identical to the vaccines used in low-income countries, but they contain some of the same components. The main non-live vaccine in the Danish Childhood vaccination programme is DTaP-IPV-Hib (vaccine targeting diphtheria, tetanus, pertussis (acellular component), polio, and *H. influenzae* type b) which is recommended in a three doses schedule at 3, 5, and 12 months of age. Since 2007, the non-live pneumococcal conjugate vaccine (PCV) have been recommended together with DTaP-IPV-Hib. The main live vaccine is MMR (vaccine targeting measles, mumps and rubella), which is recommended in a two doses schedule at 15 months of age and 4 years of age. Until 2001, the live oral polio vaccine (OPV) was recommended in a three-dose schedule at 2, 3, and 4 years of age.

The main assumption examined in the Danish data is: Having a live vaccine as the most recent vaccine is associated a lower risk of hospitalisation for infection.

Real-life data

In Denmark, real life data is continuously collected and included in national registers. For instance, the general practitioners collect information when

they see a patient, including information on the free-of-charge childhood vaccines. The data is mainly collected for administrative purposes but it is also a valuable source for researchers. It is possible to link the information from all the different registers using a unique identification number (called CPR#) given to all Danish children at birth and included in all the national registers. Figure 1 illustrates the type of information we collected.

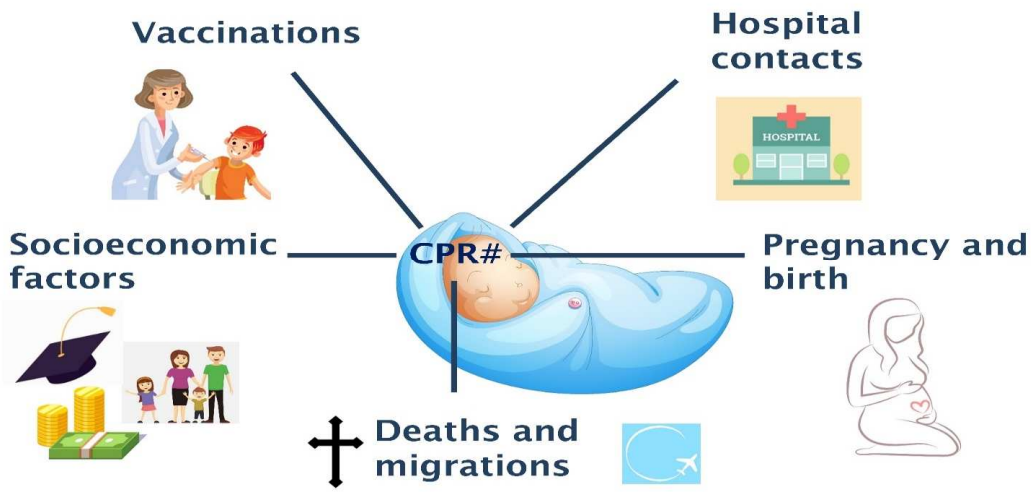

Figure 1. Illustration of the type of information from Danish national registers that is linkable using the unique identification (CPR#) assigned to all Danish residents at birth.

We identified hospitalisations for infections based on the diagnosis codes registered in the Danish National Patient Register. The main exposure in the studies was “the most recent vaccine”. For instance, on the date a child receives MMR, the vaccination status changes from the group with DTaP-IPV-Hib as the most recent vaccine to the group with MMR as the most recent vaccine. As vaccines are recommended at specific ages, vaccination status depends on age. Age is also an important risk factor for hospitalisation for infections. Therefore, we used a statistical model that ensured that we would always compare children of the same age but with different vaccines as the most recent (e.g. MMR vs. DTaP-IPV-Hib). Also, a lot of other factors could influence the association between most recent vaccine and hospitalisations for infections and we adjusted the analyses for a lot of child and family characteristics.

The main results of the Danish studies are displayed in Figure 2. The assumption that having a live vaccine as the most recent was associated with a lower risk of hospitalisations for infections was confirmed for both MMR and OPV. Similar results have subsequently been found in the Netherlands and USA.

The Danish studies also showed that the children who received MMR at the same time as DTaP-IPV-Hib had a higher risk of being hospitalised with an infection compared with the children who received MMR alone. Furthermore, we found that those who received an additional dose of MMR had a lower risk of hospitalisation for infection compared with the children who had only received one dose of MMR.

Interestingly, the associations was strongest for hospitalisations for lower respiratory infections and the hospitalisations that lasted two days or longer (probably the most severe infections). The studies from low-income countries have often found that the magnitude of NSEs differ for girls and boys. We did not find such a pattern in Denmark.

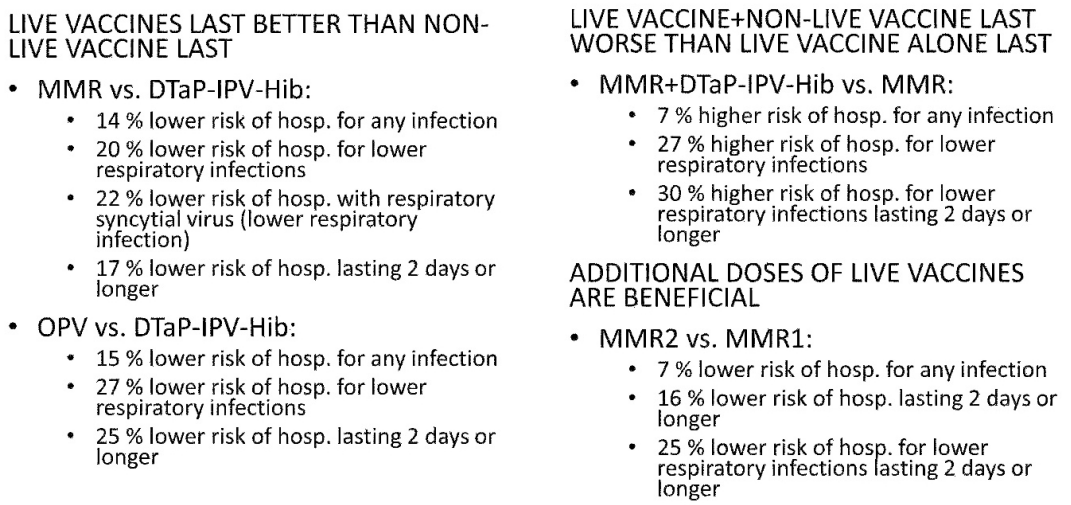

Figure 2. Overview of the main results from observational register-based Danish studies.

Abbreviations: DTaP-IPV-Hib, non-live vaccine against diphtheria, tetanus, pertussis (acellular), polio, and Haemophilus influenzae type b; MMR, live vaccine against measles, mumps, and rubella; OPV, live oral polio vaccine.

Public health implications and future perspectives

The studies from Denmark confirmed that MMR vaccination is safe in relation to unrelated infections. This might help to support the up-take of MMR vaccination among children. It is important to examine if live vaccines could reduce the occurrence of other types of diseases like allergic disease and autoimmune diseases, to get a better understanding of the NSEs of vaccines. In the long run, it would be important to test a "live vaccine last" policy in randomised trials in high income countries to get a better indication of the optimal vaccination schedule.

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23. The impact of vitamin A supplementation on child survival: Interactions with vaccines, sex and prior vitamin A

Background and assumptions

Vitamin A is important for numerous physiological functions in humans including for the immune system. Vitamin A is stored in the liver and is, as long as stores are not depleted, released to the bloodstream according to physiological needs. WHO recommends universal blanket high-dose vitamin A supplementation (VAS) to children between 6 months and 5 years of age every 4-6 months in areas with vitamin A deficiency. The recommendation was formulated in the early 1990's and was based on a meta-analysis of eight trials, which showed that VAS reduced child mortality by 23%. Subsequently, research on VAS has mainly focused on ways to optimise uptake and the only evaluation undertaken has been assessment of coverage.

Real-life data

Recent data indicates that blanket VAS may no longer reduce mortality by the estimated 23%. In a Cochrane meta-analysis from 2017, the estimated benefit of VAS was reduced to 12% (7-17%), but the conclusion remained "In populations with documented vitamin A deficiency, it would be unethical to conduct placebo-controlled trials". However, as shown in Figure 1, the effect of VAS seemed to change over time; the benefits observed in the late 1980 and early 1990s were not evident in the more recent trials.

Already in 1993, it was noted that the effects in the eight trials varied, two trials showed no benefit. Also it was pointed out that the variation in effect was not predicted by the level of vitamin A deficiency in the population. Though it did not receive much attention at the time, this was a first indication that the effect of VAS goes beyond replenishing vitamin A stores: VAS prevents and treats vitamin A deficiency, but it also has non-specific effects.

Based on the observations that vaccines have non-specific effects (Chapter 26) and that vitamin A is immunomodulatory and amplifies the specific antibody response to vaccines (1), we formulated the hypothesis that VAS am-

plifies the non-specific effects of vaccines. In other words, the effect depends on vaccines given at the time of supplementation (2,3). Observational studies of VAS campaigns in Guinea-Bissau indeed supported that VAS given with or close to measles vaccine is beneficial, while VAS with DTP-vaccine is associated with higher mortality. We have also found support for this in a reanalysis of one of the original eight VAS trials from Ghana, the only one having information on vaccination status.

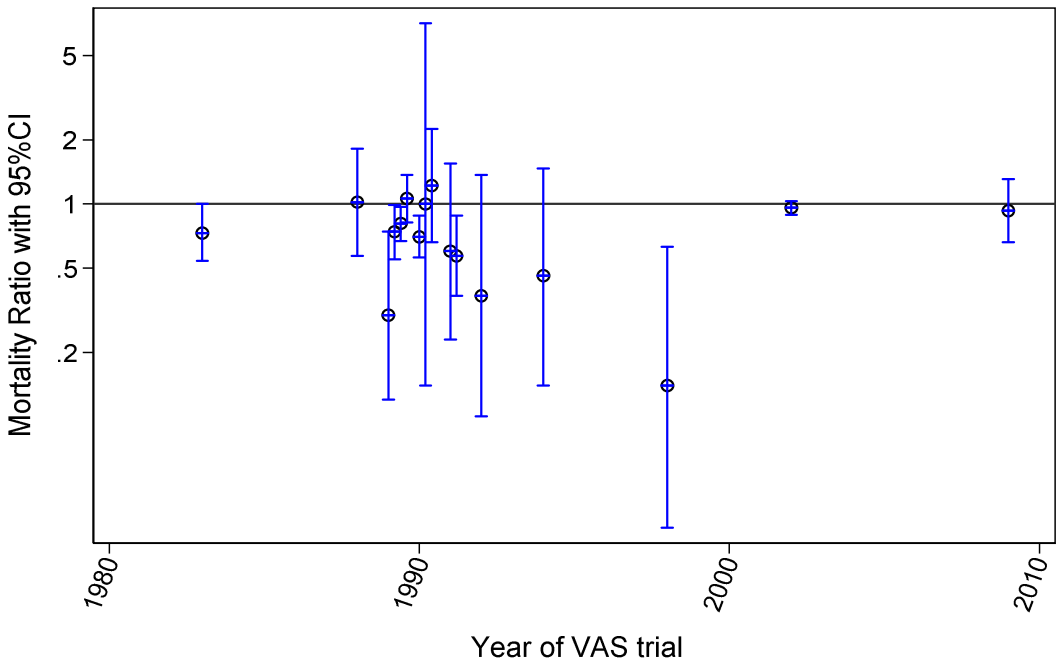

Figure 1. Mortality effects observed in trial of high dose vitamin A supplementation.

VAS is a low-cost intervention, but providing VAS to millions of children every 4-6 months is costly. To lower implementation costs, WHO recommends integration of vitamin A with immunisation services, but the effect of combining the two types of interventions was not tested. According to our hypothesis, the effect could depend on vaccine type. The recommendation is to use any vaccination contact after 6 months of age to give VAS, i.e. both with measles vaccine scheduled at 9 months and with delayed doses of DTP vaccines (Chapter 21). We conducted the first randomised placebo-controlled trial of VAS at vaccination contacts on mortality (4). In spite of two thirds of both boys and girls being vitamin A deficient, we found no

overall beneficial effect of VAS among >7500 children participating in the trial. However, we found contrasting effects in boys and girls: VAS doubled mortality in boys but halved mortality in girls (Figure 2). In this trial, the sex-differential effect was particular pronounced among children receiving a combination of live measles vaccine and non-live DTP vaccine with vitamin A or placebo.

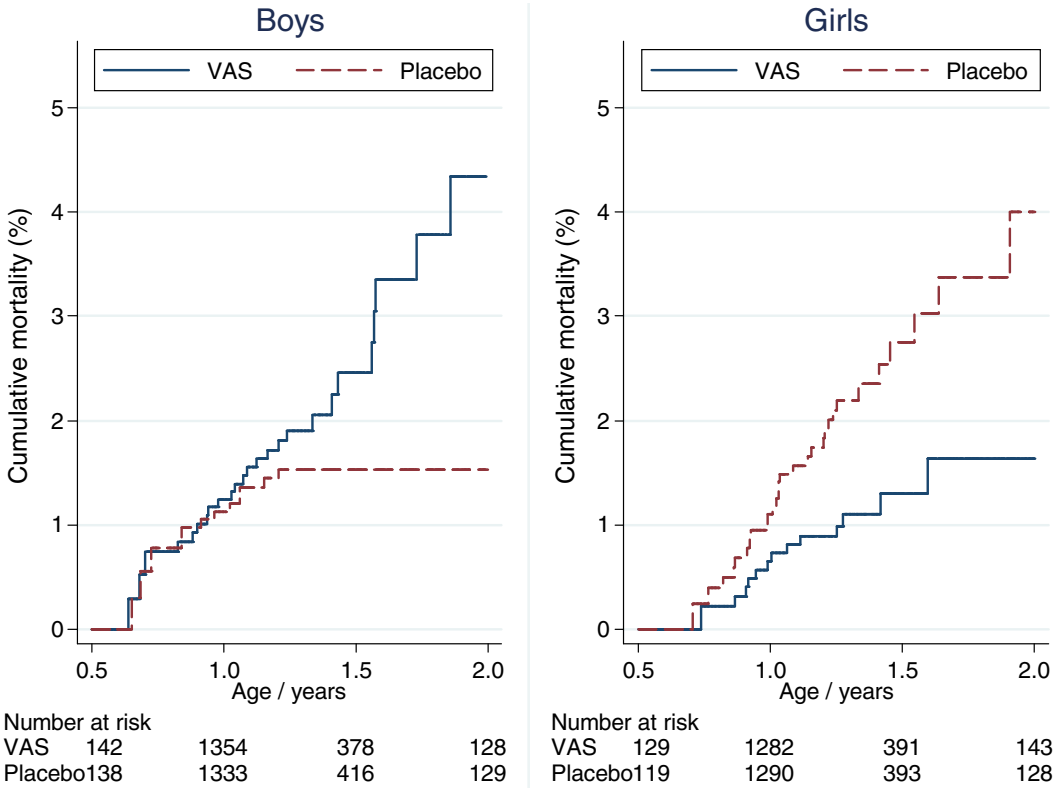

Figure 2. Sex-differential effect of high dose vitamin A supplementation at vaccination contacts.

While interaction with sex and vaccines may explain part of the heterogeneity of the effect of VAS, interactions with prior doses may also be important: In 2002, we tested giving half the recommended dose of VAS compared with the recommended dose during a campaign. Mortality was lower for children receiving the lower dose. In 2004, we repeated the trial, but found that lower doses were no longer associated with a benefit. This made us investigate factors, which could explain the contrasting effects in the two trials. Because we had implemented trials of VAS at birth and because annual campaigns with VAS had been conducted, children had been exposed

to prior doses, and that turned out to explain the variation in results between the two trials. In the trial described above (4) and in a trial of neonatal VAS (Chapter 24), we found additional support for the hypothesis that VAS is beneficial in girls who have received a prior dose.

Public health implications and future perspectives

The assumption of an overall benefit of VAS in populations with widespread vitamin A deficiency, which justifies the current vitamin A policy, has been contradicted by recent data. While VAS has the potential to lower mortality under some circumstances, providing high-dose VAS can also increase mortality. We have identified sex, vaccination status (Chapter 26), season (Chapter 31) and prior doses as important predictors of the effect of VAS. To test these findings and identify other important determinants of the effect, we urgently need large randomised trials. To optimise the VAS programme we need to identify in which subgroups VAS is beneficial and ensure that we do not give VAS when it may cause harm. For now, together with researchers from other fields, we recommend cessation of the universal vitamin A distribution (5).

Recommended Reading

1. Benn CS, Aaby PA, Bale C, et al. Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, west Africa. Lancet 1997; 350: 101-5.
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3. Benn CS, Aaby P, Arts RJ, Jensen KJ, Netea MG, Fisker AB. An enigma: why vitamin A supplementation does not always reduce mortality even though vitamin A deficiency is associated with increased mortality. Int J Epidemiol 2015; 44: 906-18.
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24. Extending the vitamin A policy to neonates

Background and assumptions

Children are born with low liver stores of vitamin A and depend on vitamin A rich breast milk during early life. The observation that vitamin A supplementation (VAS) to children between 6 months and 5 years of age was associated with reduced child mortality led to studies of VAS in younger children. A total of 12 randomised trials of neonatal VAS (NVAS) versus no NVAS/placebo have now been carried out, three of them in Guinea-Bissau.

Real-life data

We assessed the effect of NVAS by vaccination status and sex in three trials of NVAS in Guinea-Bissau (1,2). NVAS may have been beneficial in the first months of life while BCG and OPV-at-birth were the most recent vaccinations. However, the effect ceased to be beneficial and became negative for females once they started receiving the inactivated diphtheria-tetanus-pertussis (DTP) vaccine around 2 months of age, the overall effect being significantly different for the two sexes.

It has not been possible for us to get permission to analyse any of the other NVAS trials to test the hypothesis that NVAS was beneficial initially, but the effect became negative after receipt of DTP. However, an ecological analysis of the trials which provided data on mortality from 6-12 months (not just 0-6 months), shows that in females, the effect of NVAS is associated with 20% (95% CI=2-42%) increase in mortality from 6-12 months, thus supporting the hypothesis (3, Figure).

Public health implications and future perspectives

The results are in line with our hypothesis that VAS interacts with vaccines, amplifying the non-specific effects (NSEs) of vaccines (1,4). In the case of NVAS, it appeared that it could prime for a negative response to vaccines given several months after NVAS. Hence, apart from preventing vitamin A deficiency, NVAS also affects the immune system's general capacity to handle subsequent challenges in a manner, which may differ for males and females. It is imperative to understand the immunological effects of NVAS before it becomes policy.

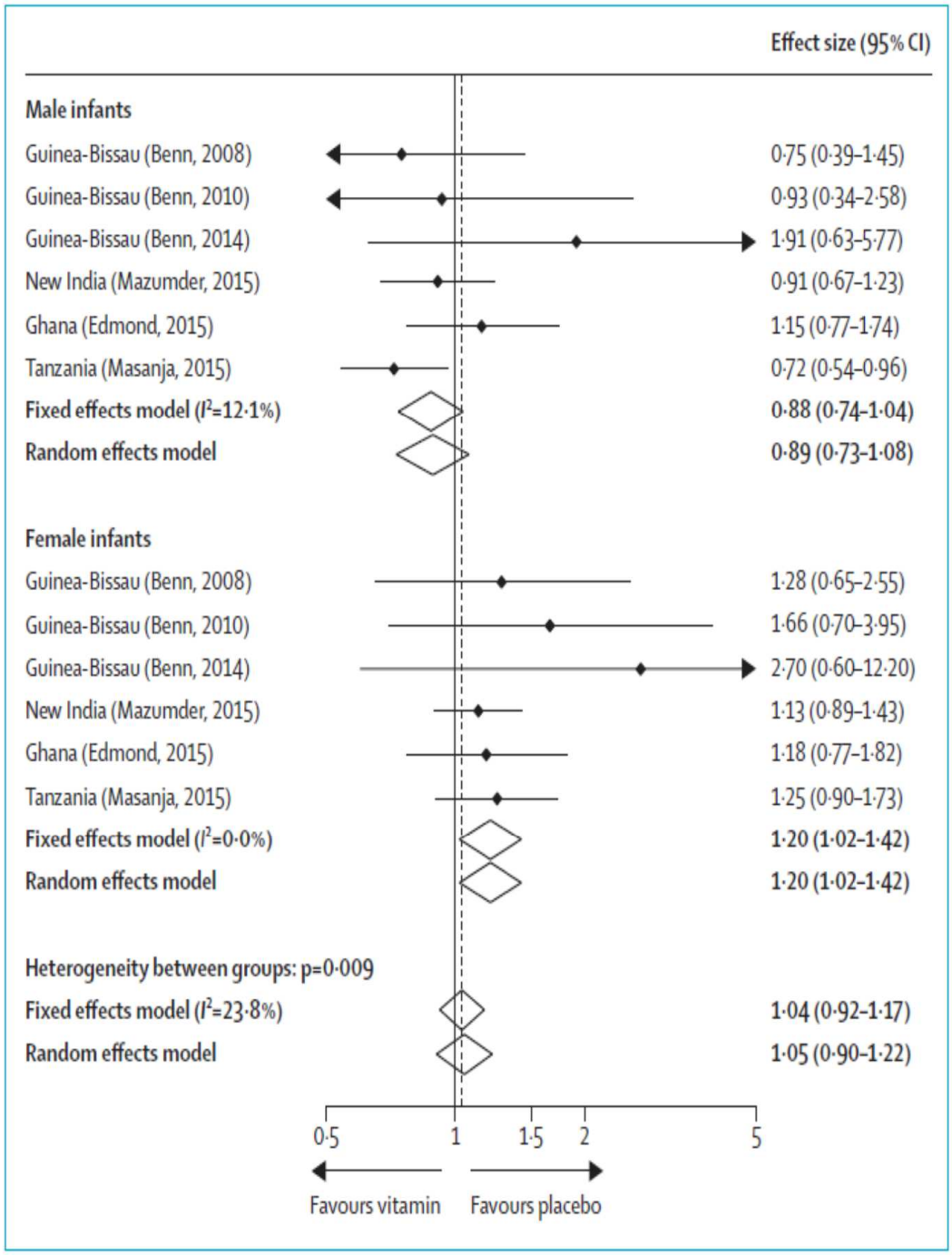

Figure 1. NVAS vs placebo effect size estimates in children 6-12 months of age. The forest plot shows the results of a meta-analysis of all studies that reported estimates by sex. The open diamonds represent 95%CI of the overall meta-analysis. P value for the same effect of NVAS in both sexes was 0-009, for the three Neovita trials alone p=0-02. NVAS=neonatal vitamin A supplementation. (from Benn CS, Aaby P, Fisker AB. Neonatal vitamin A: time to move on? Lancet 2015; 386: 132-3).

WHO is currently conducting a meta-analysis of the 12 NVAS trials, and this would be a chance to test our hypothesis of negative interaction between NVAS and DTP vaccine in females. Our group has contributed with data and commented on first versions of the paper. However, in the end we decided to withdraw as co-authors, as the other authors declined to test the hypotheses regarding negative interaction between NVAS and DTP vaccine in females.

For all we know, WHO is still considering whether NVAS should be recommended at least in parts of the world. The overall effect of NVAS may be beneficial in sub-populations with low DTP-coverage, and where the post-neonatal mortality is very low compared with the neonatal mortality. However, the available data indicates that NVAS could cause harm if implemented in areas with high DTP coverage and with a lower neonatal/post-neonatal mortality ratio. Importantly, there would be no way of knowing since it would be considered unethical to conduct randomised trials after the implementation. A sad example of the catch-22, which arises within the area of health interventions policies.

Recommended reading

1. Benn CS, Aaby P, Arts RJ, et al. An enigma: why vitamin A supplementation does not always reduce mortality even though vitamin A deficiency is associated with increased mortality. Int J Epidemiol 2015; 44: 906-18.
2. Benn CS, Diness BR, Balde I, et al. Two Different Doses of Supplemental Vitamin A Did Not Affect Mortality of Normal-Birth-Weight Neonates in Guinea-Bissau in a Randomized Controlled Trial. J Nutr 2014; 144: 1474-9.
3. Benn CS, Aaby P, Fisker AB. Neonatal vitamin A: time to move on? Lancet 2015; 386: 132-3.
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25. WHO and the non-specific effects of vaccines and vitamin A supplementation: How to handle controversial results?

Vaccines

It is easy to be wrong but it is hard to accept failure. There may be some comfort in knowing that we all fail - so the real issue is whether we fail in the right direction (1).

HTMV: When the high titre-measles vaccine (HTMV) trial in Guinea-Bissau failed and showed higher female mortality, we informed the World Health Organization (WHO)'s Expanded Programme on Immunizations (EPI) about potential problems with increased female mortality. This happened in January 1990, shortly after HTMV had been made official global policy. We suggested that the EPI ask other groups working on HTMV to test the observation. Though EPI thanked us for the interest, it was noted that we had small numbers; while the effect was clearly negative for females, the overall negative effect was only borderline significant. No further communication occurred.

Half a year later, we had made similar observations in data from an HTMV RCT in Senegal. We wrote again to WHO, expressing concerns about increased female mortality. This carried no response. However, following a personal visit to WHO in Geneva, it was decided to have an expert committee to review the evidence for HTMV. The first review panel in February 1991 dismissed the issue as having no plausible biological explanation and being an unplanned observation. However, one year later, in 1992, American researchers found similar excess female mortality after HTMV on Haiti. At that time point, WHO stopped the global recommendation of HTMV (Chapter 7).

DTP: Five years later, we wrote to WHO, because we had data suggesting that DTP might have deleterious NSEs and that BCG had beneficial NSEs. This carried no response. When a paper covering these observations was subsequently accepted by BMJ, we informed WHO again, suggesting that the potential negative effects of DTP should be tested elsewhere.

The Global Advisory Committee on Vaccine Safety (GACVS) under WHO organised an international expert field visit to Bissau to check the data collection and analysis. The experts identified no errors (2), and wrote a report calling for emergency and detailed plans "regarding the consequences of suspending DTP use globally" "if, in the coming months, Aaby's findings are confirmed by studies done in other settings". WHO sponsored other health and demographic surveillance sites (HDSSs) to examine the link between vaccination status and survival. At the preparatory meeting for these additional studies, we advised against assuming that the HDSSs had perfect data on vaccination; it had to be foreseen that some children had to be excluded from the analysis because they had no information on vaccination. Nonetheless, the WHO-sponsored studies excluded no children and thus inherently assumed that all children, for whom there was no information on vaccination, were "unvaccinated". These studies went ahead and were published in BMJ, The Lancet, and the International Journal of Epidemiology, all reaching the conclusion that DTP was associated with strongly beneficial effects on child survival. GACVS subsequently published that there was no reason to worry about DTP. An international expert group – WHO Task Force on Routine Infant Vaccination and Child Survival - reasserted that there was no credible data to support that DTP was associated with negative effects for child survival or that DTP was associated with higher female than male mortality (3).

It took us another two years to establish that the studies showing beneficial effects of DTP were flawed not only with misclassification of exposure but also with "survival bias" (Chapter 16). When the data was analysed prospectively, comparing children with known status as vaccinated or "unvaccinated", DTP-vaccinated children had 2-fold higher mortality than DTP-unvaccinated children (Chapters 16, 17). Though leading experts subsequently recognised that errors had been made, there was no follow-up from WHO and GACVS at that time to assess the possible consequences and whether new studies or policies were needed, apart from GACVS asserting that it would "keep a watch" for potential deleterious effects of DTP.

It took another 6 years before WHO and the Strategic Advisory Group of Experts on immunization (SAGE) returned to the issue of DTP in the form of a review of the NSEs of BCG, DTP and MV from 2013-2014. With regard to DTP, the review showed that DTP was associated with 38% increased overall mortality, highly significantly different from the beneficial effects observed for BCG and MV. However, the review emphasised that the studies on DTP

were not RCTs and therefore all the data on DTP could be due to bias. There was no attempt to assess the direction of the bias. The potential biases invoked by the reviewers were irrelevant or not important. However, ”bias” has apparently been the excuse for not responding to the finding; nothing has been done with respect to further investigate or ameliorate the observed negative effects of DTP.

The WHO review recommended further studies of NSEs of vaccines. The WHO’s IVIR-AC charged with implementing these studies decided to emphasise RCTs of BCG and MV. These new RCTs, which were planned without discussion or consultation with those who had previously studied NSEs, are designed so that they will not measure the NSEs of BCG alone or of MV alone, but will measure BCG followed by DTP and MV co-administered with DTP. The planned RCTs are huge in order to measure the impact on survival, involving 150,000 children. Should these trials be funded and implemented, it will take another 4-5 years to get answers to the wrong questions.

There is no way that bias can explain the increased mortality associated with DTP vaccinations. Since the most healthy children are vaccinated first, we should have expected that DTP-vaccination inherently was associated with better survival. The fact that all studies show the opposite should be a strong “danger signal”. Triangulation of all data will suggest that there is something fundamentally wrong: when all studies suggest a negative effect though a beneficial effect was expected (Chapters 16, 17); when all studies suggest increased female mortality even though there was no excess female mortality in the pre-vaccination era (Chapter 16); when all studies of HTMV suggest that DTP after HTMV explains the negative effect of HTMV (Chapter 7; when all studies of DTP administered after MV is also associated with increased female mortality (Chapter 21); and when other non-live vaccines show a similar pattern of excess female mortality (Chapter 19).

It is now more than 25 years since these issues regarding non-specific and potentially harmful effects of vaccines were first raised. Though we have come some way by WHO recommending further studies of NSEs, the knowledge has still not been used. WHO has consistently assumed that if WHO’s programmes are questioned, it is up to WHO to decide who are right or wrong and that those who raised the issue by definition are “biased”. By following this model rather than a collaborative model of finding a common answer through discussion and data analysis, WHO has consistently failed in the wrong direction.

Vitamin A supplementation (VAS): High-dose vitamin A supplementation (VAS) is recommended to children from 6 months-5 years in around 100 low and middle-income countries, where vitamin A deficiency is expected to be a public health problem. There have been attempts to see if it would benefit newborns as well. Our group has conducted studies in both age groups.

VAS to children aged 6 months-5 years: As outlined in Chapter 23, the WHO policy to distribute high-dose VAS to children aged 6 months-5 years was based on studies done decades ago. All recent evidence, including our trial in Guinea-Bissau, suggests that the effect has ceased to be beneficial. We have proposed that this may be linked to the roll-out of the vaccination program (Chapter 23). Attempts to raise that issue has been met with fierce resistance, not least from researchers from Johns Hopkins University, the group which was responsible for the first large scale vitamin A supplementation trial in 1986, which eventually led to the WHO policy.

The argument for continued use of VAS is primarily that it prevents vitamin A deficiency – an argument that has been rejected on numerous occasions (4). Nonetheless, the WHO policy continues.

Neonatal vitamin A supplementation (NVAS): Following a trial from Indonesia, showing a very beneficial effect of NVAS, we conducted three NVAS trials in Guinea-Bissau: two in normal birth weight neonates children (the first assessing 50.000 IU vs. placebo, and immediately after a trial comparing 50.000 IU vs. 25.000 IU vs. placebo), and one in low birth weight neonates receiving 25000 IU or placebo (Chapter 24)). All three trials indicated that NVAS was associated with increased female mortality, starting some months after NVAS, at the time of DTP vaccination. The results were in line with our hypothesis that VAS and vaccines interact – we had just not foreseen that this could take place if the two interventions were given months apart.

In 2008, WHO had a technical consultation regarding NVAS. We presented the results and asked for permission to test our hypothesis in the other five trials of NVAS; apart from the Indonesian trial, trials had also been done in Nepal, Bangladesh, India and Zimbabwe. The Indonesia, Bangladesh and India trials found positive effects of NVAS, but they had low DTP coverage and only followed children to 6 months of age; the two other trials found tendencies for negative effects. The request was denied by the researchers. Instead, with BMGF funding, WHO decided to carry out three new mega-

trial of NVAS, to “inform policy”. Initially it was only the plan to follow children to 6 months, but we advocated that the trials should at least follow children to 12 months, as all the studies with longer follow-up had seen worrying declines in NVAS effect and even negative effects. We also secured that they included an analysis of potential interactions between NVAS and DTP vaccine.

When the results of the new trials became available in 2014, it was clear that NVAS was not associated with benefits. All the new trials, like the former, had increased female mortality in the second half of infancy (5) (Chapter 24).

WHO arranged an analytical workshop with the aim to conduct a meta-analysis of all NVAS trials to explore reasons for heterogeneity. The explanation, which so far best explains the heterogeneity is negative interaction between NVAS and DTP in females: all the studies with beneficial effects had low DTP coverage and/or only followed children to 6 months (Chapter 24). Nonetheless, the request to analyse the data for a potential interaction between NVAS and DTP in females was declined.

In the end, we withdrew from the meta-analysis paper, which tried to emphasise that NVAS had beneficial effects in the Asian countries where there were maternal vitamin A deficiency (VAD) but no beneficial effect in the African countries where there was no maternal VAD. This would not explain why NVAS was associated with increased female mortality in the African trials and it is blind to what will happen when the coverage of DTP is increased in the Asian countries.

In the most recent NVAS trial from Pakistan, NVAS had no beneficial effect. As of today, the hypothesis about a negative interaction between NVAS and DTP-containing vaccines has not been tested in the new studies.

Conclusion

These are two histories about the interaction between research and global policy making. Research, which shows that policies may not be optimal and even harmful, should be of utmost concern. Nonetheless, WHO has not confronted the issues by documenting that DTP and NVAS in the presence of a high DTP coverage have the expected beneficial effect on child survival. Instead, methodological issues have been taken to the foreground: were the studies planned? were they biologically plausible? were they observational

studies rather than RCTs? was it only one research group? was it only in Guinea-Bissau? could it be bias?

WHO eventually withdrew the HTMV after it was documented that it was associated with negative sex-differential effects on child survival. However, it does appear that the main priority for WHO subsequently has been to maintain current policies and refute or ignore suggestions that official policies could have negative effects.

We have written now several open letters to WHO (https://www.bandim.org/Research/Non%20specific%20effects/Letters%20to%20WHO%20SAGE.aspx), suggesting that their analysis of DTP is flawed with survival bias, that RTS,S malaria vaccine is associated with increased female mortality, that OPV has beneficial NSEs and that removal of OPV will lead to higher child mortality, etc. We have received only vague responses. We will continue to send letters to WHO. In the end, these may come to serve as an unfortunate testament to WHO's lack of capacity to handle research, which questions current basic assumptions for the World's health.

Recommended reading

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2. Mulholland K, Barreto ML. Routine vaccination and child survival in Guinea-Bissau. Lessons can be learnt from this study. BMJ 2001;322:360.
3. WHO Task Force on Routine Infant Vaccination and Child Survival: Report of a meeting to review evidence for a deleterious effect of DTP vaccination on child survival. Held at the London School of Hygiene & Tropical Medicine, 20-21 May 2004. www.who.int/entity/vaccinesafety/topics/dtp/taskforce_report.pdf.
4. Mason JB, Benn CS, Sachdev H, West KP, Jr., Palmer AC, Sommer A. Should universal distribution of high dose vitamin A to children cease? BMJ 2018; 360: k927.
5. Benn CS, Aaby P, Fisker AB. Neonatal vitamin A: time to move on? Lancet 2015; 386: 132-3.

26. Optimising the implementation of the vaccination programme: Taking evidence into account

Background

Following the 2014 review of the non-specific effects (NSEs) of Bacillus Calmette Guerin vaccine (BCG), Diphtheria-tetanus-pertussis vaccine (DTP) and measles vaccine (MV), WHO's Strategic Advisory Group of Experts (SAGE) concluded that the evidence was insufficient to suggest a change in the vaccination schedule. However, while further studies may be needed to define the optimal vaccination programme, we could obtain better health effects of the current vaccination programme with simple measures taking NSEs into consideration.

Real-life data

Though the vaccination programme stipulates BCG and oral polio vaccine (OPV) at birth, 3 doses of Pentavalent vaccine (Penta: DTP-H. influenza type B-Hepatitis B) and OPV at 6, 10 and 14 weeks of age and MV at 9 months, the implementation does not emphasise the timing of vaccines. The evaluation focuses on coverage by 12 months of age, and on good management of resources, ensuring that vaccine doses are not wasted or have to be discarded. The focus on vaccine wastage creates a conflict for the live vaccines BCG, MV and yellow fever vaccine: these vaccines are freeze-dried, they have to be reconstituted with diluent before use and once reconstituted, they must be used within 6 hours of reconstitution.

By evaluating the programme by 12 months coverage and vaccine wastage, there is no incentive to provide BCG vaccination early. BCG has in randomised trial in low birthweight children (<2500 g) been shown to reduce mortality during the first month of life by 38% (17-54%) (Chapter 9), but in many settings vaccination of low birth-weight infants is deferred until they have gained weight. Vaccination of normal birthweight children are also commonly delayed: fewer than half the children in the rural areas are vaccinated within the first month of life (Figure) (Chapter 10). To maximise the impact of BCG on child mortality (Chapter 9) all vaccination opportunities should be utilised. This does not happen, because 20-dose BCG vials are not opened unless there are >10 children present to be vaccinated (Chapter 10).

As a consequence of the focus on not wasting doses of BCG vaccine, vaccination opportunities are wasted, and the coverage for both Penta1 and Penta2 rise above BCG coverage (Figure) indicating that while the contact with the health system is used for vaccination with Penta, BCG is not provided.

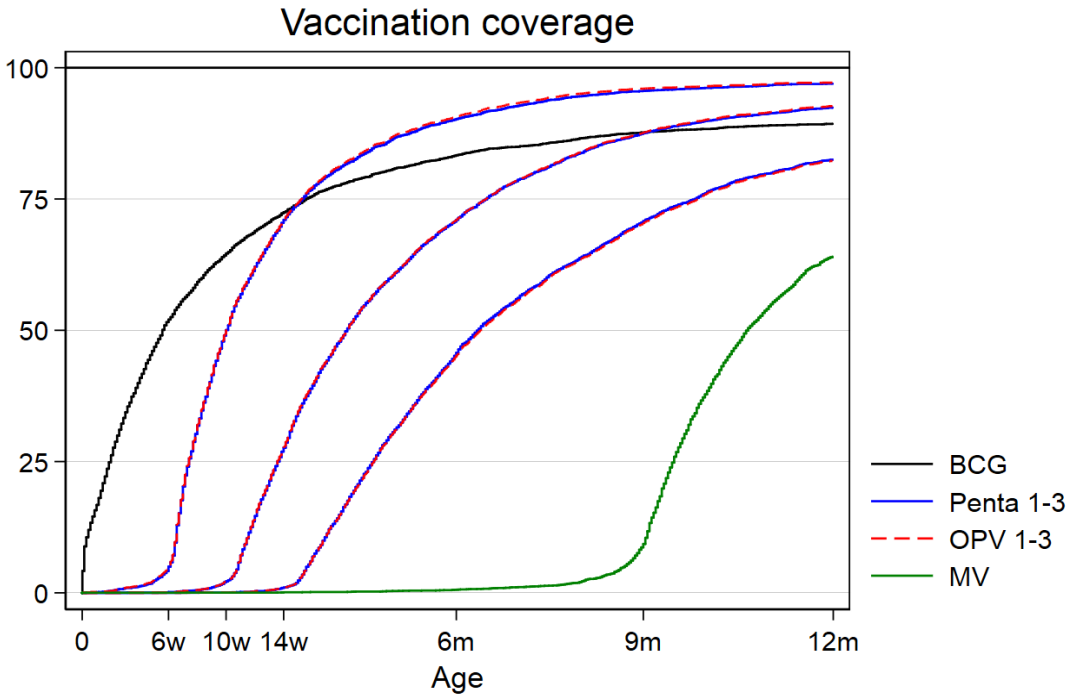

Figure. Vaccination coverage rural Guinea-Bissau 2017-18

Note: Coverage among 4470 children aged 12-23 months with a vaccination card inspected in Gabu, Bafata, Tombali, Quinara, Bubaque and Bolama.

The focus on vaccine wastage also affects how MV is given. MV is supplied in 10 dose vials, and in the light of the beneficial NSEs of MV (Chapters 6, 7), it should be a priority to ensure that all children are measles vaccinated. This does not happen – on average children have to seek MV 1.4 times to obtain MV, making it a costly and time consuming affair to become measles vaccinated (1). This is not captured in the routine statistics, but current MV coverage is much lower than Penta3 coverage in rural Guinea-Bissau (Figure). We have with collaborators shown that this is also the case in Ghana, Burkina-Faso, Kenya and Bangladesh (2). In a cost-effectiveness analyses we have shown that even if 87% of MV doses are wasted, it is cost-saving to open an MV vial and vaccinated at first opportunity (3).

Penta3/DTP3 coverage is the main indicator of vaccination programme performance (Chapter 20), and though MV is now increasingly being included in the indicator lists, the sequence of vaccines is still not considered. Receiving DTP/Penta with or after MV is associated with increased mortality (Chapter 21). Therefore, sequence of vaccination should be taken into account.

As outlined above, based on current knowledge we could presumably improve child survival just by implementing the vaccination programme according to schedule, ensuring that BCG and MV were given timely at birth and at 9 months of age and that the third dose of Penta was succeeded by MV. Further benefits could potentially be obtained by introducing an extra early dose of MV shortly after Penta3 (Chapter 7) or by revaccinating with BCG simultaneously with Penta3. Such changes will depend on further trials testing the effects and we suggest that these trials also consider how to best vaccinate when children depart from the recommended schedule.

Other changes to the vaccination programme are about to happen without prior trials of the overall effect. As part of the polio eradication endgame, OPV will be removed from the vaccination schedule and inactivated polio vaccine (IPV) be introduced. From all we know, the live OPV has beneficial NSEs and co-administering it with DTP has ameliorated the negative NSE of DTP (chapters 11, 17). Hence, by removing OPV from the vaccination schedule children will be deprived of its benefits. At the same time IPV seems to have negative effects on the survival of girls (Chapter 19). Hence, phasing out OPV and introducing IPV may increase mortality.

Public health implications and future perspectives

Assessing vaccination programme performance by coverage and wastage does not optimise the implementation. To ensure that the present vaccination programme is implemented in a manner, which optimises the impact on child mortality, we suggest the main indicator should not be DTP/Penta3 coverage, which is associated with increased female mortality (Chapter 16-21). Instead, the BCG coverage by 1 week and 1 month of age and the proportion of children receiving MV after the third dose of Penta by 12 months of age, all of which are associated with better child survival, should be used as the vaccination programme's performance indicators. We urgently need assessments of the effects of shifting from OPV to IPV, to identify remedies to counteract the predicted negative effect on child mortality.

Recommended reading

1. Byberg S, Fisker AB, Rodrigues A, et al. Household experience and costs of seeking measles vaccination in rural Guinea-Bissau. Trop Med Int Health 2017;22: 12-20.
2. Kavao M, Welaga P, Zandoh C, et al. Analysis of Coverage of Fully Immunized Child (FIC), Associated Factors, Outcomes, and Impact Using Routinely Collected Population Cohort Data 2001-2014, 2014. INDEPTH report to GAVI.
3. Byberg S, Fisker AB, Thysen SM, et al. Cost-effectiveness of providing measles vaccination to all children in Guinea-Bissau. Global health action 2017; 10: 1329968.

27. Markers of immune function: Cytokines and other blood-borne molecules

Cytokines are signalling molecules of the immune system, conveying messages of danger, inflammation, wounding, repair or convalescence from cell to cell. There is a plethora of different types of cytokines, each with a special origin of cell, target of cell, immunological message and potency. Some of these molecules are found in the blood, which is easily accessible compared to other immunological compartments (e.g. in contrast to inner organs etc.), and in addition are found in relatively high quantities making them quantifiable by standard technology.

These properties have made cytokines convenient informants of the state of the immune system since decades; hence, they have also been studied by researchers at the Bandim Health Project.

One typical way to interrogate the state of the immune system is to cultivate a blood sample with molecules known to tease the immune cells, e.g. molecules resembling pathogen-derived particles, often known as pathogen-associated molecular patterns. The encounter and recognition of these molecules signals danger to the immune cells, and an intracellular process ensues often leading to the production and release of cytokines in order to alarm other cells of the danger. The relative quantity and composition of the released cytokines can inform us on the state of emergency and the defence capability of the interrogated immune cells. It remains, however, only a proxy measure, as cytokines *per se* rarely have any protective mechanism. A more profound test would be a functional assay, in which the ability of the immune cells to kill or neutralize microbes is analysed; and the ultimate test would be an *in vivo* infectious challenge with time to recovery or survival as the outcome. Whereas the latter infectious challenge is obviously not possible in most studies involving human subjects, and the functional assay often requires a considerable amount of blood, advanced technology, is very time-consuming and quite expensive, the *in vitro* cytokine response assay is more feasible, particularly for studies involving children and larger study populations.

In the interpretation of the cytokine response data produced by such studies, the researcher may ask whether a high or a low cytokine response is a

hallmark of a “good” immune system. Intuitively, an impaired immune response fails to combat the infection, but also excessive immunological reactions to a microbial insult can ultimately lead to the death due to immune-pathological events. Prospective cohort data to investigate this link is, however, very rare, not least in infants. A study in low birth infants from the Bandim Health Project quite interestingly found that the cytokine response can indeed be too low as well as too high (1). Linking cytokine responses to mortality data collected during a vaccine trial, we found that the optimal immunological responsiveness for several cytokine outcomes was at a robust, but not excessive level, yielding a U-shaped mortality risk curve of the immune response (Figure). While acknowledging the above limitations, the results underscored that cytokine responses are indeed relevant markers of relative immune competence.

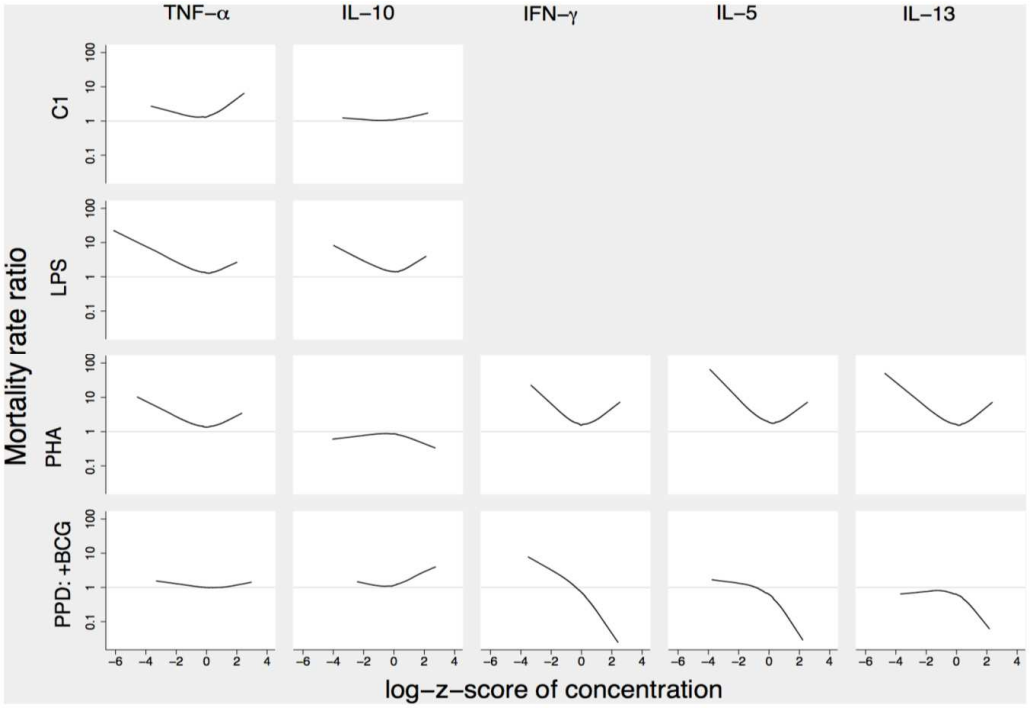

Figure. Flexible shapes of the associations between the cytokine log-z-scores and the mortality rate. Notes: The curves show mortality rate ratios comparing the mortality rate of children with a given log-z-score and age to the mortality rate of children aged 38 days at bleeding with a log-z-score equal to zero. For PPD the association is only shown in the group randomized to BCG at birth (BCG-vaccinated) as there was little cytokine production induced by PPD in the BCG non-vaccinated control group (Published in 1).

Albeit not a cytokine, suPAR (soluble urokinase-type plasminogen activating receptor) is a non-specific marker of inflammation, and studies from BHP and elsewhere have found strong associations between elevated suPAR levels in the blood and overall mortality risk to a number of infectious diseases and cancer. Hence, suPAR has been included in the monitoring of TB, HIV and malaria patients in a number of cohort studies from the BHP (2).

BHP has published 14 articles reporting cytokine data from Guinea-Bissau. These *in vitro* cytokine studies have investigated whether vaccines or micronutrient interventions did cause non-specific perturbations of the immune system, as fundamental evidence of non-specific immunological effects to corroborate the epidemiological evidence. The aim was secondarily to give pathway-specific or functional clues for further investigations. Having now established good evidence of non-specific effects of particularly the BCG vaccine, the agenda has moved on to further characterise the underlying molecular mechanisms, which requires expertise and technology from leading immunological experts. We have found fruitful collaborations via our international Optimmunize network.

What ultimately constitutes a “good” immune response is context dependent, conditioned by age, type of infection, and probably also sex. Our collaborators in Nijmegen, The Netherlands are involved in unravelling these associations on genetic, epigenetic, transcriptomics and metabolomics levels in large consort undertakings, also using data from the BHP surveys and clinical trials.

Our collaboration with professor Kollmann's laboratory in Vancouver is currently focusing on unravelling important early signatures of a protective BCG response in newborns, attempting to nail what constitute a frail vs. a thrifty phenotype in the most vulnerable window immediately after birth. The systems biology approach generating e.g. metabolomics and *ex vivo* cytokine data will be linked to mortality data in the follow-up period.

Hence, the good old Bandim Health Project Health and Demographic Surveillance System site built on sweat, perseverance and hard footwork carrying along a SECA scale, pen and paper in the 'zones' and to the health centres has moved into the 21st century of -omics and big data. However, we will continue to carry a SECA scale, pen and paper around to detect unexpected epidemiological patterns.

Recommended reading

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28. Markers of Immune Function – Atopy

Background and assumptions

With evidence that vaccinations and micronutrient supplementation have non-specific effects on child survival, efforts have been made to investigate the immunological impacts of these interventions. BCG, measles vaccination, diphtheria-tetanus-pertussis (DTP) vaccination, and high dose vitamin A supplementation (VAS) are all known to impact on cytokines and T helper cells of the immune system which are also implicated in the development and expression of allergy.

There is limited burden of allergic disease in Guinea-Bissau. However the global pattern is of increasing burden in most countries, especially as child mortality falls and poor countries transition to patterns of disease seen in high-income countries. Many routine interventions with vaccinations and VAS are administered during childhood in Guinea-Bissau with the aim of reducing child mortality, and there is an underlying assumption that these interventions have no impact on allergic disease. The following research challenges this assumption.

Real-life data

Atopy was first studied in Guinea-Bissau in 1993-1994 to investigate the relation with measles infection. Children and young adults aged 14 to 21 years were studied with skin prick tests (Figure) to common airborne allergens (1). A positive test (atopy) indicates that the child is sensitised to a substance and has increased risk of allergic disease, but does not imply that the child has allergic disease. In this study, children with a history of measles infection had considerably reduced risk of atopy. This finding offered support for the “hygiene hypothesis” of allergic disease, indicating that infection, not just exposure, might protect children from allergy.

Similar to many natural infections, BCG vaccination is known to be an inducer of T helper type 1 cells, which are associated with protection against atopy. To investigate BCG, 400 children aged 3 to 14 years were skin prick tested in 1994 (2). A marked reduction in atopy was seen amongst those vaccinated with BCG in infancy with greatest reduction amongst those vaccinated earliest in life. Alarmingly, DTP vaccination was associated with increased risk of atopy.

Other studies internationally indicted that BCG was either protective or had no effect on atopic disease. The randomised control trial (RCT) of BCG for low weight infants in Guinea-Bissau provided a way to re-examine this question. 281 children randomised to early or the usual delayed BCG at birth were revisited at age 3-9 years (3). Skin prick tests did not find a difference between children who received BCG vaccination early versus late, but a pattern emerged of lowest risk of atopy amongst those vaccinated earliest. Again, there was a suggestion of increased risk of atopy associated with DTP vaccination.

Vitamin A deficiency is associated with a T helper 1 dominant immune profile, suggesting potential for VAS to suppress T helper 1 cells and cause atopy. Three RCTs conducted in Guinea-Bissau have provided opportunity to answer this question, with worrying results. In the first RCT, high dose VAS given with measles vaccination at 9 months of age had no impact on atopy at age seven years of age (3). However, the results of second study were more alarming. High-dose VAS or placebo was randomly allocated with or without BCG vaccine as part of the low weight RCT mentioned above. Children who received VAS had almost three-fold increase risk of

atopy, with the association strongest amongst girls and amongst those, who concurrently received BCG (4).

To confirm or refute this finding, the study was repeated on a much larger scale amongst a cohort of children who were randomly allocated VAS or placebo at birth. 1530 children were re-visited at age 9-11, and VAS was associated with almost twice the risk of atopy amongst girls who had also received BCG (5). Similarly, the risk of wheeze, a clinical manifestation of atopy, was increased almost two-fold amongst VAS recipients.

Figure. Testing for atopy.

Public health implications and future perspectives

By using existing cohorts to study the associations between vaccinations, VAS and atopy, we have demonstrated that early BCG appears to be associated with reduced atopy, while neonatal VAS increases the risk of atopy

amongst girls. In studies of vaccinations, VAS and all-cause child mortality described in other chapters, a remarkably similar pattern is demonstrated, whereby early BCG reduces neonatal mortality (Chapter 9), and neonatal VAS increases female mortality (Chapter 24). Similarly, there is an indication that DTP vaccination may increase both atopy and all-cause child mortality (Chapter 16). These similarities suggest that there may be a common immunological mechanism underlying effects on mortality and atopy, which is yet to be elucidated.

Neonatal VAS is unlikely to be introduced as an intervention given that recent international trials demonstrated no beneficial impact on child mortality.

As more non-live (like DTP) vaccines are developed and live vaccines (such as BCG and measles vaccine) are phased out, the immunological consequences of vaccinations on atopic disease may become more prominent. Furthermore, as child mortality declines in Guinea-Bissau, other markers of immune function and morbidity may be necessary to study the non-specific effects of vaccines. Further studying atopy may be useful to understand the unintended impact of vaccinations on the immune system.

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5. Aage S, Kiraly N, Da Costa K, Byberg S, Bjerregaard-Andersen M, Fisker AB, Aaby P, Benn CS. Neonatal vitamin A supplementation associated with increased atopy in girls. Allergy 2015;70:985-94.

29. Markers of immune function: thymus

Background and assumptions

In a country with a high burden of infectious disease, crowding and limited healthcare facilities, a fit immune system is crucial for infant survival. Over the years the Bandim Health Project (BHP) have used various markers of immune function as outcomes in randomised intervention trials in order to assess the immunological impact of the health interventions under scrutiny.

One marker of immune function is the size of the thymus gland, an organ essential for the development of the cellular immune system. Poor nutritional status and infections are known to reduce thymus size, while breastfeeding increases its size. Ultrasound is a simple method for estimating thymus size and can be performed under simple conditions (Figure 1).

Figure 1. Measuring thymus size using ultrasound.

Real life data

The first studies of thymus size in Guinea-Bissau were conducted between 1995-1997 at the health centre in Bandim. Here it was first demonstrated

that having a higher birth weight and having a thymus size above the median independently were associated with lower infant mortality. Birth weight was strongest associated with mortality the first 6 months after birth and thymus size strongest associated with mortality the next 6 months (1) Also having a thymus size below the median at 6 months of age was a strong predictor of subsequent mortality (Figure 2)(2)

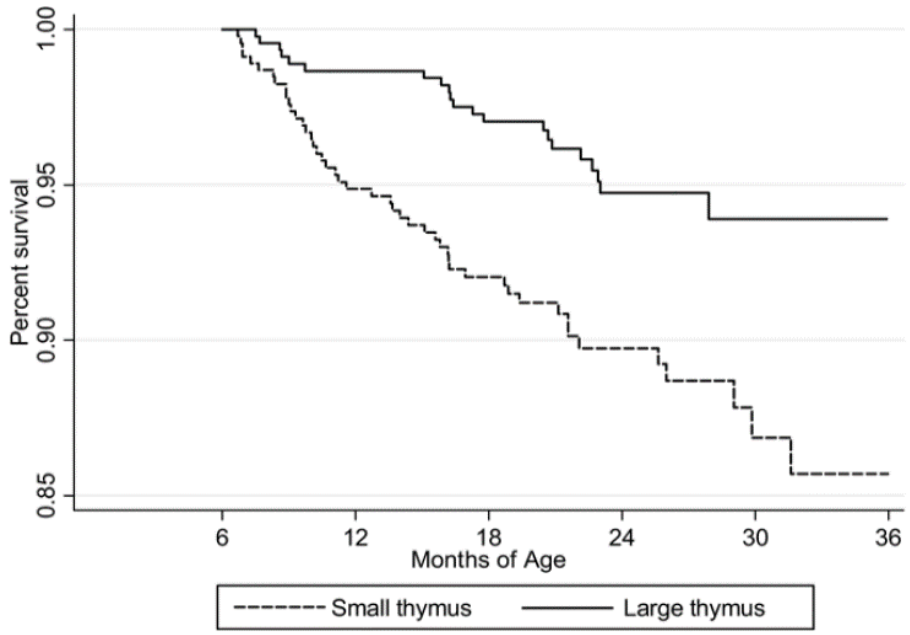
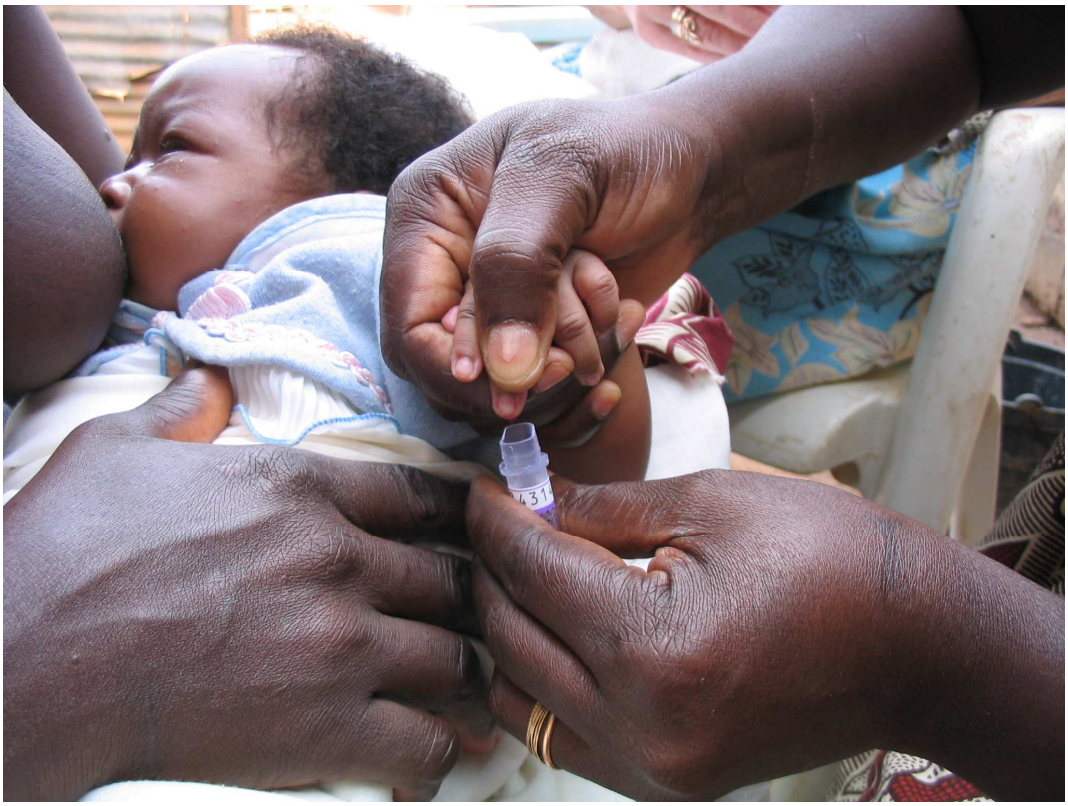


Figure 2. Kaplan-Maier plot of survival from inclusion to 36 months of age by thymus size. P = .001 (log-rank test). (From 2)

Factors associated with a small thymus at birth were prematurity, lower birth weight, female gender, Pepel ethnic group and infants born during the rainy season (1). At 6 months of age, female gender was still associated with a small thymus, as was having severe malaria or having been treated for malaria. Infants with malnutrition (small mid-upper arm circumference, small weight for height, small weight for age) also had a small thymus (2).

Between 2009 and 2012, new thymus studies were conducted at the Bandim Health Project. In line with previous studies, small thymus size at birth was associated with increased mortality in infancy in a cohort of 940 newborns. Factors associated with a small thymus could mainly be related to stress and infections and included pathological amniotic fluid, birth in the

dry season, elevated maternal body temperature, antibiotic treatment at the time of labour, infant Apgar score and infant convulsions (3).



With thymus size being a surrogate marker for infant mortality, this was used as an outcome in three randomised vaccine trials. Within a randomized trial of early measles vaccination (MV) at 4.5 months in addition to the MV at 9 months, there was no effect of an early measles vaccination on thymus size 4 weeks later (4). Within a randomised trial of not providing oral polio vaccine (OPV) at birth, children who had not received OPV had a larger thymus relative to weight after 2 weeks, but not after 4 and 6 weeks (5). Within a randomised trial of low weight infants receiving BCG at birth compared with the usual delayed BCG, there was no effect on thymus size after 4 weeks (unpublished results). Overall vaccination did not appear to have any convincing effect on thymus size.

Public health implications and future perspectives

Although infant mortality has declined from 1995 to 2012, a small thymus at birth remains associated with increased mortality. While vaccines with beneficial non-specific effects had no convincing effect on thymus size,

other factors associated with malnutrition, stress and infection may be important. Future research could focus on identifying other risk factors for a small thymus size, e.g. congenital and neonatal infections, preeclampsia, gestational diabetes. Interventions to reduce these conditions could possible increase thymus size and improve infant immune function and survival.

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30. Training of innate immunity

The epidemiological discoveries of the non-specific effects (NSEs) of vaccines described in the previous chapters have been met with deep scepticism in the medical world. One often raised criticism is the seemingly lack of a biological mechanism fitting the ruling paradigms of vaccinology and immunology, making the NSEs of vaccines appear implausible. However, recent ground-breaking immunological research may hold the key to a conciliating explanation.

A new understanding of the innate immune system: The immune system has classically been conceptually divided into the innate and the adaptive immune system, with distinct divisions of function and capabilities, although with a tight collaboration and mutually dependent for keeping a competent defence system. Whereas the adaptive immune system learns to recognise very specific patterns of the pathogens encountered during an infection, the innate immune system has been described as static or unchangeable, and based on the recognition of conserved and common pathogen-associated patterns, e.g. particular cell wall components found in bacteria.

The last decade of immunological research has challenged this dogma. Today we know that not only the adaptive immune system, but also the innate immune system can learn from previous encounters, and take upon a different character in the wake of an infection. This may manifest as a more powerful and faster responsiveness to secondary infections, or a tolerant, dampened responsiveness (1).

BCG trains the innate immune system: It has long been known that BCG vaccine stimulates and activates cells of the innate immune system. Animal model studies also found that BCG could improve the resistance to non-tuberculous pathogen infections, even in animals manipulated to have a depleted adaptive immune system and only a functional innate immune system. Hence, it was demonstrated that BCG was able to teach the innate immune system to fight an unrelated infection. However, the molecular mechanisms were not elucidated and the findings seemingly had no implications to the field of vaccinology.

In 2012, a ground-breaking study was published showing that BCG vaccine renders monocytes, an important cell type of the innate immune system, more responsive to unrelated pathogens BCG (2). This state of increased alertness of the monocytes was observed up to a year after vaccination, underscoring that BCG had profound immunological imprinting effects. The altered responsiveness was the effect of epigenetic remodelling of the monocytes, in which the translation of specific genes is changed, but not the genes (genetic code) themselves. The molecular basis of the epigenetic remodelling of the monocytes after BCG is the addition (or removal) of tiny carbon-based molecules (methyl- or acetyl-groups) to the proteins (histones) around which the DNA is wrapped, modulating the physical conformation of the DNA strand, which in turn affects the accessibility of the DNA reading machine (the RNA polymerases). Further studies soon found that BCG also affected other cell types of the innate immune system, including the so-called Natural Killer cells. The training of the cells may manifest as an altered production of core cell signalling molecules (cytokines), and a change in the glucose metabolism such as a shift from oxidative phosphorylation toward aerobic glycolysis, which may reflect the higher demand of fast energy in immune activated cells (3).

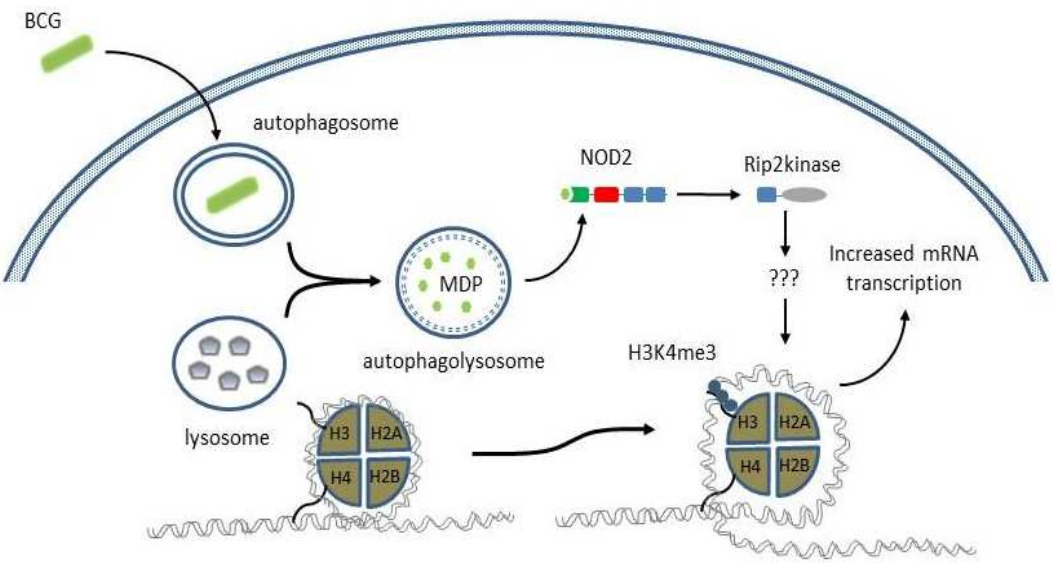

Figure. Putative events leading to BCG-induced training at a cellular level. BCG is internalised, its cell-wall component MDP triggers NOD which leads to activation of Rip2kinase and (through unknown mechanisms) epigenetic reprogramming, resulting in increased responsiveness and potentially other functional changes. (1).

In an immunological study of BCG nested within a randomised trial in low weight babies in Guinea-Bissau, we found that BCG at birth increased the innate cytokine responses, showing for the first time that the innate training effects was indeed also taking place in human infants (4).

The innate training by BCG fitted the picture emerging from the epidemiological studies from Bandim Health Project (Chapter 9), hinting at a potential biological explanation of the observed reductions in infectious mortality in babies receiving BCG at birth. The fact that BCG enhanced the innate immunity aligned well with the extraordinarily rapid protective effect of BCG in babies, showing a reduction in mortality already within few days of vaccination – a time course that excludes the adaptive immunity as the main mechanism.

BCG improves resistance to viral disease – a human model study: Following the above mentioned study in 2012, Bandim Health Project has had a close collaboration with the laboratory of the founding father of the new trained innate immunity paradigm Professor Mihai Netea from Radboud University Medical Center in Nijmegen, The Netherlands. Whereas the use of experimental animal models can help researchers interrogate the functional aspects of the immune system, the direct translation into human clinical evidence is compromised by the obvious ethical constraints of submitting human study volunteers to infectious challenges. However, in an ingeniously designed proof-of-concept study using the live attenuated viral vaccine against yellow fever as an infectious disease model in man, we showed that receiving BCG vaccine prior to yellow fever vaccine reduces the viremia and inflammation that naturally occurs in the host after vaccination. This observation can be interpreted as experimental evidence of the non-specific protection against heterologous infections as found in numerous epidemiological studies.

Trained immunity – relevance to other vaccines*?* Whether other vaccines than BCG do perform trained immunity has yet to be investigated in full. We have good indications from our laboratory experiments that the smallpox vaccine, *vaccinia,* renders monocytes more responsive to secondary innate stimulation (5), which seem in agreement with the epidemiological evidence of beneficial NSEs of smallpox vaccination (Chapter 12). Whereas it may already be too late to undertake these studies for oral polio vaccine (OPV), as OPV is being phased out and now increasingly subjected to restrictive biosecurity measures, Bandim Health Project and its collaborators are

underway with studies of potential innate training effects of the measles vaccine. In contrast to the live vaccines, we have indications that non-live influenza vaccine and non-replicating smallpox vaccine (5) cause innate tolerance. If this pattern of innate immune training after live vaccines but tolerance after non-live vaccine is consistent, this could help explain the observed differences in mortality effects after live and non-live vaccines. The coming years of immunological research are expected to substantially improve our knowledge.

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31. The weather or not – non-specific effects and the seasons: Mortality

Background

Guinea-Bissau has two distinct meteorological seasons, the rainy season from June to November and the dry season from December to May. Most rains fall from July to September. The weather conditions an associated seasonality in many aspects of daily life in Guinea-Bissau, including migration patterns, accessibility to different locations in the country, availability of fruits and vegetables, and pathogen exposures. As elsewhere in Africa, the "rainy season" has been synonymous with the "malaria season" both in popular and in medical culture. Interestingly, a startling seasonality in mortality risks have been identified in studies in both urban Bissau and the rural areas.

Real-life data

Mortality: Accumulated data from our repeated rural population surveys covering the period from 1990 to 2013 shows that while the overall mortality steadily declined, the under-five child mortality remained considerably higher in the rainy season than in the dry season throughout the study period, averaging 51% higher (95% CI: 45–58%) (1).

In urban Bissau, the seasonal difference was evident though less pronounced in 1991 to 1996 with 15% (95% CI: 4–28%) higher under-5 mortality in the rainy season vs. the dry season (2). Also, hospitalization rates and in-hospital deaths were higher in the rainy season. A survey during 2008 to 2013 among newborns admitted to the neonatal intensive care unit at the main hospital in Bissau found the lowest mortality risk for babies born in December to February, equivalent to the early dry season compared with the rest of the year. However, the seasonal pattern of a higher mortality in the rainy season has not been found in all studies; e.g. an analysis of infant (<1 year) mortality in rural Guinea-Bissau in 1992-3 and 2002-3 did not find marked differences by season (3). A study from the rural surveys in 2002 even found that delivery during the rainy season was a risk factor for maternal mortality. The excess mortality in the rainy season is in alignment with studies from other African populations. In the rural areas, the seasonal

difference in mortality risk was larger in girls than in boys, and increased with increasing age (1).

There can be multiple underlying explanations for seasonal differences in mortality, but very likely seasonal pathogen exposures are a key driver of these patterns. The fact that endemism of some infectious disease differ by age-group may help explain the deviations from the general trend of a higher rainy season mortality as discussed above. Most interestingly, in addition to the seasonal differences *per se* in mortality risk, we have also observed that the non-specific effects of some health interventions may depend on the season in which they are first administered, which will be discussed in the following.

Differential intervention effects*:* A large trial investigating the effect of providing early measles vaccine (MV) at 4.5 months of age in addition to the routine MV dose at 9 months (Chapter 8) found a slightly larger beneficial effects on all-cause mortality up to 3 years of age in children measles vac-

cinated in the dry season vs. the rainy season. In addition, there was significant reductions (47%) in hospitalisations between 4.5 months and 9 months exclusively for early MV recipients vaccinated in the dry season. This seasonality of the beneficial effect of early MV on hospital admission rates persisted into the follow-up period from 9 months to 18 months. Along the same line, early MV was associated with larger mid-upper arm circumferences at 24 months in girls receiving MV in the dry season but not in the rainy season.

Several studies from the BHP have identified a scar amongst BCG-vaccinated as a determinant of increased survival probability and it is therefore important that BCG vaccination in the dry season was associated with a higher chance of yielding a scar at 6 months. In addition, we have neonatal mortality data from low weight cohorts enrolled from 2002 to 2011 indicating a consistent seasonal effect of BCG vaccination on mortality, with a remarkably larger beneficial effect of BCG in December to February compared with the rest of the year. An immunological sub-group study nested within a large trial of providing BCG at birth to low weight infants found that BCG at birth increased innate cytokine responses to TLR2 agonist stimulation of the blood, and this association tended to be more pronounced for infants vaccinated in the dry season than in the rainy season.

BHP has conducted several trials of vitamin A supplementation (VAS), either at birth or together with routine vaccines in later infancy. One randomised trial in 2002-2004 found that neonatal VAS administered in the dry season was associated with a lower infant (<1 year) mortality risk but a higher mortality risk when administered in the rainy season (4). However, two subsequent trials of neonatal VAS did not reproduce these seasonal patterns, and neither did trials of VAS in later infancy reveal strong seasonal differences in the effect of VAS on mortality.

Public health implications

The dichotomous definition of seasons based on precipitation is somewhat arbitrary, as e.g. pathogen epidemics may follow a different pattern. Nonetheless, the rainy season has quite consistently been associated with negative health outcomes. In addition, the beneficial NSEs of health interventions such as MV, BCG and VAS, seem to be most pronounced in the dry season. Our recommendation is that season should be taken into account in future studies of mortality patterns and effects of health interventions.

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32. The weather or not – non-specific effects and the seasons: Infections, immunological markers

Background

Guinea-Bissau has two distinct meteorological seasons, the rainy season from June to November and the dry season from December to May. Most rains fall from July to September. The present chapter discusses the evidence on seasonality in infectious diseases and immunological markers from studies conducted at Bandim Health Project (BHP).

Real-life data

Infection seasonality: In a community-based study in Bissau, all-cause diarrhoea in <4-year-old children was considerably higher in the rainy season. The parasite *Cryptosporidium parvum* is one organism causing diarrhoea. It is most prevalent in Guinea-Bissau in the early rainy season and an important cause of death in the youngest children (1). Another important diarrhoea-causing enteropathogen, rotavirus, also exhibits a seasonal pattern, with annual epidemics occurring during the relatively dry and cooler months, from January to April (2).

The magnitude of the malaria burden is yet unclear, as studies from BHP find that malaria is significantly over-diagnosed in the health care system. Malaria has gradually shifted from being endemic to epidemic, peaking in October to December, but remains an important disease (Chapter 38).

Seasonality in immunological markers. The BHP has also investigated possible seasonality in immunological mechanisms and markers. T-lymphocyte subsets in 0 to 5-month-old infants have been found significantly associated with season, with a positive shift in the CD4+:CD8+ T cell ratio from the rainy to the dry season. Another study found slightly higher eosinophil and platelet counts, and slightly lower red blood cell counts and haemoglobin concentrations in the rainy season in 5-month-old infants.

In a skin prick test study at 7.5 months of age using common allergens and vaccine antigens, season was significantly associated with anergy (absence of a local skin reaction) to tuberculin, the prevalence of anergy being higher in the rainy season (Odds Ratio = 1.67 (95% confidence interval: 1.25–2.23)). There was also a tendency for higher prevalence of anergy to tetanus and

diphtheria antigens in the rainy season, indicating a relative suppressed immune response at this time of the year.

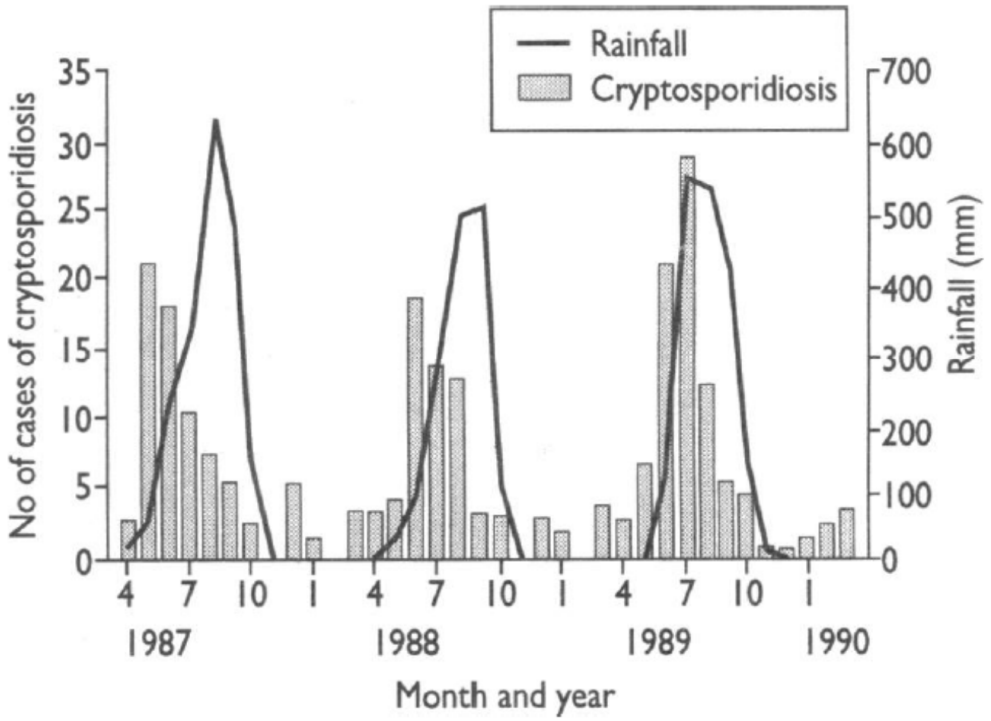

Figure. Monthly rainfall (Institute of Meteorology, Bissau) and number of episodes of cryptosporidium diarrhoea among 3215 episodes of diarrhoea in children in Bissau, Guinea Bissau, West Africa (1).

Using ultrasound scans in 1995-1997, we identified a tendency for a smaller thymus size at birth for babies born in the period from September to October, i.e. the end of the rainy season in Bissau, whereas a parallel study measuring the thymus size in 6-month-old infants found the largest measures in children born in December to February, i.e. the early dry season, with less variation over the remaining calendar year. In contrast, a third thymus scan study conducted in 2014 found that the rainy season was associated with a larger thymus size at birth. The three studies, however, all found a positive association between thymus size and survival.

The risk of having undetectable pre-vaccination maternal measles antibody levels at 4-6 months was higher in the rainy season than in the dry season.

There was no seasonal difference on the effect of neonatal vitamin A supplementation on *in vitro* cytokine production, leukocyte subsets, or retinol-binding protein (RBP) levels 4-6 months after supplementation. The latter outcome is used as an indicator of vitamin A stores in the body. One study found that RBP levels 6 weeks after NVAS was slightly higher for children born in the dry season vs. the rainy season, perhaps reflecting a differential metabolism of the supplied vitamin A or differences in diet-derived vitamin A. To the latter end, a study in rural Guinea-Bissau among children aged 6 months to 2 years found a higher risk of vitamin A deficiency (VAD) in the rainy season vs. the dry season, the proportion ratio being 1.64 (1.49-1.81). The lowest risk of VAD was found at the end of the dry season, from February to May. Seasonal differences in background VAD levels are probably mainly reflective of seasonality in vitamin A rich foods as well as infectious disease pressure.

Public health implications

The seasonality of common infectious diseases in Bissau is evident from a number of studies. However, the patterns may change over time as seen e.g. for malaria due to improved vector control, bed net use, treatment or perhaps even climate change (Chapter 38).

Most immunological studies have been conducted over a period of one year or shorter, and the generalisability can be questioned. In addition, observed seasonal differences are sometimes rather small with unknown implications for health and disease. One remarkable exception is thymus size, which is positively associated with survival in early life. It is noteworthy, however, that the changes in immunological parameters generally mirror the changes in mortality (Chapter 31).

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33. HIV-1 and HIV-2 in Guinea-Bissau 1987 - 2016: two crossing epidemics

Human immunodeficiency virus type 2 (HIV-2) was discovered in 1985, two years after the identification of HIV-1. The first patients originated from Guinea-Bissau and Cap Verde, respectively, which suggested that West Africa could be a hotspot for this new virus.

HIV research at the Bandim Health Project (BHP) began soon after the discovery of HIV-2. In 1987, a survey was done in 100 randomly selected houses within the BHP area of Bissau, and the results were daunting; 8.9% of the adults and 0.6% of the children were infected with HIV-2 (1). This is the highest prevalence found anywhere in the world. In contrast, no study participant was infected with HIV-1.

The first case of HIV-1 infection in Guinea-Bissau was found in 1989 in a 25-year-old man with HIV-1/2 dual infection, and HIV-1 quickly spread in Bissau. Including HIV-1/2 duals, 2.3% of the adult population was HIV-1 positive in 1996 (2), rising to 4.6% in 2006 (3). But lately the HIV-1 epidemic seems to be stabilizing or slightly decreasing with 4.0% of the adult population being infected in 2016 (4).

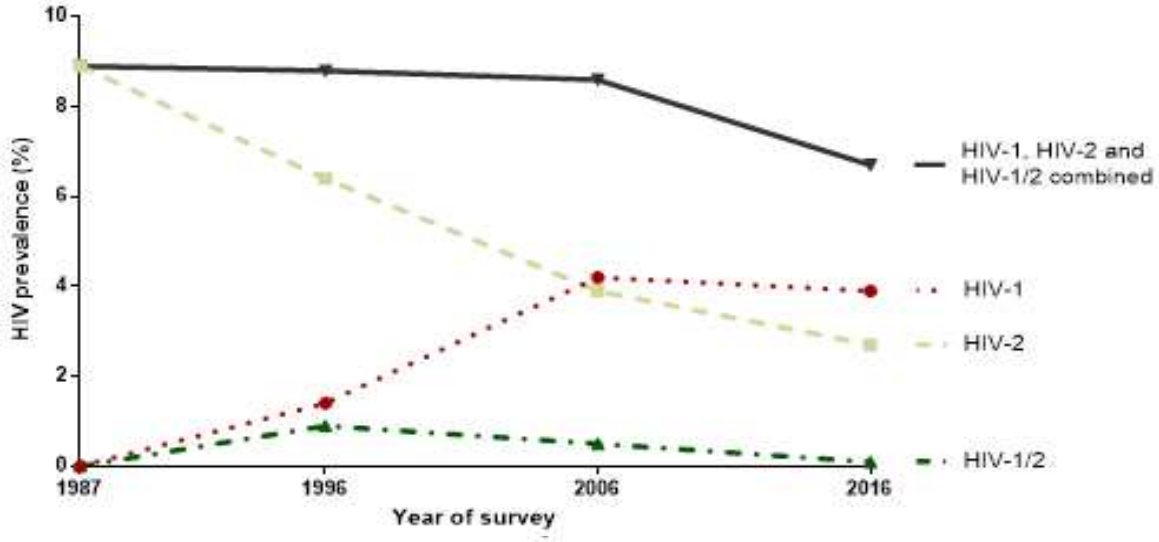

Figure 1: The prevalence of HIV-1, HIV-2 and HIV-1/2 dual infections among adults in Bissau during the 4 surveys performed at BHP 1987-2016. The upper line represents the combined prevalence of HIV-1, HIV-2 and HIV-1/2 dual infections.

While HIV-1 has been increasing in Bissau for the last 30 years, the HIV-2 prevalence has been steadily declining. In 2016, the adult prevalence had dropped to 2.9% (4), and a mathematical model even predicted that HIV-2 is on the way to become extinct in Guinea-Bissau (although this may not occur until next century).

The explanation for the crossing epidemics of HIV-1 and HIV-2 is not fully elucidated. HIV-2 has been characterised as being a `milder´ virus than HIV-1. The mortality rate among HIV-2 infected individuals is much lower than for HIV-1, but still higher than for the background population. HIV-2 is also characterised by lower levels of circulating virus in the blood stream, and lower transmissibility.

HIV-2 originates from the Sooty Mangabey, a primate indigenous to the forests of West Africa, whereas HIV-1 was transmitted from chimpanzees in Central Africa. The early HIV-2 epidemic was likely driven by blood transfusions, prostitution, and unhygienic circumcisions and health care related procedures. HIV-2 transmission was also frequent during the independence war in the 1960s and early 1970s. HIV-1 was only introduced to Guinea-Bissau at a later time point when these risk factors were partly reduced.

Antiretroviral treatment (ART) was introduced in Bissau in 2005, and became more widely available in 2007 with the support from international donors. As well-treated individuals with HIV does not transmit the virus further, ART may give a significant impact on the HIV epidemics in Guinea-Bissau, but this needs to be elucidated in future studies.

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34. The Bissau HIV Cohort - Challenges facing HIV treatment in Guinea-Bissau

In sub-Saharan Africa, the introduction of antiretroviral therapy (ART) for patients with HIV infections has improved the lives of millions of people and decreased mortality. However, despite support from international donor organisations, the infrastructure for delivering ART in low-resource settings is still affected by substantial problems. Moreover, as the use of ART has increased, there have been reports of drug stocks running out because of insufficient human resources or poor infrastructure. Also, frequently the means of monitoring the effects and side effects of ART are not available.

In 2005, a national HIV program was implemented in Guinea-Bissau by the Ministry of Health. However, it was only during 2007 that the program led to an increase in the number of patients being treated. The Bissau HIV Cohort was established in 2007 by the Bandim Health Project in Guinea-Bissau and Aarhus University Hospital in Denmark in collaboration with nurses and physicians from the Hospital Nacional Simão Mendes (HNSM), which is Guinea-Bissau's main hospital and is located in the capital Bissau.

All patients with HIV infections who attend the HIV clinic at HNSM are eligible for inclusion in the Bissau HIV Cohort. Demographic, clinical and laboratory data are collected at each visit and entered in a database. Blood samples from the patients are stored in a biobank. The purpose of establishing the cohort was to help study how clinical, virological and immunological parameters influence the effectiveness of therapy (1).

Today, data has been collected for more than 10,000 HIV-infected patients. The cohort is the world's largest single-centre cohort of HIV-2 and HIV-1/2 dually infected patients. Other co-infections are prevalent in this study population, including HTLV-1, hepatitis B and C, cytomegalovirus and cryptococcal infections. Thus, this cohort represents a unique opportunity to study how co-infections alter immune response, disease progression and response to treatment. Despite difficult working conditions, inclusion and follow-up have been maintained in this large cohort over 10 years.

The cohort is characterised by patients presenting late for diagnosis, a high rate of loss to follow-up, low treatment adherence leading to major problems with treatment failure and development of resistance as well as high

mortality rates (1-3). The cohort has been used as a platform for randomised controlled HIV treatment trials and HIV vaccine trials. Several studies have demonstrated that the most commonly used rapid tests for discriminating between HIV-1 and HIV-2 are not performing well (4).

The largest HIV clinic in Guinea-Bissau is facing numerous obstacles in delivering ART at a sufficiently high quality and, as a result, patients' lives are put in jeopardy. These difficulties may have been exacerbated by the frequent recurrence of political instability in the country. If similar issues are encountered by the many ART facilities in Africa that report few data, it is likely that the implementation of ART in affected areas will be impaired.

The management of people with HIV infection in vulnerable countries still faces many challenges. International research collaboration can help identify problems and solutions, as well as enhance the capacity of the healthcare system (5). Future research by the Bissau HIV Cohort study group will investigate whether our identification of problems with the delivery of ART has led to measurable benefits, such as fewer patients being lost to follow-up, lower mortality, better diagnosis of opportunistic infection, more frequent detection of treatment failure and better-educated local staff.

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35. The Bandim TBscore – development and applications

Background and assumptions

Clinical prediction rules (CPRs) use clinical findings to diagnose a disease or predict an outcome. They are useful when clinicians fail to identify relevant but under-diagnosed conditions and clinical decision-making is complex. Further, they may guide less experienced examiners through the right diagnostic pathway.

Most CPRs for pulmonary TB (PTB) published over the recent years were developed to aid the clinician to decide if patients admitted to hospitals in low- and medium-incidence settings should be placed in isolation. Others are used on initially sputum smear negative (SN) patients to improve and accelerate diagnosis of PTB. Few have tried to combine signs and symptoms into a CPR to screen for PTB, and only two CPRs to monitor TB-treatment response have been proposed.

The Bandim TBscore was developed in 2008 by Wejse *et al* (1) to enable objective monitoring of tuberculosis patients on treatment. The score consists of five symptoms (cough, haemoptysis, dyspnoea, chest pain and night sweats) and six signs (pale inferior conjunctivae, pulse>90 per minute, positive finding at lung auscultation, temperature >37°C (axillary), Body Mass Index (BMI) <18/<16 and Mid-Upper-Arm-Circumference (MUAC) <220mm/<200mm). Each variable contributes with one point while BMI and MUAC contribute with an extra point if <16/ <200mm, hence the maximum score 13 (Figure 1). It has later been refined and shortened to the 2013 proposed TBscoreII, validated in Guinea-Bissau and Ethiopia (2, 3).

Real-life data

The Bandim TBscore has been researched thoroughly; assessing its ability to predict outcome of TB treatment in different cohorts in Africa and its inter-observer variability in African and Indian cohorts (2).

While carrying out our TB research in Guinea-Bissau, refining and validating the score further, our focus shifted towards improving the currently low TB detection rates. The WHOW estimated in 2017, that 4 million persons with

TB remained undiagnosed in 2016. Through our close collaboration and daily interaction with the local health centres, it became apparent to us, that to improve the low case detection rates we had to increase health care personnel's awareness towards TB and engage the basic structures already in place to enhance the number of patients investigated for possible TB infection. This has inspired us to build cohorts of patients with signs and symptoms suggesting possible TB disease (i.e. presumed TB, preTB) - a unique group of patients not studied before.

Through our studies, we have learned that the TBscore, alone or in combination with a biomarker, can help us guide overworked health personnel in public health centres to refer relevant patients to diagnostic work up for TB. (4, 5).

Bandim TBscore

Symptoms	
Cough	1
Haemoptysis	1
Dyspnea	1
Chest pain	1
Night sweat	1
Kliniske fund	
Anemia	1
Pulse > 90 beats/min	1
Unormal lungestetoskopi	1
Temperature > 37°C (axillary)	1
BMI <18	1
BMI <16	1
MUAC <220 mm	1
MUAC <200mm	1
Total points possible	13

Figure 1. The Bandim TBscore - Selfreported symptoms and clinical signs.

Public health implications and future perspectives

In under-resourced over-burdened health facilities with a large patient load presenting with co-morbidities, many patients with TB remain undiagnosed and untreated. Simple clinical tools at points of healthcare, which can pick up patients with active TB have great potential for diagnosing patients with TB early and preventing TB-associated morbidity and mortality. Targeting delayed TB diagnosis and TB-related mortality will add to the agenda of reducing poverty-related diseases.

A major strength of the Bandim TBscore is the possibility to follow up patients during treatment, as has also been shown by our group. Thus, the score may be used both to enhance the number of confirmed TB cases among presumed TB patients and serve as an easily adaptable monitoring tool during the treatment or re-evaluation of these.

Currently, we are completing a large trial running simultaneously in Guinea-Bissau and Ethiopia using the Bandim TBscoreII to guide the local health centres in which patients to refer for TB diagnostics.

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36. Tuberculosis in Bissau – the incidence changes but the burden remains

Background and assumptions

Research in tuberculosis (TB) epidemiology has been a part of the Bandim Health Project (BHP)'s research portfolio since 1996- During the past 10 years, the TB research has expanded into more areas also involving case-finding (Chapter 35) and double-burden epidemics of emerging Non-Communicable-Diseases linked with tuberculosis, such as diabetes and hypertension. Some studies have had a focus on characteristics and outcome of patients with TB disease, others have involved the study population with general characterization of disease or risk factor prevalence.

Real-life data

Understanding why some develop TB disease and others do not, has been an ongoing research focus. In collaboration with groups in Italy and the US, the previous work on TB genetics has been continued with a description of potential candidate genes for TB risk. The potential role of biomarkers to predict TB risk and disease severity has also been a focus point. suPAR, Procalciton and p24 are various markers which have been shown at high levels to predict mortality in TB patients (1); all of the markers also correlated with the TBscore. A major TB prevalence survey has been conducted in the same households as the HIV survey, and this study revealed a large burden of undetected TB in the population, with 25% of cases found being previously undiagnosed. The TB incidence previously described for the 1990s was followed over a 10 year period in the 2000s (2), revealing a declining incidence of overall TB over the years, but an unchanged incidence of smear positive TB, Figure 1a-b. These studies showed a marked increase the year the prevalence survey was conducted, likely because of increased awareness of TB in the study area. The unchanged rate of smear positive TB indicates that there was no increased effect of TB by the TB treatment program, but rolling out Anti-Retroviral Therapy for HIV may have played a role in the falling TB incidence, Figure 1c.

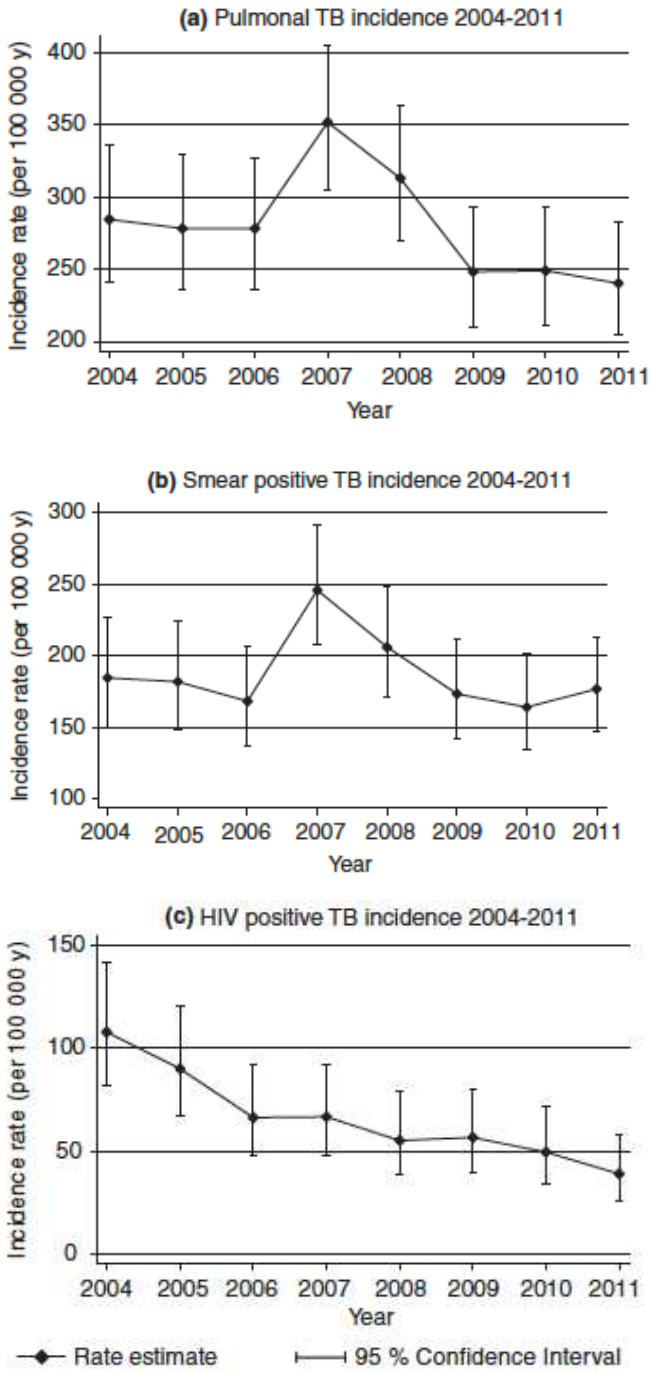

Figure 1. Tuberculosis incidence in the study area 2004-2011.

(a) Pulmonary Tuberculosis (TB) incidence 2004–2011.
(b) Smear Positive TB incidence 2004–2011.
(c) HIV positive TB incidence 2004–2011.

Many factors have been shown to be important for the TB epidemiology in Bissau, and sometimes simple interventions may have considerable impact. Patients suspected of TB but not initially diagnosed with TB have been followed and it was shown that the diagnostic yield can be increased by 5% with follow-up visits from a field assistant a month later. A number of other risk factors for TB have also been described, such as diabetes (3) and both HIV-1 as well as HIV-2 co-infection.

Currently studies are ongoing on the impact of smoking, alcohol and hypertension on TB risk. A risk factor for poor outcome was shown to be treatment delay, which is no surprise and also shown in many other cohorts. Yet, with a large cohort of 973 TB patients, it was demonstrated conclusively and for the first time that delay also had impact on the severity of the clinical presentation at diagnosis. Somewhat surprisingly, malnutrition was not found to be a major risk factor for development of TB (4), although clearly a strong risk factor for poor outcome. The protein content in the diet in Bissau is low and this may be a possible future intervention to improve nutritional status of TB patients on treatment, as they still have a very high mortality.

The unique possibilities of having Health and Demographic Surveillance System population-based data has further made it possible to describe the mortality of children in households with TB patients and documenting the increased overall child mortality in houses with a recent TB case. If the mother of the child had TB, it was associated with a 7-fold increased mortality risk (5).

Attempts have also been made to reduce the current high burden of TB in the study area, with studies investigating the effect of isoniazid prophylaxis on the children, which showed that it was possible to achieve a high adherence at 79% and a marked reduced child mortality at 70%. A randomised controlled trial (RCT) comparing two different strategies for preventive therapy has just been completed.

Public health implications and future perspectives

Detailed knowledge on the epidemiology of TB and the burden of disease in Bissau is a prerequisite for intervening, and the current research agenda focuses on how it is possible to increase the case-finding, in order to reduce the risks of the patient and surroundings associated with delayed access to

treatment. The mapping of important risk factors is an important mean to highlight where case-finding activities may yield active TB disease.

The specific focus on patients suspected of having TB (so-called "presumed" TB patients) is a field few research groups work with, and the results delivered in this area may carry large public health implications, because this is where a major part of the undetected TB is to be found (1).

A consistent focus has also been interventions to target the TB burden, which also has been addressed through attempts to prevent TB in children in exposed families. This has been a WHO recommendation for years and further enforced in the 2018-updated guideline on latent TB, but still not implemented in many low income countries. The results from Bissau show an example for the world in terms of feasibility and dramatic effects, which hopefully will lead to a higher degree of implementation of preventive TB therapy in other low-income countries. Another future perspective may be to support TB patients with protein-rich supplements to improve outcome, and currently a large RCT is testing this hypothesis among TB patients in Bissau.

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37. Malaria treatment: which treatment schedules to use?

Background

In Guinea-Bissau as in all of Sub-Saharan Africa, the treatment of malaria has changed during the last decades. Chloroquine and quinine were previously the two most important antimalarials, but due to high levels of chloroquine resistance this old and well-known drug was abandoned. Quinine is still recommended for third line treatment, but should not be used as first or second line treatment (1). Following WHO recommendations, the National Malaria Programme in Guinea-Bissau abandoned chloroquine in 2008 and now recommends artemether-lumefantrine, an artemisinin based combination, as first line treatment.

Despite and probably because of extensive use of chloroquine, levels of chloroquine resistance were uniquely low in Guinea-Bissau compared to the rest of Africa. The recommended chloroquine dose is 25 mg per kg bodyweight split into single daily doses over 3 days. In Guinea-Bissau, a practice of treating with approximately three times standard dose chloroquine, split into three daily doses for 5 days, had arisen and was in use concurrently with the low prevalence of chloroquine resistant parasites (2, 3). Several studies have shown that chloroquine at a dose of 50 mg/kg given over three days is effective (4,5), and as effective and well tolerated as the recommended treatment with artemether-lumefantrine, both regimens achieving the WHO´s recommended efficacy for antimalarial drugs (3). Thus, chloroquine resistant parasites can be effectively treated when higher doses of chloroquine are used.

The standard 25 mg/kg dose of chloroquine for treatment of malaria was established in 1946 in trials on adult American soldiers. However, many drugs should in children be dosed according to body surface area. Nevertheless, in daily practice and even in studies evaluating the efficacy of chloroquine and other antimalarials dosing are based on bodyweight irrespective of age, which could have implication both on the treatment outcome in the individual child and on the interpretation of the level of resistance towards an antimalarial. We therefore studied whether using body surface

area instead of bodyweight had a significant impact on chloroquine blood concentrations and treatment outcomes.

Real-life data

The day 7 concentrations in children less than 2 years of age taking 50 mg/kg and in children aged 10 to 14 years taking 25 mg/kg were 825 and 758 nmol/l, respectively. Consequently, children less than 2 years of age need approximately double the dose per kg to obtain chloroquine concentrations comparable to those obtained in older children. Thus, young children are underdosed when treated by body weight. However, we found that chloroquine concentrations, normalised for dose taken in mg/m^2, were unaffected by age (4). In other words, there was a linear correlation between the amount of chloroquine taken per m^2 body surface area and the chloroquine concentration obtained in the blood. That this also has clinical implications was shown when studying the outcome of treating children infected with *P.falciparum* genotypes conferring chloroquine resistance, as the adequate clinical and parasitological responses were 14%, 38%, and 39% after standard-dose and 66%, 84%, and 91% after double-dose chloroquine in children aged <5, 5-9, and 10-14 years, respectively. In parallel, median chloroquine concentrations were 471, 688, and 809 nmol/L for standard-dose and 1040, 1494, and 1585 nmol/L for double-dose chloroquine as shown in the Figure (5).

Interestingly, we have also participated in a multicentre study, which showed that treating with artemether-lumefantrine in the recommended doses gives 24% and 13% lower lumefantrine concentrations in children weighing less than 15 kg and 15 to 25 kg, respectively as compared to non-pregnant adults. These results suggest that revised artemether-lumafantrine dosing regimens for young children are required. Simulations using the lumefantrine pharmacokinetic model suggest that lengthening the dose regimen to e.g. twice daily for 5 days would be more efficacious than using higher individual doses in the actual regimen.

Public health implications and future perspectives

In Guinea-Bissau, we have shown that artemether-lumefantrine and dihydroartemisinin-piperaquine are both efficient with successful treatment rates on day 42 of 95% and 100%, respectively. However, in other malaria areas an increase in the level of resistance and tolerance towards the com-

monly used antimalarials has been registered. Therefore, it would be an advantage to have more treatment options. As double dose chloroquine was shown to be as efficient as artemether-lumefantrine (4) it would be obvious to continue to consider chloroquine as a possibility.

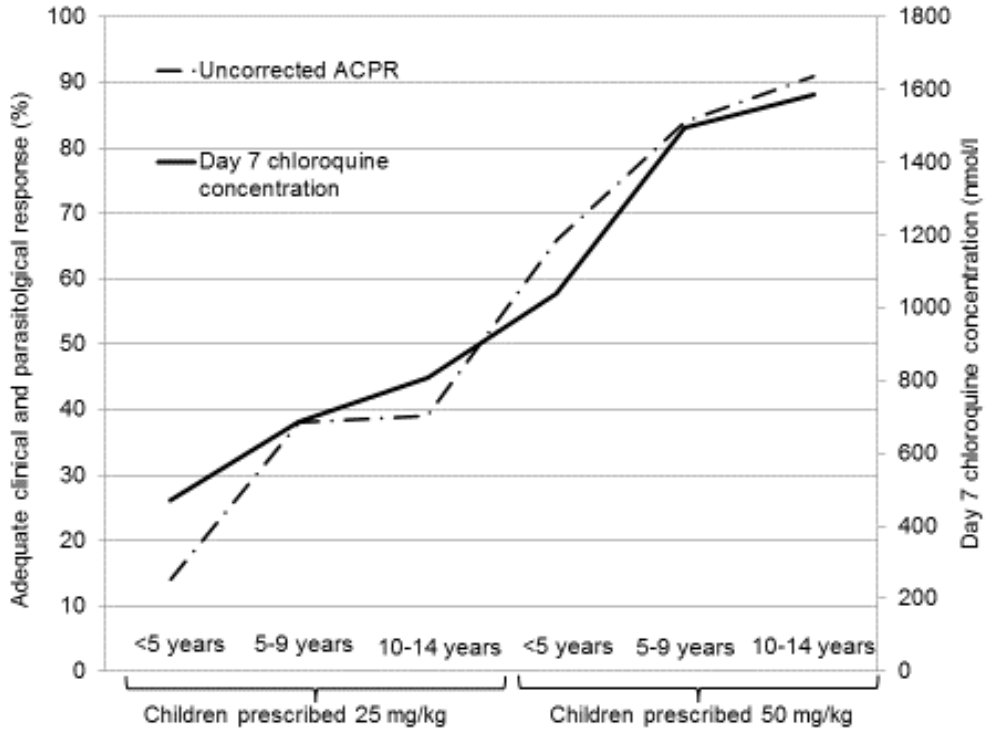

Figure 1. Adequate clinical and parasitological response (ACPR) and chloroquine concentrations among children aged <5, 5–9, or 10–14 years prescribed 25 or 50 mg/kg of chloroquine.

Usually, the efficacy of an antimalarial is evaluated by performing *in vivo* studies with currently recommended doses in children below 5 years of age. Our studies on chloroquine concentrations indicate that when evaluating whether chloroquine is still working clinically, this would give a pessimistic and unfair result. The studies should be performed using dosing by body surface area, which would allow for high-dose regimens to be evaluated. The study on the lumefantrine concentration indicates that the same holds true for other antimalarials as well.

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38. The epidemiology of malaria and the development of resistance

Background

In Guinea-Bissau, the epidemiology of infections with *Plasmodium falciparum* has changed significantly over the past decades. Prevalence of malaria decreased from 59% in 1990 to 3% in 2004, whereas the incidence of uncomplicated malaria was stable with the highest frequency in children under 5 years until 2003, after which it fell sharply until 2007 as shown in figures 1 and 2 (1). One of the reasons why the malaria burden reduced despite the presence of chloroquine resistance in Guinea-Bissau in the late 1980s was the use of an effective high-dose chloroquine treatment (Chapter 37). From 2003, campaigns with mass distribution of impregnated mosquito nets have also been organised. After 2008, the malaria incidence in children has increased again (Figure 1), especially for the 10-14 year olds that now account for the majority of malaria in children aged < 15 years. This shift can probably be explained by the declining prevalence and thereby lack of development of immunity since the mid-90s (1). The decline in malaria incidence and prevalence thus took place before artemisinin-lumefantrine was introduced for the treatment of uncomplicated malaria in 2008 (1).

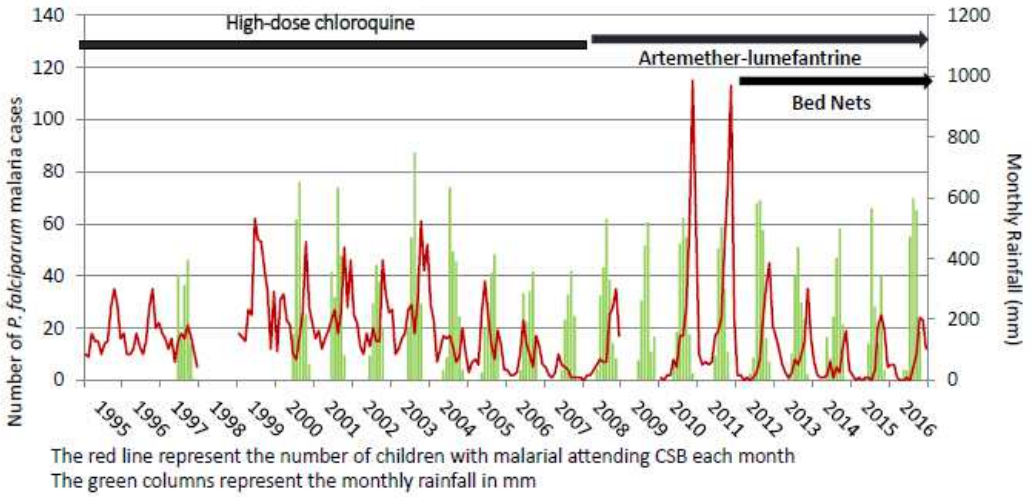

Figure 1. Monthly frequency of *P.falciparum* malaria in children aged less than 15 years attending the Bandim Health Centre and monthly rainfall (mm).

As shown in several studies conducted by the Bandim Health Project, a considerable overtreatment took place at the health facilities in the country (2, Chapter 37) thereby increasing costs, but more importantly also increasing the risk for development of resistance in the malaria parasites. In order to minimize the treatment of microbiologically unconfirmed malaria the Ministry of Health introduced rapid diagnostic tests, which are much less labour-demanding, faster and a more reliable way of diagnosing malaria than the traditional microscopy.

The development of resistance to the malaria drugs used is worrying and close monitoring is important. *In vivo* studies are difficult and expensive to perform, but an alternative could be to monitor the development of resistance of the parasites on a molecular level, as more and more mutations associated with less sensitivity or even resistance are described.

Real-life data

Probably due to an effective high dose treatment, the frequency of genetic markers for CQ resistance remained constant from 1990 to 2007. However, since the replacement of chloroquine by artemether-lumefantrine in 2008 an increase in mutations known to cause chloroquine resistance has been observed with the frequency of *pfcrt 76T* increasing from 24% to 57% (3). One explanation might be that quinine is often used as an alternative to chloroquine, but in insufficient dosages. Mutations have also been found indicating increasing resistance to sulfadoxin and pyrimetamine, but unlike in Southeast Asia no increased tolerance or resistance to artemisinin has been observed.

In order to monitor the development of resistance on a molecular level there is a need to collect blood samples from infected patients, including from areas with poor infrastructure and without electricity. The rapid diagnostic tests now routinely used for the diagnosis of malaria in all health centres and hospitals can to some extend be used for sequencing well-characterized polymorphisms that cause resistance. However, studies have shown that blood samples collected and dried on filter paper and stored at room temperature give a higher output than the 5 µl blood obtained from the rapid diagnostic tests and can be used for full sequencing of the parasites, allowing for monitoring of genetic development across the entire genome over time (4, 5).

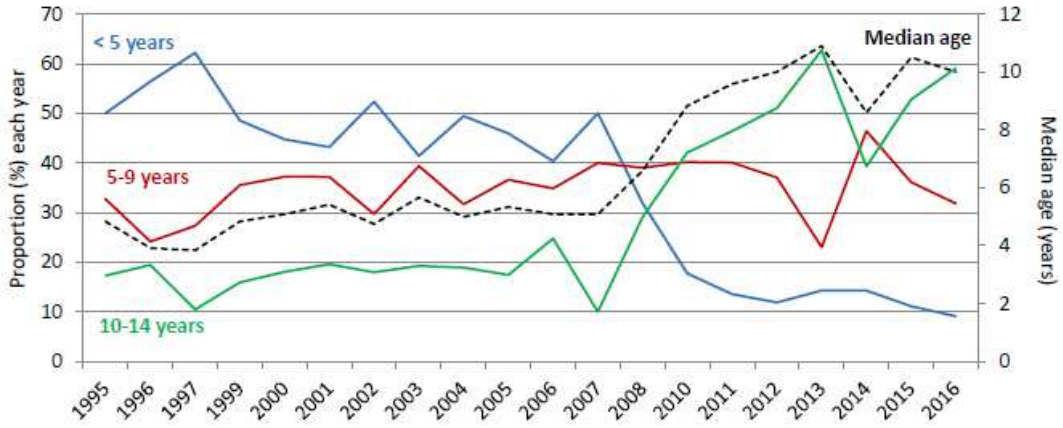

Figure 2. The annual proportion of children aged less than 5, 5-9 and 10-14 years with malaria and the median age of children aged less than 15 years attending the Bandim Health Centre and diagnosed with malaria.

Public health implications and future perspectives

The immunity of the children is decreasing causing a shift in the age-groups getting malaria. Until now, the higher incidence in the older age-groups has not caused an increase in mortality, but the development should be carefully monitored. That the incidence of malaria is decreasing could imply that it is even more important to have an effective treatment. With the increasing knowledge of the molecular basis for resistance in the parasites a cheap, easy and effective procedure for monitoring the development of resistance is in sight. We have shown that dried blood spots can be collected and stored even under difficult conditions and without electricity. Rapid diagnostic tests have been shown to also yield an output sufficient for identifying the most important mutations, but the methodologies still need to be refined. Collecting and using the rapid diagnostic tests for monitoring is, however a very promising possibility.

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39. Diabetes

Background

Diabetes rates are rising rapidly across the globe, with sub-Saharan Africa experiencing the biggest relative increase. Diabetes has thus become one of the major contributors to mortality and morbidity in the region. Efforts to reduce the burden of diabetes are, however, often hampered by a lack of country specific data, along with good treatment options.

Real-life data

In Guinea-Bissau, the International Diabetes Federation currently estimates an overall diabetes prevalence of 2.4%, though recognising paucity of data. At the Bandim Health Project, we found a diabetes prevalence of 5.8% among patients attending the local HIV clinic in Bissau (1). A study investigating whether diabetes contributes to the development of tuberculosis found a prevalence among randomly selected population controls of 2.1% (2). Recently, a Portuguese group found a very high diabetes prevalence of 14.3% among personnel at four military complexes (3).

Figure 1. The national diabetes clinic in Bissau city.

For many years, diabetes received little attention in Guinea-Bissau. This, however, changed in 2006 when Dr. Luis Carlos Joaquím returned from Russia, where he had been working for two decades, specialising in diabetes. With the help of Bandim Health Project (BHP) and a small dedicated staff, he established the only diabetes clinic in the country. In 2011, at the height of his career, he received a large World Diabetes Foundation grant to further increase local diabetes awareness. Sadly, Dr. Joaquim died the following year after a short illness, but his clinic continues to attend patients.

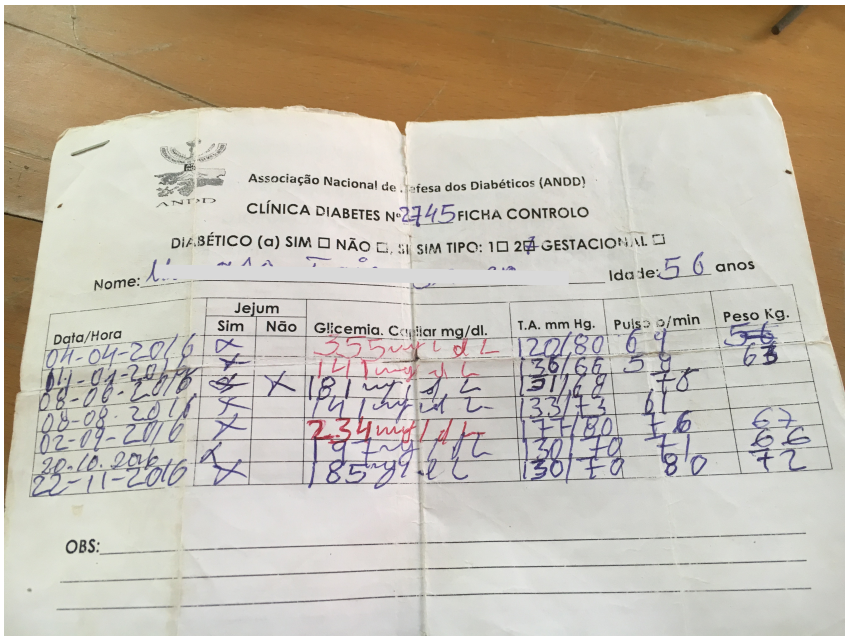

Figure 2. A patient record from a person attending the clinic.

In 2013, the BHP undertook a study aimed at characterising patients who attended the diabetes clinic and comparing them with age and sex matched controls recruited from the BHP study area (4). An astonishing 20.4% of the randomly selected community controls had high fasting glucose (FPG) levels, 8.7% within the diabetic range. A significantly higher proportion of diabetes patients had a family history of diabetes and hypertension as well as elevated waist circumference, compared with the community controls with normal FPG. Worryingly, 39% of the diabetes patients were severely dysregulated with FPG levels above 15.1 mmol/l. Approximately 65% of the patients were prescribed anti-diabetic medication, though often only taken if the patient could afford it. Mortality was 3.6 times (95%CI: 1.96-6.68) higher

among diabetes patients than among community controls over an observation period of 3.3 years.

Insulin was until recently only available in Bissau through parallel import from neighbouring Senegal. This meant that the supply of insulin was unstable and the prices were twice as high as the prices in Senegal. In August 2018, after several negotiations with Novo Nordisk, insulin is now sold directly to Guinea-Bissau at an affordable price.

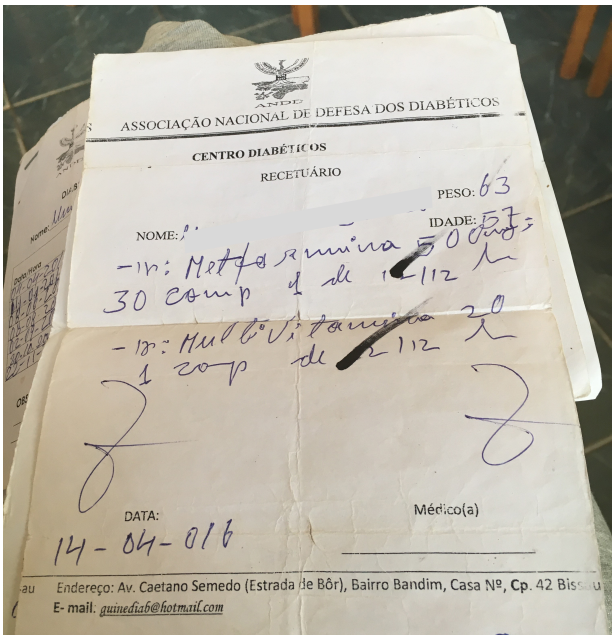

Figure 3. A medicine prescription for a diabetes patient in Guinea-Bissau.

Future perspectives

Several new studies are under development. In a recent randomised trial conducted in the US, two doses of BCG vaccine, given 4 weeks apart to patients with type 1 diabetes, lowered hemoglobin A1c (HbA1c) to near normal levels after 3 years, and the effect was maintained for the next 5 years (5). We aim to conduct a study in Guinea-Bissau, where we will investigate the effect of providing two BCG vaccines 4 weeks apart on the HbA1c levels and FPG levels in individuals with prediabetes. BCG is a cheap intervention, and if it has the intended effects, it can provide a better glycemic regulation for currently poorly treated diabetes patients.

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40. Stillbirths

Background

Stillbirth rates remain high in sub-Saharan Africa, with an estimated average of 32 per 1000 births (1). Half of these are estimated to be intrapartum, i.e. stillbirths, which occurred after the onset of labour and before birth (2). Until recently, international efforts largely focused on the reduction of neonatal deaths (2). As an example, stillbirths did not feature in the 2015 Millennium Development Goals. Whilst more attention is now given to stillbirths, the dearth of reliable country-specific data has severely hampered efforts to prevent stillbirths(3).

Real death data

The global estimates of stillbirth rates are largely based on cross-sectional surveys, where women of fertile age are interviewed regarding their obstetric history. However, it is well known that pregnancies not resulting in a live birth may be selectively underreported. Bandim Health Project (BHP)'s Health and Demographic Surveillance System data (which includes pregnancy registration) creates a reliable platform to measure the real stillbirth rate, basing the estimates on prospectively followed pregnancies rather than retrospectively captured stillbirths.

Figure 1. Maternity ward, National Hospital Simão Mendes, Bissau.

We studied stillbirth rates in the BHP study area between 2007-2013, in order to determine the community stillbirth rate (1). At the same time, we investigated stillbirths at the National Hospital Simão Mendes, with particular focus on fresh (intrapartum) stillbirths, as these are often preventable through low-cost interventions. The two cohorts were partly overlapping, as many women from the Bandim study area gave birth at the National Hospital.

We found a very high stillbirth rate of 50 per 1000 births in the community, indeed one of the highest observed in Africa. Stillbirth was strongly correlated to lack of antenatal care. At the National Hospital, the overall stillbirth rate was 81/1000 among women from Bissau, with 70% of these being preventable intrapartum deaths (based on maternal reporting of foetal movements). The higher hospital stillbirth rate likely reflected selected referrals of complicated deliveries, but may also have been the result of sub-standard care. Specifically, the risk of fresh stillbirth was correlated to the time of delivery, with the highest rate observed around 8 pm (82/1000) and the lowest around 2 am (30/1000). Interestingly, the hospital caesarean section rate increased significantly during the study (from 120/1000 in 2007 to 178/1000 in 2013, $p<0.001$); this did not result in fewer stillbirths.

Public health implications and future perspectives

In summary, we identified an alarmingly high stillbirth rate in Bissau (1). Most of these deaths were likely preventable through a combination of affordable community interventions (e.g. better antenatal care and maternal education) and improvement in hospital procedures (e.g. improved emergency obstetrical training and labour management). Additional focus should be on the judicious use of caesarean section, as the immediate benefits of the procedure must be balanced both against significant short-term risks (e.g. infection, bleeding, anesthetic complications) and long-term risks (uterine rupture or *placenta accreta* in a future pregnancy). Careful labour management and increasing local capacity to perform assisted vaginal deliveries may be equally important. Finally, more data is needed concerning fatal delays during labour and birth, including “patient delays” in seeking help and getting to the hospital, but also “doctor delays” in preventing, recognising and acting on birth asphyxia and maternal and foetal compromise.

Figure 2. Outside the maternity ward.

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41. Twins

Background

Twins are intriguing from biological, sociological and cultural perspectives. As a result, numerous twin registries have been established, mainly in high-income countries. The classical twin study typically aims to estimate the relative influence of familial factors, including genetic and shared environmental factors. Another strength of twin research is the ability to study factors influencing traits and disease phenotypes, controlling for familial confounding. Also, twins have a high frequency of intrauterine growth restriction and prematurity, accompanied by increased mortality and morbidity around birth, but also possibly connected with long term detrimental effects ("thrifty phenotype hypothesis")(1, 2). Finally, twin studies can be used in "natural experiments" regarding the effect of crowding and infectious disease exposure.

Figure 1. Twin pair and their mother.

Though Africa has the highest natural twinning rate in the world, there has been little research on twins. This constitutes a gap, not least since twins now account for 11% of the overall child mortality in the region (3). This estimate is expected to increase, making African twin studies pertinent.

Real-life data

In 2009, we established one of the first African twin registries (1). Briefly, twins accounted for 3.5% of all newborns in Bissau, with newborn twins having an extremely high perinatal mortality of 22%, compared to 8% in singletons (RR=2.71, 95% CI: 1.93-3.80). In 65% of the twin pregnancies, the mother was unaware of carrying twins. Excess twin vs. singleton mortality continued until the first 3 months of life (4). Among newborn twins, the most important risk factors of mortality after hospital discharge were birth weight<2000 g, death of co-twin perinatally and severe maternal illness during pregnancy, particularly HIV infection. Worryingly, twin hospitalisation rates were similar to singletons, suggesting underlying barriers to hospital care in emergency situations. Together, these findings underscore the need for a comprehensive strategy during pregnancy, intrapartum and postnatally to reduce twin mortality, including prevention of malnourishment (5).

Figure 2. Field examination of infant twin.

Apart from describing the twinning rate and infant twin mortality in Bissau, we also studied whether low birth weight among twins predisposes them to various dysglycaemic disorders. This is of interest, since foetal conditions may be quite different in low-income settings, for example, due to other types of food intake and frequent infectious diseases. In a cohort of participants aged 5-32 years, we did not find a higher rate of diabetes, metabolic syndrome or impaired glucose tolerance among twins, compared to singletons. This occurred despite the average twin birth weight being 680 g lower than for singletons. Twins did, however, have higher median glucose levels in both fasting and in the post-prandial state after glucose intake, which could potentially relate to conditions during foetal life (2). Recent analyses of our data also suggest a higher body fat percentage among twins.

Noteworthy, due to the potentially harsher conditions during foetal and early life, the metabolism in twins in Guinea-Bissau could be comparatively more affected later on, than what is seen in e.g. Western populations. However, it could also be argued that twins surviving beyond childhood in Guinea-Bissau are strong individuals, thereby exhibiting resilience towards e.g. dysglycaemic disorders. In fact, it has been suggested that twinning in Africa (which has a strong genetic component) may even be protective against certain diseases in adulthood (1).

Figure 3. Preparing for oral glucose tolerance test.

Future perspectives

The most important aspect of our work has been the creation of a registry that can be used in international twin comparisons, and also in examining variations in health and disease presentation in Guinea-Bissau, using the different types of twin study designs. The registry will likely also be very valuable in determining life-course health among twins in the population. The cohort is currently part of an international collaboration between twin registries, and a biobank has been established. One obvious future study here would be to repeat the above glycaemic investigations in 10-20 years.

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PETERARBY