BANDIM HEALTH PROJECT

2003-2008

IMPROVING CHILD SURVIVAL

BANDIM HEALTH PROJECT 2003-2008

Projecto de Saúde

BANDIM

Thanks

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. They have supported the project ever since. We pursued the implications of this observation but often failed in our subsequent attempts to improve child survival. However, the mothers continued to collaborate with the project appreciating the interest in the health of their children. Hopefully we can continue to demonstrate this concern.

To the many individuals who have worked for the project over the 30 years, more than 400 Guinean assistants, drivers, accountants, lab technicians, nurses, and physicians, and more than 120 expatriate students, volunteers and experts.

To those who supported our work but also to those who claimed our results were impossible, unplanned, and biologically implausible and obliged us to document even better that most of our observations are reproducible. New knowledge does not start by planning to see the expected and biologically plausible. New knowledge starts by pursuing the contradictions in our common assumptions and seeing the same pattern again and again. To those who helped seeing something new.

Content

Childhood interventions

Maternal Mortality

HIV and Tuberculosis

Bandim Health Project 2003-2008:
Improving child survival

This is a collection of highlights of major research themes investigated by the Bandim Health Project (BHP) during the last 5 years, as well as a bibliography of what has been published. The background to this research has been described in the previous booklet from the first 25 years: 1978-2003. That booklet also contained a description of how Health and Demographic Surveillance System (HDSS) research is conducted in Bissau.

The main objective of the BHP has remained the same: through the HDSS to assess the overall impact of the common interventions sponsored by international donors. The last 5 years has been a period of both consolidation and expansion with respect to geographical and institutional study area, capacity building, research themes, and local and international collaboration.

Geographical and institutional research areas

In 2002, BHP included the two adjacent districts Cuntum 1 and Cuntum 2 (see Map) in order to follow a population large enough to study the impact of interventions on child survival. By now the BHP's HDSS covers slightly more than 100,000 people in the capital, or around 30% of the capital's population. In the interior the cluster sample surveillance system has been extended from the original 5 regions to include all 10 health regions in the country. Hence, we now cover the whole country with two mobile teams which visit the 20 clusters in each region every six months to collect information on important health indicators. This expansion was made possible by support from the Ministry of Health and a grant from the World Bank through a two year period to collect information on health indicators for the national plan of health. The challenge now will be to secure funding to maintain this national coverage.

We have also expanded in terms of institutional collaboration. Going back to 1990, BHP has registered the children from the study area who were hospitalised at the national hospital's paediatric ward. This collaboration has gradually been extended in 1997 to include all children hospitalised at the ward and more recently to registration of all outpatient consultations. BHP has become more and more involved in research of how to manage the paediatric ward as described in Chapter 9. During the last 6 years we have also expanded the collaboration with the national hospital's maternity ward including most of the children born at the hospital in various trials to assess the impact of the first interventions in life like BCG vaccination and administration of oral polio vaccine. Around 60% of the deliveries in the project area occur at the maternity ward of the national hospital. Since 1990 we have registered all children who were born at the ward. Now we administer BCG vaccination to all children and have recently helped with starting HIV screening for the mothers giving birth at the ward. It has always been possible to follow the children from the study area long-term. Low-birth-weight (LBW) children are a high risk group with a very high mortality. In order to have a sufficiently large group and to be able to follow them long-term we are enrolling all LBW children born at the ward and drive them home wherever they live in Bissau in order to have proper identification of their residence.

Though child mortality has been the main theme of BHP, we have gradually expanded research to questions of adult health, particularly tuberculosis (TB) and HIV infection. This has also involved increasing collaboration with the national programmes for control of TB and HIV. As will be apparent from Chapter 32 we have become intensively involved in the national programme for administration of antiretroviral treatment.

Capacity building

DANIDA's ENRECA programme has funded a significant proportion of the BHP's operations in order to strengthen research capacity in Guinea-Bissau. The programme has involved

English language training since English is the language of science. Computer literacy is uncommon in Guinea-Bissau and a good deal of training has gone into improving the capacity to use computers. All the 12 candidates have gone for master courses in international health or epidemiology abroad, in Denmark, Sweden, UK, or Brazil. However, the key theme of the training has been that they should study health issues in Guinea-Bissau so all have been involved in data collection in Bissau. Those who have gone on to conduct PhD research have all done their data collection and most of the analysis in Guinea-Bissau. PhD candidates have taken part in intensive courses abroad and have been to Denmark for several months for the write up of their thesis. So far Amabelia Rodrigues has defended her thesis on the protective effect of lime against cholera and became the first PhD in health in Guinea-Bissau. Two candidates have submitted their theses. Two are in the process of writing up and a further three are enrolled.

BHP has also been intensively involved in research training for Europeans. Without getting outside capacity and funding for such research project it would not have been possible to secure the critical mass of researchers in Bissau and to maintain our research agenda. During the last 5 years 16 candidates from Denmark, Norway, Sweden and Holland have defended their theses based fully or partly on date collected in Guinea-Bissau. A further five candidates are currently enrolled in PhD programmes.

Research agenda

Most of the interventions sponsored by international donors are based on a very simple model of looking at one health problem at a time - for example, measles infection, breastfeeding, malaria treatment, malaria control, or vitamin A deficiency - and designing a solution to that particular problem. Time and again we have experienced that the impact of the interventions on child health may be totally different from what was anticipated because many of the interventions are immuno-stimulatory and may have an impact on how the child can handle other infections but also because different immuno-stimulatory interventions may interact producing unexpected results. This lesson was learnt during the first years of the project when we saw the dramatic effect of measles vaccination on child mortality, mortality declining with more than 50% from one year to the next (see Chapter 2). It took 15 years to understand that this effect could not be explained with current concepts. It was not protection against acute measles infection or prevention against long-term negative effects of measles infection which explained the impact on survival. The experience with high-titre measles vaccine (HTMV) increasing female mortality even though the vaccine was fully protective against measles infection provoked the idea that vaccines may have non-specific immune stimulatory effects with a major impact on child survival. We have gradually through the 1990s and early 2000s extended this idea to the other routine vaccinations like BCG, oral polio vaccine (OPV),

diphtheria-tetanus-pertussis (DTP) vaccine, hepatitis B vaccination (HBV), inactivated polio vaccine (IPV), and smallpox vaccine (Chapters 13-23). In our observational studies the patterns have been very systematic, the vaccines often producing sex-differential effects. Interestingly the idea has also been extended to micronutrients. The impact of vitamin A supplementation (VAS) can not be explained by the common vitamin A-deficiency hypothesis. VAS is also an immuno-stimulant and it interacts apparently with vaccinations, producing totally unexpected results (Chapters 25-29).

These observations have been very controversial because they question major assumptions underlying the current standard intervention programmes to reduce child mortality. Apart from withdrawing HTMV, the international public health community has not taken the observations on non-specific effects of vaccines and vitamin A into consideration and mostly dismissed this as observational studies from Guinea-Bissau. We have therefore during the last 5-6 years extended collaboration with other HDSS to examine the data they had on vaccination. In studies from Malawi, Senegal, The Gambia, Sudan, Congo, and Ghana we have been able to document similar patterns of non-specific and sex-differential effects of vaccines and VAS. However, importantly we have also started several randomised clinical trials to document the existence and importance of non-specific effects of several different vaccines and VAS. As indicated in later chapters, these studies have documented non-specific and sex-differential

effects. However, it has not always been the results we had planned. The main reason is that there are far more interactions than is normally appreciated; VAS campaigns and administration of other vaccines may have an impact on the survival of the vaccinated children enrolled in a trial. For example, the negative effect of HTMV for girls was not due to HTMV per se but the DTP vaccinations administered after HTMV (Chapter 18).

There has recently been more interest in non-specific effects of vaccines and the Global Advisory Committee on Vaccine Safety of the WHO has declared that it will keep a watch on non-specific effects of vaccines but also that observational studies will not be sufficiently convincing evidence if they stand alone. Hence, further randomised trial will be needed. Given what we know now about interactions between different interventions and the fact that mortality levels are declining (Chapter 2), this will be very demanding.

- 70% recebeu anti-paludico
- 64% recebeu antibiotico
- Dos q receberam Anti-paludico 5% tambem recebeu antibiotico

We have also used the population basis to conduct other intervention trials for example, of the use of prophylactic antibiotics in the management of measles infection (Chapter 5). Though such use of antibiotics has been controversial, it did in fact reduce severity of measles infection. Following a previous unplanned observation of no benefit from encouraging exclusive breast-

feeding, we conducted a randomized clinical trial of intensive promotion of exclusive breast-feeding. The control group was visited in a similar way as the intervention group but was left to follow the usual practices for introduction of water and supplementary feeding. Though the intervention group did follow advice and delayed the introduction of water and supplementary feeding, they experienced no health benefit from being compliant. Children in the intervention group grew worse and had slightly, but not significantly, higher mortality than the control group (Chapter 24). The long-term perspective has made it possible to follow the girls we vaccinated against measles 20 to 25 years ago and see that mothers who have been vaccinated in childhood have much lower antibody levels than women who have natural measles infection. These antibody levels are transferred to the children and children born to immunized mothers are therefore becoming susceptible much earlier than before. This became clear during a recent measles epidemic in Bissau, nearly 20% of the children getting measles before 9 months of age the current recommended age for measles vaccination (Chapter 19). During the outbreak we managed to show that it is possible to vaccinate effectively against measles already at 4½ months of age.

Following a large adult population we have in the recent decade started looking at risk factors for adult morbidity and mortality (Chapter 22, 30-35). So far we have conducted two trials to examine whether postpartum haemorrhage could be reduced with misoprostol (Chapter 30)

and whether vitamin D improved efficacy of TB treatment (Chapter 34). More such trials are likely to be conducted in the future.

Local and international collaboration

After 30 years, BHP is finally – with support from the Danish National Research Foundation and the Novo Nordisk Foundation - getting it own buildings for housing the research activities and archives (see Photo). The health sector in Guinea-Bissau is becoming increasingly aware that BHP is an important provider of trained people and of data on the current health situation in Guinea-Bissau. BHP is therefore also one of the key institutions in the National Institute of Health recently formed in Guinea-Bissau.

Through the last 5 years the international collaboration has grown in importance. There are signs of increasing donor interest in strengthening local research capacities and in collaboration with the MRC, The Gambia, we are involved in plans to strengthen research training and collaboration between different West African institutions. The research collaboration with MRC in the areas of retroviral infections and non-specific effects of vaccines has also been expanded.

BHP has been part of many research initiatives within the framework of the INDEPTH Network and we have reanalyzed data from several INDEPTH sites. We have also established a working group within INDEPTH which will

deal with overall impact of interventions in childhood, particularly vaccinations and micro-nutrients. In a longer perspective these sites will hopefully be interested in measuring the impact of interventions in randomised trials. Ideally the INDEPTH Network has the potential to measure the overall impact of donor interventions in different settings with interaction with different interventions. This could make a real difference for child survival if interventions were properly tested before they were introduced.

Bandim Health Project 2008-2013

Five years ago we predicted that vaccine issues will dominate the research agenda for years to come. Our results have often been considered impossible, unplanned and biologically implausible. However, they are repeatable and that is where scientific inquiry should start. With all the results we have obtained in the last five years, it can safely be predicted that vaccines will continue to dominate the research agenda. However they will do so in interaction with other immuno-stimulants like vitamin A and other micronutrients. It will also become increasingly important that boys and girls react differently to many of these interventions. Hence, it might be better in some situations to treat boys and girls differently. It will be very important to conduct more research to understand the immunological mechanisms involved in both beneficial and negative non-specific effects. Though we have used mortality as the main outcome in most of our trials, mortality is luckily declining

and it will be necessary to develop a consensus within the international health community about which morbidity and growth patterns and immunological reactions can be used as proxies for beneficial and negative non-specific effects. New vaccines – for example rotavirus vaccine, malaria vaccine, pneumococcal vaccine, mono-valent poliovaccine and HPV - are becoming increasingly important for the international public health agenda. Given our previous observations such new vaccines will have to be tested for possible non-specific effects and for interactions with other vaccinations if we are to gain the full benefit of their targeted effects.

The new office of the BHP

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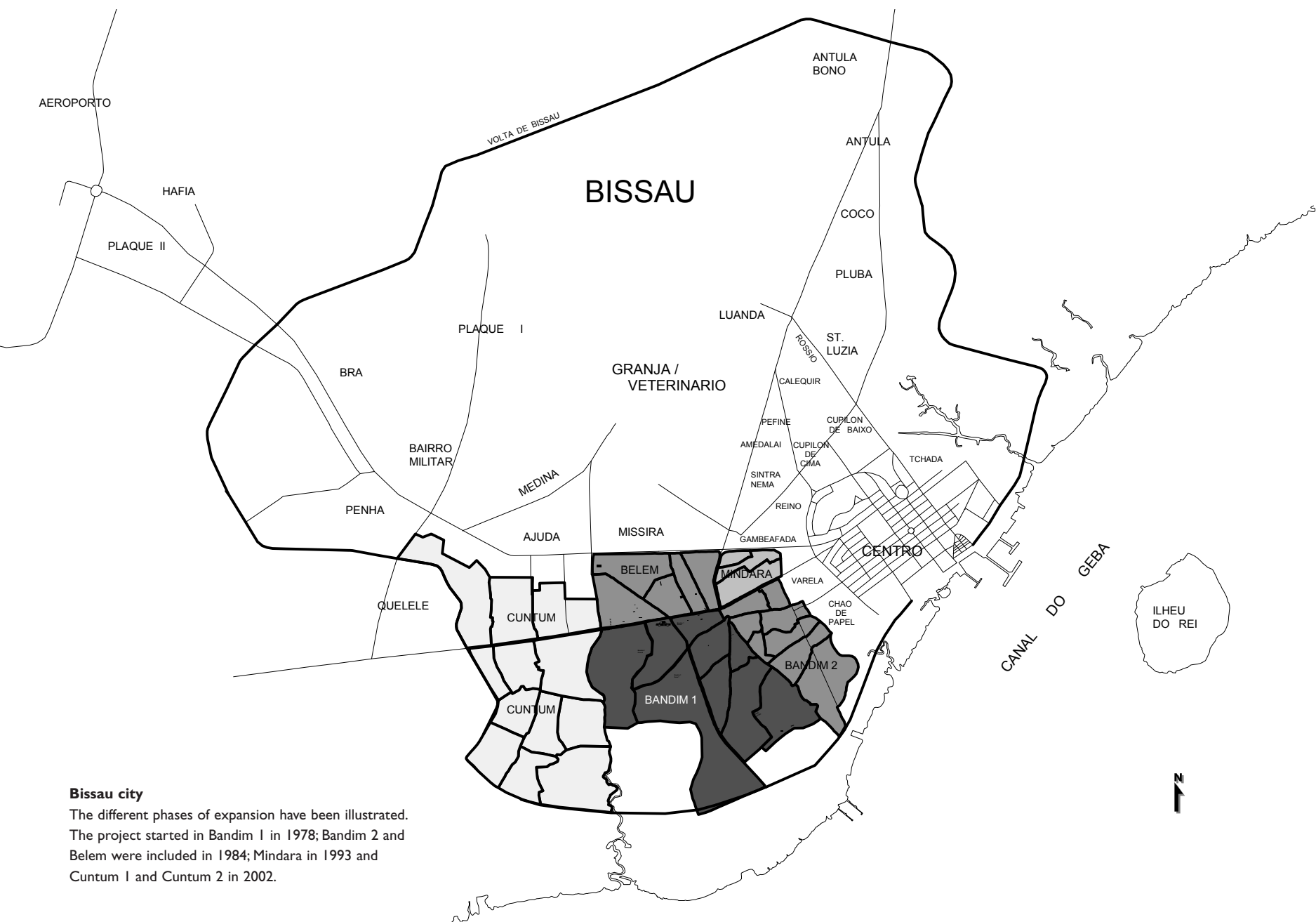
Bissau city

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.

Child mortality in Bissau
– the last 30 years

The Bandim Health Project (BHP) has followed the population in the district Bandim 1 in the capital Bissau since December 1978. At the time the population was 6,200. The population followed in Bissau has gradually increased to more then 100,000, both because the population concentration has increased dramatically – Bandim 1 has now more than 30,000 inhabitants - but also because we have gradually included the neighbouring districts. Bandim 2 and Belem were included in 1984, Mindara in 1993, and Cuntum 1 and Cuntum 2 in 2002 (see Map in Chapter 1).

Over the 30-year period from 1978 to 2008, child mortality has changed in major ways (Figure 1), both due to improved public health interventions like immunisation and improved general living conditions. But there has also been counter trends as for example during the civil war in 1998-99. A major component of under-five mortality is neonatal mortality (Figure 2). The stillbirth rate (Figure 2) is not counted in child

mortality but is a major health problem. It should be noted that the combined rate of stillbirths and neonatal deaths (peri-neonatal mortality) has remained very stable around 9-10% over the last 20 years. It seems that peri-neonatal mortality increased in the 1980s. It is possible
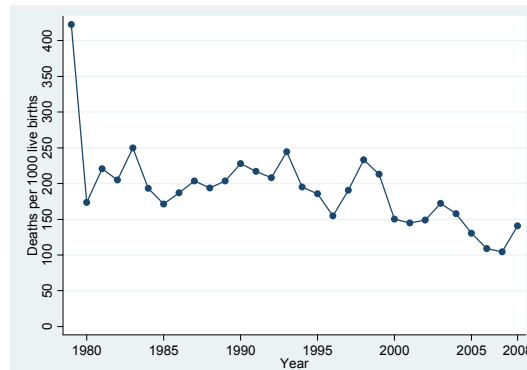
Figure 1. Under-five accumulated mortality in the study area in Bissau. 1978 to 2008.
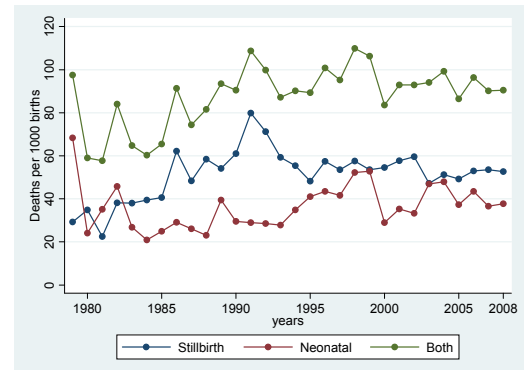
Figure 2. Stillbirth rate and neonatal mortality rate in the study area in Bissau. 1978 to 2008.

that this is partly a registration problem. We have become better in registering the pregnancies and we may therefore simply have found more of the early stillbirths. However, it is worth noting that neonatal mortality also increased from the late 1980s to the mid-1990s.

Pattern in child mortality over the last 30 years

Initial immunisation campaigns: 1978 – 1980. After independence in 1974 the under-five mortality in Guinea-Bissau was extremely high (around 500/1000). A census was conducted in Bandim 1 in the fall of 1978 and a survey to study the nutritional status and child health was carried out in December 1978. During the first year we did register an under-five mortality of more than 400. From the very beginning of 1979, we organised antenatal consultations and anti-tetanus vaccination to the pregnant women. Hence, the neonatal mortality of 70/1000 we registered during the first year was probably lower than it was prior to the introduction of childhood interventions. At the time there was no routine childhood vaccination programme in Bissau. Following the experience with a very severe measles epidemic with a case fatality of 21% for children less than five years, we arranged a general measles immunisations campaigns for all children aged 6 months to 6 years of age when we re-examined the children nutritionally in December 1979. A similar measles vaccination campaign was organised again in December 1980. It will be seen that the decline in mortality

was dramatic (Figure 1). The under-five mortality dropped to around 200/1000, a level which has been maintained through most of the 1980s and 1990s.

Routine immunisation by BHP: 1981 – 1986. Having seen the impact of measles vaccination we started from 1981 introducing routine vaccinations, including OPV, DTP and measles vaccine, through 3-monthly community sessions in each sub-district of the study area in which all children less than 3 years of were weighed and vaccinated. Malnourished children were identified. Initially the central vaccination programme was not willing to let the nurses in the study area use BCG as this vaccine was considered to difficult to administer correctly. The mothers had to go to the central vaccination office in the city. Few did. Only from 1983-1984 did the health authorities start using BCG at the main maternity ward and we were allowed to use BCG in our community vaccination sessions. The coverage for BCG increased dramatically. There was no indication that mortality declined in this period, if anything it increased slightly.

Routine immunisation: 1986 – 1997. In the latter part of the 1980s the national immunisation programme had become more established and BHP stopped organising the outreach vaccination sessions in Bandim. Hence throughout this period the public health agenda was defined by the many UNÌCEF and WHO-sponsored programmes to reduce mortality, including immunisations, essential drugs, and diarrhoea management. The impact on under-five mortality levels

was limited (Figures 1 and 2). There was no trend towards declining mortality levels.

Through the 1980s and 1990s BHP was involved in a series of measles vaccination trials and breastfeeding promotion studies. These studies helped secure a high coverage for measles vaccination in the study areas.

Civil war: 1998 – 2000. In 1998-1999, Guinea-Bissau was hit by a civil war. For several months all people fled from the capital and lived under miserable conditions as internally displaced people in the interior of the country. The public health system broke down. It will be seen that both neonatal and under-five mortality increased in this period. Though the health care system had not become fully operational, there was a major drop in mortality in 2000. In fact the under-five mortality seems to have shifted from 200/1000 to 150/1000 around 2000. We have no clear explanation for this change. During the war we provided impregnated bed nets to all pregnant women and young children in the study area as well as in large parts of Bissau. This could have been important. The general measles vaccination campaign for children under five years of age in the end of 1999 and the oral polio vaccine and vitamin A campaigns organised from then on may also have been important.

Intensive health interventions: 2001 – 2008. Prior to the war the intervention studies conducted by BHP had mainly focused on measles vaccination. After the war BHP initiated several other projects related to BCG vaccination, micro-nutrient supplementation to pregnant women and vitamin A supplementation to children both at birth and later in life. This means that most newborns and infants are recruited for a trial. As part of these studies we have also provided free consultations and some essential drugs. This may have been important for the decline in mortality that seems to have occurred in the last 7 years. In 2003-2004 there was a small peak in mortality probably related to a large and severe measles epidemic.

Public health implications

In conclusion, mortality dropped dramatically when we introduced tetanus vaccinations to the pregnant women and measles vaccination. Through the 1980s and 1990s with the many programmes to improve child care and child survival, mortality changed very little. Surprisingly mortality started declining after the war before the public health system had become fully operational. Since neonatal mortality has remained stable, the decline in post-neonatal mortality is even more dramatic. The better health services provided to children in the study area as well as the many campaigns and decline in malaria morbidity may have contributed to this result. It will be one of the challenges to determine the relative importance of these interventions. It will also be a major challenge to reduce the very high peri-neonatal mortality.

ROTAVIRUS

Background

There is currently a global effort to accelerate the introduction of new vaccines against rotavirus, one of the most severe diarrhoeal diseases. These attempts, which are supported by major health organisations including WHO and GAVI, are explained by the fact that infants and young children worldwide are heavily affected by rotavirus disease; unlike other diarrhoeas, the common hygienic measures appear to be ineffective in preventing this infection and no specific treatment is available for rotavirus disease. Thus, vaccination is the cornerstone strategy to control rotavirus infection. Recent estimates suggest that diarrhoea accounts for 13% to 21% of all under-five deaths. Globally, it is estimated that 20-70% of all hospitalisations due to diarrhea in young children and 20% of diarrheal deaths are caused by rotavirus. Although high-income as well as low-income countries are affected by rotavirus disease, the death-toll is substantially higher in developing countries, and in Sub-Saharan Africa alone, approximately 110,000 to 210,000 children die each year from rotavirus infection.

Results

Rotavirus hospital surveillance: In Guinea-Bissau, rotavirus infections exhibit a seasonal pattern with annual epidemics occurring during the relatively dry and cooler months, from January to April, with few cases registered from May to December. In the hospital setting rotavirus accounts for a high case-fatality ratio (8%) and a high rate of nosocomial transmission: during the rotavirus season 23% of all children admitted for non-rotavirus diarrheal disease acquired rotavirus infection during admission (>48 hours upon admission). These results cor-

roborate the idea that rotavirus is one of the most contagious pathogens within the paediatric wards and underscore the need for prevention of disease prior to hospital admission (168,204)

Prophylactic vitamin A supplementation (VAS) and rotavirus morbidity: VAS administered to children above 6 months of age in low-income countries has been associated with reduction in mortality from diarrhoea. However, studies of the prophylactic effect of VAS on diarrhoea morbidity have provided diverging results. During the rotavirus season 2005, we examined the effect of VAS on diarrhea disease in infancy in a randomised trial and in particular whether VAS would alter rotavirus colonization and morbidity. Contrary to expected, VAS did not reduce rotavirus colonization and rotavirus diarrhoea; VAS was associated with a significantly higher incidence of rotavirus colonization and diarrhea in the youngest children (Figure). There was no overall effect of VAS on non-rotavirus diarrhoea but the effect differed significantly by sex, being beneficial in boys but not in girls (205).

Future perspectives

In order to study the impact of rotavirus vaccines on morbidity and mortality among Guinean children we attempt to administer rotavirus vaccines to infants in the near future. Assuming that the rotavirus vaccine proves to be as protective against rotavirus disease and mortality in

Guinea-Bissau as elsewhere, and therefore a likely candidate to be introduced to the childhood immunisation programme in Guinea-Bissau, it will be competing with several other effective interventions against childhood diseases. Therefore the vaccine cost effectiveness still has to be demonstrated, and upcoming plans are to conduct a rotavirus cost-effectiveness study.

References on rotavirus:
21, 22, 37, 168, 204, 205

Figure.
Cumulative incidence curves, by sex and presence of rotavirus.

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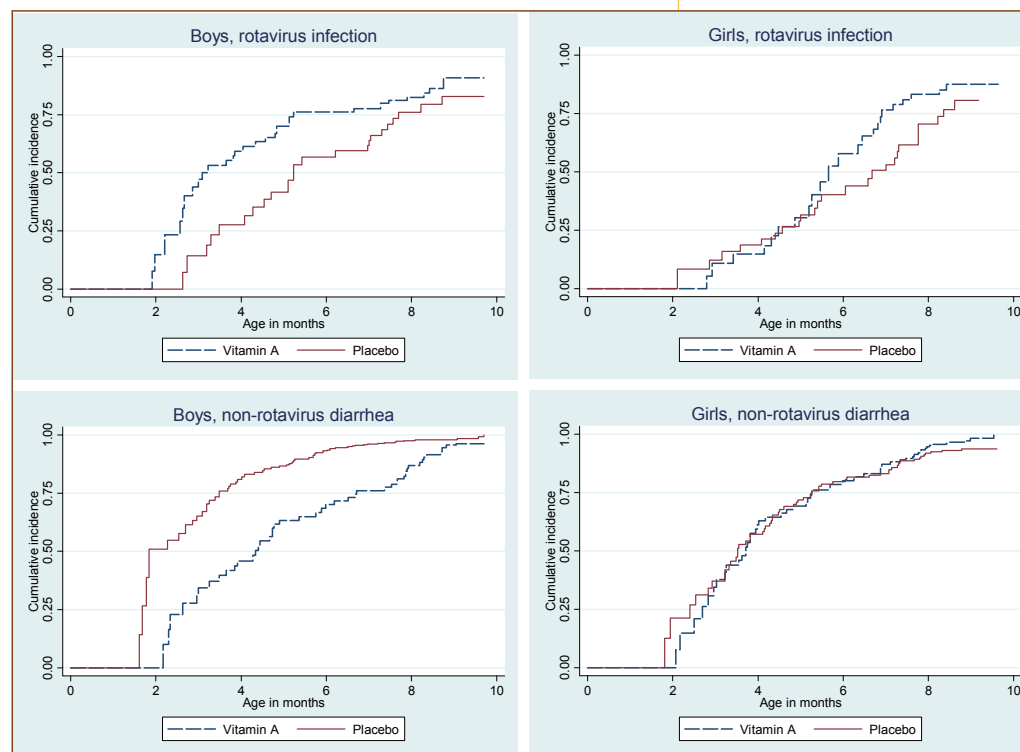
Long-term consequences of Chickenpox in Guinea-Bissau

Background

There are virtually no studies of chickenpox in Africa. Based on a large outbreak of chickenpox in Bandim in 2000-2001, we described how the age distribution of chickenpox cases was similar to high-income countries. As for measles infection, intensity of exposure was an important determinant of severity of chickenpox. Although overall acute mortality was low many children had pneumonia and skin infections.

In contrast to previous ideas, community studies of measles have suggested that the long-term consequences of infection may be beneficial among the children who survive the acute phase of measles infection. We therefore examined the long-term impact of chickenpox infection.

Results

A total of 111 cases and 111 matched controls were examined in a 6-month follow-up study. There were no significant differences in background factors for these two groups. Weight, height and mid-upper-arm-circumference (MUAC) did not differ for cases and controls at the time of chickenpox infection or prior to infection. However, six months after chickenpox infection, cases had grown better than controls with respect to weight, MUAC and height. For all three anthropometric measurements, significant differences were found only for girls; there were no differences for boys (see Table).

The frequency of consultations within the last month had been high. In both groups 42% had been to a health centre or the hospital; more controls (16) than cases (6) had consulted the paediatric ward. In the month before the exami-

Anthropometry for cases and control at the time of chickenpox diagnosis and at follow-up

	Cases	Controls	Paired Analysis
TIME OF CHICKENPOX			
Mean age at inclusion (days)	334 (305-364)	332 (301-362)	P = 0.70
Mean weight at inclusion (kg)	8.6 (8.2-8.9)	8.5 (8.2-8.9)	P = 0.96
Mean MUAC at inclusion (mm)	145 (143-148)	143 (140-145)	P = 0.12
FOLLOW-UP: 6 MONTHS AFTER CHICKENPOX			
Mean age at follow-up (days)	499 (469-529)	504 (473-536)	P = 0.45
Mean time of follow-up (days)	165 (162-168) F: 168 (164-173) M: 161 (157-165)	173 (166-179) F: 173 (165-181) M: 172 (162-183)	P = 0.01 P = 0.21 P = 0.02
Mean weight at follow-up (kg)	9.5 (9.2-9.8) F: 9.5 (9.1-10.0) M: 9.5 (9.1-9.9)	9.2 (8.9-9.5) F: 8.8 (8.5-9.2) M: 9.5 (9.1-9.9)	P = 0.04 P < 0.01* P = 0.96*
Mean growth (g/day)	5.8 (4.8-6.7) F: 5.5 (4.0-7.0) M: 6.0 (4.8-7.2)	3.4 (2.2-4.6) F: 2.6 (0.9-4.3) M: 4.2 (2.4-6.0)	P < 0.01 P < 0.01 P = 0.07
Mean MUAC at follow-up (mm)	147 (145-149) F: 147 (144-151) M: 147 (144-150)	143 (141-146) F: 141 (139-144) M: 145 (141-148)	P = 0.01 P = 0.01 P = 0.27
Mean growth in MUAC (100*mm/day)	1.3 (0.4-2.2)	0.5 (-0.6-1.6)	P = 0.23

Note: interaction between the effect for boys and girls, p<0.04

nation, cases had used antibiotics (p<0.03) and chloroquine more frequently (p<0.09). Skin infections were more common in cases (p<0.06). For case children, the occurrence of skin infections or use of antibiotics could not be related to the initial severity as measured by number of pox, maximum fever response, or being a secondary case (infected at home) (p<0.70).

We also examined the impact on hospitalisations and mortality in the total cohort of children in the community including the 1539 children who had had chickenpox in the outbreak. The hospitalisation rate ratio (HRR) before 3 years of age was 1.16 (0.77-1.74) for previous chickenpox cases compared with children who had not had chickenpox. There was no difference in the type of diagnoses reported for previous chickenpox cases and community controls; for both groups 64% of the hospitalisations were reported to be due to malaria.

Among the 293 chickenpox cases less than 2 years of age in March 2001, 11 died before reaching 3 years of age. One of the chickenpox deaths occurred within 30 days and was considered an acute chickenpox death. If acute mortality was excluded, the mortality ratio between cases and non-cases was 0.74 (0.39-1.41)) adjusting for significant background factors. The epidemic was too small to test other factors, but the effect may have been better for children who were breast-fed at the time of chickenpox infection.

Conclusion

Weight, height, and MUAC were significantly better for female cases at follow-up. In Guinea-Bissau weight gain is undoubtedly a benefit. This growth benefit may be due to non-specific effects of chickenpox infection. We have previously documented that chickenpox have an immunostimulatory effect increasing the levels of eosinophils and in other studies we have found high eosinophils levels to be associated with better survival. Chickenpox could potentially have a beneficial impact on resistance to other infections.

Another possible explanation for the differences in growth was the use of antibiotics. If cases had

received more antibiotics after infection, it might have had a positive influence on general health in an area with a very high frequency of intestinal infections. At least for malnourished children, metronidazole has a positive influence on growth, and long-term use of antibiotics given to animals increases growth velocity.

In spite of severe infection in the acute phase, many having pneumonia, long-term mortality was slightly lower after chickenpox infection.

Future perspectives

The existing vaccine against chickenpox has not been examined for possible beneficial non-specific effects. However as this vaccine is live attenuated, it may have some of the same beneficial effects which have been documented for measles vaccine. However, there are no planned studies to confirm this.

References on chickenpox: 5,80,85,89,117

Measles infection and prophylactic antibiotics

Background

The management of measles and its complications has always been a challenge for doctors worldwide. Measles is a highly contagious and severe viral infection leading to profound immuno-suppression and bacterial super-infection (e.g. pneumonia, otitis media, conjunctivitis, diarrhoea).

More than 45 years of vaccination have dramatically reduced measles incidence, however there is still an estimated 30 to 40 million cases each year resulting in more than half a million deaths mostly among children in the developing world.

Since antibiotics became generally available, they have been used to treat measles and measles complications. However, it has remained controversial whether measles cases should receive prophylactic antibiotics to prevent bacterial complications. Though it has generally been recommended not to give prophylactic antibiotics, it has been a very common practice among physicians in low-income countries often due to fear that the patient might not come back.

At the Bandim Health Project (BHP), there is a long-standing tradition to perform measles research, and in collaboration with IDR in Senegal we conducted an observational study in rural Senegal suggesting that children who received antibiotics in the early phase of infection had less risk of being seen with severe measles later on. Therefore, in 1996, we took up the challenge to perform a randomised double-blind placebo-controlled trial on prophylactic treatment with antibiotics in measles.

Results

The study enrolled 84 measles cases in 1998. When war broke out in early June 1998, the study had to be stopped. Sulfamethoxazole-trimethoprim was used as active drug as it had been the recommended first-line drug against community-acquired pneumonia by the WHO when the study was planned. The main outcomes were pneumonia and admission to hospital.

As can be seen from the table 1/46 (2%) of the cases in the active group developed pneumonia, and 6/38 (16%) in the placebo group. Furthermore, the group that received prophylactic antibiotics had less conjunctivitis and significantly higher weight gains in the month after inclusion than the placebo-group.

Public health implications

A recent Cochrane review has incorporated our results. Thus it is now recommended to administer antibiotics to children with active measles in geographical areas with a high case fatality rate or with a high incidence of post-measles pneumonia.

Publications on prophylactic antibiotics:
132,157,158,162

Table. Intention to treat analyses of outcome measures

	Co-trimoxazole (n=46)	Placebo (n=38)	OR$^\$$ (95% CI)	Adjusted OR¤ (95% CI)	Adjusted OR¤ (95% CI) Laboratory confirmed cases
Main outcome measures					
Pneumonia after inclusion	1 (2%)	6 (16%)	0.08 (0.00-0.56)#	0.14 (0.01-1.50)#	0.11 (0.00-1.22)#
Hospitalised with measles after inclusion	0 (0%)	3 (8%)	0 (0-1.03)§	-	-
Other outcome measures					
Diarrhoea after inclusion	3 (7%)	5 (13%)	0.27 (0.04-1.39)#	0.17 (0.01-1.55)#	0.10 (0.00-1.04)#
Severe fever after inclusion	6 (13%)	11 (29%)	0.32 (0.10-1.07)	0.36 (0.09-1.43)	0.34 (0.08-1.53)
Oral thrush after inclusion	0 (0%)	3 (8%)	0 (0-1.03)§	-	
Stomatitis after inclusion	4 (9%)	7 (18%)	0.37 (0.09-1.50)	0.43 (0.08-2.26)	0.35 (0.06-2.12)
Conjunctivitis after inclusion	12 (26%)	17 (45%)	0.36 (0.14-0.96)*	0.31 (0.10-1.03)	0.25 (0.06-0.96)*
Otitis media after inclusion	1 (2%)	2 (5%)	0.38 (0.02-4.42)#	0.72 (0.05-10.6)#	0.44 (0.01-6.93)#

* p<0.05, # Profile-likelihood confidence interval, § Exact test, $^\$$ Controlled for age group, ¤ Adjusted for age group, weight-for-age z-score at inclusion, time since rash and measles vaccination status

24

Malaria treatment and development of resistance

Background

During the last 15 years the Bandim Health Project has studied the efficacy of different treatment schedules for malaria, focusing on the antimalarials available and/or recommended by the National Malaria Programme. Quinine continues to be a good and efficient choice for third-line treatment, however, the effectiveness when used as second-line treatment is more questionable (195).

Sulphadoxine-pyrimethamine (S/P), which proved to be an efficient antimalarial, was until recently recommended as second-line treatment. However, a high frequency of mutations coding for S/P resistance was found in Bissau (53), stressing the importance of monitoring the efficacy.

We have previously shown that while treating with chloroquine in the recommended dose of 25 mg/kg bodyweight had a high treatment failure rate, increasing the dose to 50 mg/kg turned chloroquine into an efficient antimalarial. That 25 mg/kg is insufficient has been observed in all of Africa. Therefore chloroquine has almost been completely abandoned and the much more expensive artimisinine combination therapies have been recommended and introduced in most sub-Saharan countries, including in Guinea-Bissau in the summer of 2008. Since we found chloroquine to be still efficient in the higher dose of 50 mg/kg and the drug is cheap and well-known, we monitored the genetic background for resistance in Guinea-Bissau.

Results

Both amodiaquine and chloroquine have been suggested to be combined with artemisinine. We therefore examined the efficacy of different doses of these two drugs. Amodiaquine was very efficient both in the dose of 15 mg/kg and the dose of 30 mg/kg. As previously found chloroquine 25 mg/kg had a high failure rate, whereas 50 mg/kg proved to be efficient (138). In several studies using chloroquine between 2004 and 2008, we did not find any decrease in efficacy; hence, chloroquine resistance as measured by in-vivo tests has not increased during the last 15 years. The WHO in-vitro micro test to assess chloroquine resistance has been used almost annually since 1990. A slight decrease in resistance was found during this period confirming the in-vivo results (194).

Our analyses of parasites indicate that the genetic basis of chloroquine resistance is the same in Guinea-Bissau as in the rest of Africa (193). The prevalence of the mutation pfcrt 76T was found to vary between 13% and 38%, which is low compared to most other African countries. Furthermore, we have found that the prevalence of single nucleotide polymorphisms (SNP) at pfcrt positions 76, 271, 326 and pfmdr1 position 86 did not change between 1992 and 2005, indicating that the prevalence of mutations coding for chloroquine resistance did not increase during this period (194).

To study the efficacy of treating with chloroquine 50 mg/kg compared to 25 mg/kg we genotyped the parasites on the day of inclusion and in case of re-parasitaemia following treatment and found that the efficacy of the higher dose was 78% as compared to 34% for the standard dose when treating parasites carrying the mutation indicating resistance (pfcrt 76T) (193). An argument against using higher doses has been that the risk of re-infections with resistant parasites would increase significantly. However, the risk of re-infection with a parasite carrying the 76T mutation was only 9% for both the two groups treated with 25 mg/kg or 50 mg/kg.

Between 1994 and 2003, we monitored the malaria treatment given at a health centre in Bissau 4 to 8 weeks per year. We found that a median chloroquine dose of 63 mg/kg was prescribed, in spite of the official recommendation being only 25 mg/kg (194). Also we monitored chloroquine prescriptions and reported intake of chloroquine in 102 children. The median total chloroquine dose prescribed and reportedly taken was 81 and 77 mg/kg, respectively. No severe adverse events were reported and no adverse events were associated with higher chloroquine concentrations. Interestingly, only 3 of the 102 children had *P. falciparum* in the blood

indicating that diagnostics are poor leading to massive over-prescription (224).

As many parasites have one or more mutations coding for resistance to S/P we compared the efficacy when using S/P for children with treatment failures to the first line drug. S/P remained efficient with no difference in treatment success rates from 1995/1996 to 2002/2004 (145).

Public health implications

During a period of approximately 15 years the resistance to chloroquine has not increased in Bissau, if anything it has decreased slightly. Treating with 25 mg/kg is insufficient, but increasing the dose to 50 mg/kg turns chloroquine into an efficient antimalarial also for treating parasites harbouring the mutation coding for chloroquine resistance. Recent studies suggest that chloroquine resistance is mediated by an energy-dependent saturable chloroquine efflux carrier, alternatively mediated by a channel. In Guinea-Bissau high doses of CQ are commonly taken and well tolerated suggesting that chloroquine resistance can be overcome by the higher doses probably due to a loss of fitness of the parasites (194,197). That loss of fitness plays a role is supported by the fact that chloroquine resistance has disappeared from Malawi 7 years after abandoning the drug. If and when chloroquine is reintroduced, alone or in combination with artemisinine, this should be done with the higher dose of 50 mg/kg to limit the spread of chloroquine-resistant parasites (159).

The fact that S/P remained efficient for second-line treatment over a period of 10 years indicates that the drug can still be used for selected groups of patients. As it is the best antimalarial for intermittent preventive treatment of pregnant women it has been reserved for that, thus raising the question as to which antimalarial to use for second-line therapy. As an artemisinine combination therapy has been adopted as first line treatment this can not be used for treatment

failures. Therefore the National Malaria Programme has recommended quinine given three times a day for seven days – a treatment schedule which is difficult and for which one could expect a low adherence suggesting that the effectiveness should be evaluated (195).

Future perspectives

The studies suggest that the common use of high dose chloroquine in combination with a loss of fitness associated with chloroquine resistance explains the continued efficacy of chloroquine in Guinea-Bissau. Our data are observational and cannot be taken as proof. In addition the data available on adverse events, though encouraging, does not rule out serious adverse events. Despite the flaws, the situation in Guinea-Bissau warrants further research into dosing strategies, adverse events and possible combinations of a drug that is cheap and available and which regains efficacy when it is removed from the environment.

As lumefantrine-artemether (Coartem) has now been introduced as treatment for uncomplicated malaria we are now evaluating the efficacy. As over-treatment for malaria is common (200,224) and as the treatment used now is expensive, the strategies for improving the diagnostic procedures should be followed closely, especially considering the lower incidence of malaria at present. We have shown that frequent treatment protects the children from clinical malaria (40). As the present over-treatment can be compared to intermittent preventive therapy it will be important to study which effect improved diagnostic procedures will have on the incidence of clinical malaria.

References on malaria:
11,40,43,53,67,136,138,145,146,193-197

Is malaria disappearing?

Background

Malaria has always been considered the biggest public health challenge in Guinea-Bissau, both due to the number of cases and deaths caused, but also due to socio-economic implications. Malaria transmission is present all year around, and reports from the 1980s and 1990s pointed to a prevalence of malaria parasites of 44-79% among children aged 2-9 years in rural communities. Several public health interventions and large amount of funds are available for malaria control. Environmental changes are occurring. Prioritisation of these interventions to maximise the use of resources requires better knowledge of the current situation.

Results

Recent epidemiological studies on malaria have included community surveys and collection of better quality data at health facilities in Bissau. The recent data have shown a decline in malaria. In a community survey carried out in Bissau in 2003, only 3% of the individuals living in the randomly selected houses had malaria parasites, contrasting with 26% in 1994. At the health facilities malaria is grossly over-diagnosed. Reports from the MOH health information system indicate that malaria is responsible for 50-70% of all consultations. In 2003/2004, malaria surveillance was established by the BHP at the national paediatric ward and three health centres in Bissau. As expected, 64% of the outpatient consultations among children < 5 years of age were clinically diagnosed as malaria, however only 13% had malaria parasites detected by microscopy. Among hospitalised children, only 44% had malaria parasites even though 73% had a clinical diagnosis (200) (Figure). The proportion of malaria-positive cases increased with increasing age. For 82% of the cases, the labora-

tory results were available to the clinicians before prescription. However, anti-malarial (96%) and antibiotic drugs (65%) were prescribed to patients with negative slides (200).

Recent figures from 2006/2008 shows that around 11% of children seen at health centres in Bissau with suspected malaria has a malaria-positive slide. Several factors may have had an impact on the decline in malaria infection. Untreated bed nets are used by around 90% of children less than 5 years of age. Since the adoption of the policy of re-impregnation of nets in 2004, the use of impregnated bed nets increased from 5% to more than 70% in 2006. Since nearly everyone consulting at a health centre has been treated with chloroquine, the health system has in fact instituted intermittent prophylactic treatment of malaria. Resistance to chloroquine has been stable, but the common use of double doses of chloroquine has been found to be effective in around 88% of the cases (138, 192, 194). The decision to change to coartem as first line drug was taken in 2007 and its implementation started in May 2008.

Public health implications

The decrease in malaria transmission will probably shift the peak of cases to higher age groups and we have started observing this trend. If further declines occur, a good surveillance system for early warning will be necessary and we will need to be prepared for possible epidemics in the future. The introduction of coartem, a more expensive drug than chloroquine, should be accompanied by attempts to improve the diagnosis of malaria by using both microscopy and rapid diagnostic tests. If implemented, it will improve the diagnosis and treatment of both malaria and other conditions that might otherwise have been ignored. Thus, several unneeded doses of coartem and the scarce money of users would be saved. However, this will reduce the intermittent prophylactic effect of chloroquine given to almost all cases seen with fever at a health facility. Hence, in a worst case scenario, treatment of individual malaria case will be improved with coartem but malaria might reappear because there is less chloroquine prophylaxis in the high risk age groups, although we recognise that coartem might also have a preventive effect as it acts also on gametocytes preventing maintenance of transmission. The decline of

malaria has occurred, but control is vulnerable and malaria might be reappearing.

Future Perspectives

Malaria is decreasing in several parts of the world including the neighbouring countries in West Africa. This has revived the hope for elimination and an expert meeting was convened in January 2008 by WHO to revise previous positions. In West Africa, joint collaboration on a common programme for monitoring elimination is being discussed between The Gambia, Senegal and Guinea-Bissau. This will require reinforcement of existing interventions, testing the most cost-effective combinations. It will be very important to continue monitoring the changes in the epidemiology of malaria using simple methods as well as good entomological data. For this reasons, the BHP is creating sentinel sites all over Guinea-Bissau in order to collect good quality data.

The implications of the different policies should be studied. Health personnel are resistant to use laboratory results in deciding on the use of anti-malarial drugs. We are currently studying the consequences of treating or not treating a child

with a malaria-negative slide with coartem or chloroquine for clinical outcomes and preventive effects against further attacks of malaria.

References on the disappearance of malaria:
200
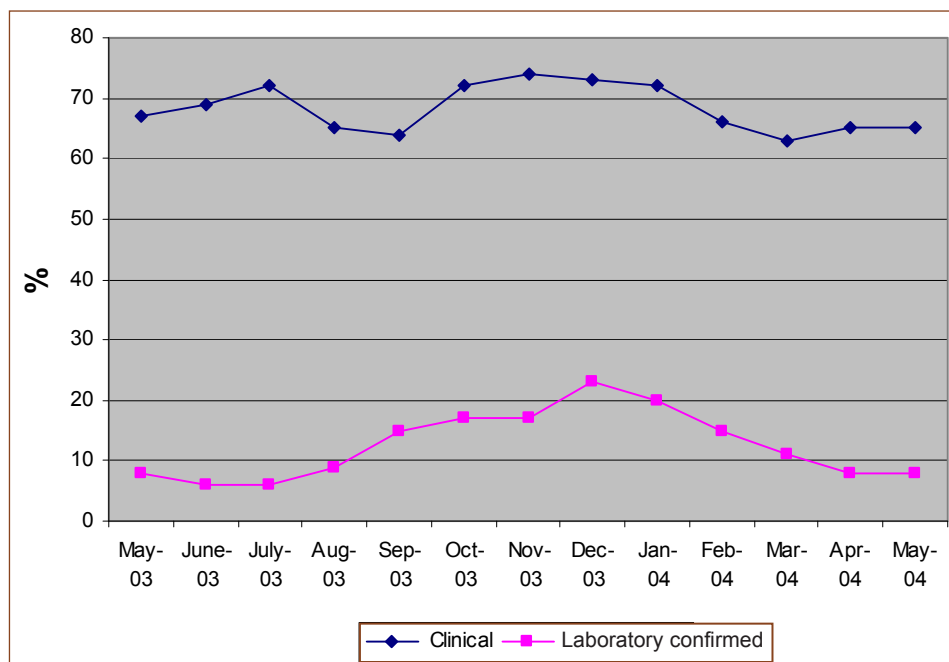
Figure. The prevalence of clinical and laboratory diagnosis among children less than 5 years of age at the health facilities in Bissau, 2003/04

Respiratory syncytial virus: Taking the observations to Denmark

Background

Respiratory syncytial virus (RSV) infection leading to early childhood hospitalisation is still a major paediatric concern globally. The incidence of RSV hospitalisation appears to increase (6, 92). In high–income countries, the incidence of atopy has been increasing steadily for decades, and wheezing and asthma have become the most frequent chronic diseases in childhood (118). Severe RSV infection often present with wheeze and a major part of RSV-infected children wheeze months after their primary RSV infection. Therefore, an important question in RSV research has been to find out whether severe RSV infection cause wheezing and atopy.

During the past 5 years, we have studied risk factors for severe RSV infection in both Guinea-Bissau and Denmark. Furthermore, we studied the association between RSV hospitalisation, wheezing and atopy, RSV hospitalisation in children with congenital heart disease, and the association between RSV hospitalisation and invasive pneumococcal disease in the Danish child population. Using cord-blood from newborns in the Danish National Birth Cohort (www.bsmb.dk), we studied the seasonal variation of maternally derived RSV-neutralising antibodies and the association to RSV hospitalisation as well the influence of maternally derived RSV-neutralising antibodies on RSV hospitalisation and on recurrent wheeze. In the Danish twin population born 1994 to 2004 we further examined the associations between RSV hospitalisation, wheezing and asthma.

Results

In Guinea-Bissau, BCG immunisation protected against severe RSV infection (88), and we obser-

ved that mother-to-son cross-sex transmission might be part of the explanation as to why boys are more frequently severely infected with RSV (60).

In Denmark, both recent hospitalisation for RSV infection and for non-RSV respiratory infection increased the risk of invasive pneumococcal disease among children less than 2 years of age (202). We observed a 2-fold increased risk of RSV hospitalisation in Danish children with congenital heart disease. Among the children with congenital heart disease, risk factors for admission were Down syndrome, cardiomyopathy, and haemodynamically significant heart disease. Young age and cardiac de-compensation were associated with more severe course of RSV disease (227).

In the general Danish child population, male gender, medical non-atopic risk factors, the presence of other children less than 12 years of age in the home, day care attendance, and maternal smoking were associated with an increased risk of RSV hospitalisation. Interestingly, atopic disposition and a history of wheezing increased the risk of RSV hospitalisation; the factor associated with the largest risk increase for RSV hospitalisation was early recurrent wheeze (141).

When we then examined the associations between RSV hospitalisation, wheezing and asthma in Danish twins, we found a bi-directional association between severe RSV infection and asthma. Severe RSV infection was associated with a short-term increase in the risk of subsequent asthma suggesting RSV induce bronchial hyper responsiveness; and asthma was associated with a long-term increased susceptibility for severe RSV disease, suggesting a host factor

being responsible for the severe response to RSV infection. This suggests that severe RSV infection and asthma may share a common genetic predisposition and/or environmental exposure (219). We observed an increased concordance of severe respiratory syncytial virus infection in identical twins, pointing to genetic factors being important for the severity of respiratory syncytial virus infection (222). Hence, RSV infection, severe enough to warrant hospitalisation, does not cause asthma but is rather an indicator of the genetic predisposition to asthma (228).

Studying maternally derived RSV-neutralising antibodies, we found a clear temporal association between the RSV antibody level in cord blood and RSV hospitalisation in infancy suggesting that RSV-neutralising antibody level plays a role in the seasonal pattern of RSV infection (220). In addition, we observed that maternally derived RSV-neutralising antibodies protect infants against RSV hospitalisation, also when the infant has recurrent wheeze. However,

to our surprise high maternally derived RSV neutralising antibody levels were associated with an increased risk of recurrent wheeze (221).

Future perspectives

Our future studies will focus on risk of RSV hospitalisation in children with chronic diseases; on genetic markers of severe RSV infection, wheezing, asthma and eczema; and on studies on maternally derived RSV-neutralising antibodies, lung function, wheezing and asthma. To link back to the Guinean studies which generated this line of research on RSV, we also intend to explore whether vaccinations are related to the major change in the relative female-male incidence of RSV which occurs in the first year of life in Denmark (141).

References on RSV: 6, 60, 88, 92, 106, 118, 131, 141, 202, 219-222, 227, 228

Improving hospital care

Background

Mortality at the paediatric ward in Bissau is usually high. However, during the war in 1998-1999 it dropped to almost half (RR=0.58 (0.50-0.68)). As soon as the war was over, it increased again (54). The hypothesised reasons for this change were availability of free drugs from humanitarian aid during the war, some personnel living at the hospital and therefore being available fulltime, relief food from aid donations to the staff, and high "morale" during the war situation. Perceiving free drugs to be the main reason, the BHP and then WHO provided free drug kits for urgent malaria treatment to the paediatric ward after the war; however, mortality did not decrease. Therefore, further reasons and mechanisms to reduce mortality have been sought.

Results

In the search for new ways to reduce mortality at the hospital, we performed a randomised trial including 951 children to test whether a strict follow-up of the protocol for malaria case management plus improved motivation with a small subsidy would add to the efficacy of free drug and training of the personnel. In the control group, the personnel received the same training and the patients had the same free drugs. Mortality among hospitalised malaria cases was 11% in the control group against 5% in the group receiving supervision of the treatment protocol plus a small monetary incentive (RR=0.48 (0.29-0.79)) (Figure 1) (187). In-hospital mortality was reduced, the length of stay was shorter and children absconded less in the intervention group (0.7% versus 2.9%). Mortality among children with a malaria-negative slide was much higher than among the malaria-positive children and the effect of the intervention was more pro-
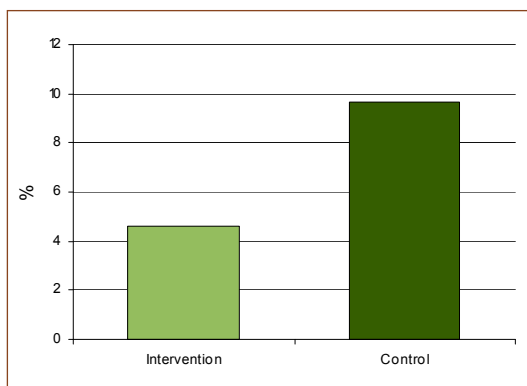
Figure 1. In-hospital mortality
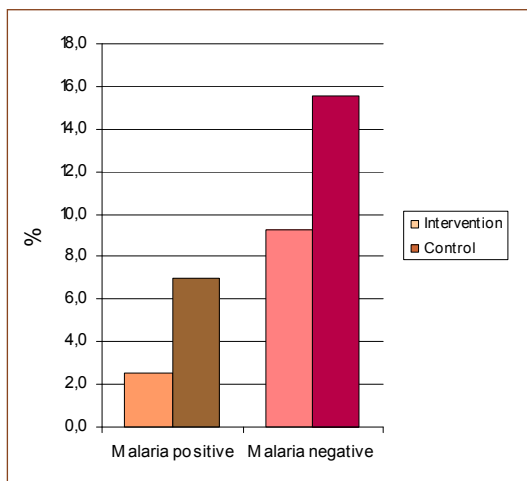
Figure 2. In-hospital mortality according to presence of malaria infection

nounced among malaria-positive patients (RR=0.36 (0.16-0.80)) (Figure 2). Apart from the effect on mortality, adherence to the health personnel advice to come for a control visit was more likely to be followed-up by the intervention group (55% versus 25%, p<0.0001). The supervision and the monetary incentive were critical in reducing the level of mortality. With current standard interventions in the health care system which mainly include training and sometimes free drugs such a change had not been possible.

We recognise that these findings are not easy to implement, particularly because subsidies are not "negotiable" with both the Government and the donors, even if mortality at the hospital may be reduced by half. Thus, the BHP is currently testing the implementation in a day-to-day working situation. We use locally generated funds from the cost recovery system to pay incentives, based on performance indicators, and for improvement of services. This study is on-going. However, we have already noticed that the major challenges are to change the attitude of the health person-nel, to have strong leadership/authority at the hospital, and to keep track of new and informal procedures created by the personnel to gain additional cash income.

Public health implications

The quality of care at hospitals influences not only the in-hospital case fatality, but may contribute to reduce mortality in the community (181, 190). Reforms of hospitals are envisaged. However, it is clear that interventions to improve care will need to address several "non-fashionable" but critical problems. These would include leadership, authority at the ward, disciplinary measures, and administration of subsidies. A huge impact may be achieved in a study setting and could probably be replicated in real life if everyone were employed by the project. However, this might be hard to achieve with all the health staff which is not used to strict control.

Re-thinking the way money for health is spent might also be needed. Large amounts of money

are available for training. Sometimes the same people are trained several times and similar topics are taught through different MOH programmes. Per-diems are paid during these seminars, motivating the staff, but also keeping them away from their working place, thus not providing services to the users. In most cases, follow-up is absent after the seminar and the new knowledge is not implemented. Training is an example of expenditures in the health sector which does not impact the results, but cannot be used in other more effective ways.

Future perspectives

The BHP will continue the search for ways of reducing mortality at the hospital and after discharge through easily implemented and affordable interventions. The effectiveness study of a composite intervention, including staff motivation, supervision, and disciplinary measures and building authority at the main paediatric ward will be continued.

Reference on hospital care: 13, 54,181,187,190

Hypothermia of newborns is associated with excess mortality

Background

In low-income countries, child mortality declined during the 1980s, but neonatal mortality did not improve. Hypothermia (HT) in newborns is known to increase perinatal mortality both among those born in hospital and those born unattended at home. African studies have found the prevalence of HT ranging from 22-70% depending on birthplace and case fatality rates among newborns were increased 3-fold by HT. The question is whether HT children develop HT because they are more prone to develop HT than other children (co-morbidity, cooling by handling sick neonates) and therefore have a higher mortality or whether their immature immune system is modified by exposure to HT leading to a higher mortality. However, a number of studies of hypothermia associated with mortality linking community and hospital data is very limited and there is no community study of longer term survival of newborns that develop hypothermia within the first hours of life. We used the clinical surveillance system at the maternity ward of Hospital Nacional Simão Mendes to assess long-term survival of newborns according to the axil temperature measured within 12 hours of birth. We investigated whether there was a long-term excess mortality among infants exposed to HT immediately after birth and for how long these infants experienced an excessive mortality risk.

Results

From 1997 to 2002 all newborns had their axil temperature measured with an electronic axil thermometer by the same maternity nurse within 12 hours of birth (50% within 1-6 hours after birth); 2926 newborn children were recorded at the maternity ward as residents in the

Table. Mortality by age group according to birth weight and temperature measured within 12 hours of birth. Bissau, Guinea-Bissau 1997-2003

Age group	Birth weight (g)	Mortality (dead/persondays)		Mortality ratio (hypothermia versus normal)
		Normal (>34.5 ^{0}C)	Hypothermia (33.0-34.5 ^{0}C)	
Perinatal 0-7 days	< 2500	0.006 (13/2244)	0.030 (21/701)	4.98 (2.50-9.95)
	>=2500	0.002 (38/17664)	0.010 (8/796)	4.60 (2.15-9.87)
	All	0.003 (51/19908)	0.019 (29/1497)	7.36 (4.67-11.6) 4.81[*] (2.90-8.00)
Neonatal 8-56 days	< 2500	0.001 (13/13179)	0.003 (12/3740)	3.21 (1.47-7.05)
	>=2500	0.000 (23/107309)	0.000 (1/4699)	0.99 (0.13-7.34)
	All	0.000 (36/120488)	0.002 (13/8439)	5.12 (2.71-9.65) 2.55[*] (1.29-5.04)
Infant 57-182 days	< 2500	0.000 (5/32549)	0 (0/8833)	-
	>=2500	0.000 (34/271508)	0.000 (2/11602)	1.37 (0.33-5.72)
	All	0.000 (39/304057)	0.000 (2/20435)	0.76 (0.18-3.14) 0.76[*] (0.18-3.27)

* Controlled for birth weight

study area at delivery. We observed 177 deaths before six months of age and 214 were lost to follow-up. There was a mean ambient outdoor temperature of 24 degrees Celsius with a minimum temperature of 15 degrees in December-January. Temperature in the maternity ward ranged from 26 to 30 degrees Celsius.

Based on the association between birth temperature and relative mortality rates, we identified three temperature risk groups: normal temperature or mild HT (≥34.5 0C), moderate hypothermia (33.0-34.5 0C) and severe hypothermia (<33.0 0C). There were 15 (0.5%) children with severe hypothermia, 223 children (7.6%) with moderate hypothermia and 2688 (92%) newborns with a normal birth temperature. We inve-

stigated potential con-
founding by baseline
characteristics as well as
age-dependent potential
confounders: introduc-
tion of weaning food,
breastfeeding and BCG
vaccination. Birth weight
was a confounder but
control for birth weight
did not modify the HT
mortality risks. There
was no effect of time of
temperature measure-
ment within the 12 hour
period after birth.
Controlled for low birth
weight, hypothermia
was found to be associ-
ated with a 4.81 (2.90-
8.00) times increase in
mortality from 0 to 7
days (Table) and a MR of
2.55 (1.29-5.04) from 8 to
56 days after birth. The long-term increased
mortality risk was mainly a problem among low
birth weight newborns (MR=3.21 (1.47-7.05)).
Beyond two months of age, HT was not associ-
ated with an increase in mortality.

Public health implications

Our definition of HT with a cut-off point of 34.5
0C was based on subsequent mortality risk. We
found a prevalence of nearly 10 % in a hospital
setting in a relatively hot climate which is com-
parable to prevalences reported in other African
hospital studies. We found HT within 12 hours
of birth to increase mortality up to two months
of age after birth, especially among low birth
weight children. We have been unable to iden-
tify other community studies of newborns expo-
sed to HT and with long term household follow-
up after discharge from the maternity ward.
In Guinea-Bissau there was no increased morta-
lity risk among newborns born in hospital with
a temperature in the range 34.6-36.5 °C; the cut-
off point of 34.5 °C identified the newborns with
a significantly high mortality risk and in need of
special care. Hence, the WHO definition of HT
as a temperature below 36.5 °C is not supported
by our data. In terms of public health priorities,
it is advisable that definitions of HT are defined
with neonatal mortality as outcome and adju-
sted to local climatic conditions. In our data
there are 108 neonatal deaths of which 29 were
related to HT. Hence, 27% of neonatal deaths
could possibly be averted by correct manage-
ment of HT. However, Ministries of Health and
donor organisations rarely consider reduction of
neonatal mortality to be a priority.

The immunological short or long term effects of hypothermia have apparently never been considered in neonates. In neonatal pigs, exposure to a cold environmental temperature can inhibit the ability to cope with an exogenous endotoxin challenge. When combined cold stress and exposure to exogenous endotoxin induce a rapid and potentially dangerous loss of body temperature. Finally hypothermia induces pro-inflammatory cytokine production in human monocytes. A partly immunological explanation for the long term excess mortality after HT is therefore theoretically possible.

Future implications

The excess mortality arising from a single HT episode extends beyond the neonatal period into the second month after birth. Hypothermia among low-birth-weight children is more severe than among newborns weighing more than 2500 g. Hence this simple measurement identifies LBW children in need of special care. Previous cost-benefit estimates of the effect of improved management of HT may have underestimated the benefits of improved management of HT. Our mortality analysis was based on a single measurement of temperature within 12 hours of birth. Any episode of HT within the first 24 hours is likely to have a long-term negative effect on infant survival. In light of our finding, long term immunological effects of neonatal HT need to be investigated. Low-cost interventions with simple care of newborns are likely to have a substantial effect and could potentially reduce some of the observed unwarranted differences in quality of care and significantly reduce neonatal and early infant mortality. We are presently carrying out an intervention study to evaluate whether intensified HT management can reduce the long term morbidity and mortality effect.

Maternal vulnerability and child mortality

Background

There is a clear sense among demographic health surveillance researchers that the present indicators of social and economic status are insufficient or indirect and sometimes not applicable in a low-income country setting where the majority lives under the same poor conditions. Yet we observe significant differences in mortality within the group of similarly poor people. Our ideas of child mortality in developing countries are simplistic and its distribution has often been misunderstood because of insufficient attention to the context.

High rates of child mortality in developing countries have variously been attributed to child neglect, economic scarcity, cultural traditions of child care, population pressure, low maternal education levels, lack of medical care, and insufficient basic resources. The relation between

maternal education and child mortality is undisputable and health interventions may have a greater beneficial impact for less educated mothers (54). Under these circumstances a mother can be extremely poor and still be able to handle her sick child rationally because she has autonomy and a strong social network.

A rational process of care includes disease and severity recognition, reasonable home-medication, access to health care and ability to obtain contact with a health worker and perseverance in follow-up until health has been regained. Women's autonomy is gaining increasing interest; greater freedom of movement, own income, say over purchases or over the number of children increase the likelihood of her choosing better antenatal care and delivery care. There is a need to investigate, on one side, the interface between rational maternal behaviour, access to health care institutions, and alternative measures of wealth and on the other side, the established set of socio-economic indicators (wealth and schooling) used in studies in poor countries. The objective of the present study was to identify alternative socio-economic indicators, and test these together with the well-known socio-economic indicators of child mortality. We also wanted to test the hypothesis that social capital and/or psychological health beliefs may explain variations in mortality risk.

Results

Using information from focus group discussions on alternative indicators, we initiated a case control study with mothers who lost a child before three years of age in the study area between 1999 and 2000 and controls matched on place of residence, mother's age, and parity. 136 matched case-control pairs were included in the analysis. The interview consisted of three different sections: one section with 70 statements to which the mother could express her level of agreement. In the second section the interviewer rated the appearance of the household and house surroundings. In the third section the interviewer characterised the impression of the mother had given through a choice of 34 personality descriptions divided into 5 groups of personality dimensions. Traditional socio-economic and health indicators are collected routinely through the child health surveillance and census. We focused both on the difference between cases and controls but also on the poorest versus the better off mothers.

Classic socio-economic background factors: Apart from a difference between Muslim and non-Muslim mothers, socio-economic status, maternal education and ethnic group did not distinguish cases and controls. There was no significant effect of maternal school education.

Favouritism: None of the indicators of favouritism distinguished cases and controls. However, the poorest mothers were more concerned that it was important to know a health person at the

hospital and were more likely not to know any health worker.

Religion - belief in spiritual powers or God: Cases and controls were equally afraid of spirits and witchcraft. However, cases tended to be more confident that they could prevent childhood illness even when the disease could be God's will. Cases were more likely than controls to agree that the survival of a severely ill child was a question of fate. Poor mothers were more likely to rely on health as a matter of luck than richer mothers.

Belief in own power: More than 80% of cases and controls agreed that it required a persistent and stubborn mother to see a doctor at the hospital but only 50% were confident that they would always be able to obtain a medical consultation if they felt it was necessary.

Belief in power of the health sector: Two-third of cases and controls agreed many diseases couldn't be treated at the hospital but also that paediatricians at the hospital would be able to cure their sick child. The poorest mothers were less likely to choose private clinics as first choice in case of child illness and tended not to use pharmacies as first choice for advice and treatment.

Disease prevention and management: Cases less often administered weekly chloroquine than controls. Children of case mothers were less likely to always sleep under a bed net. The poorest mothers were less likely to have chloroquine in the house, less likely to use anti-mosquito spray or mosquito coils, less likely to let their children sleep under a bed net and tended to be less likely to let a sick child drink and eat any type of foods. The poorest case mothers were the only mothers that anticipated problems borrowing medicine from neighbours.

Vulnerability: Case *mothers* more often anticipated care taker problems in case of child illness. Cases were significantly more likely than controls to let a less than 10-year-old child look after younger siblings. Cases complained more often that their husbands interfered too much in the care of their children. The poorest mothers were more likely to be short of money in the rainy season, more likely to rely on children to look after their siblings, less likely to be able to borrow medicine from their neighbours and less often received help or advice from their family.

Alternative methods of income: Having a family member in Portugal or France was not associated with being in control. There was no difference in the proportion of cases and controls who received money or goods regularly. There was no difference between the poorest and the rest of the mothers.

Ethnicity: Mothers belonging to the largest ethnic group in the study area, Pepel, were more likely to let a child die if this was the destiny of the child.

Interviewer's characterisation of mother: Psychological characteristics of the mothers were assigned by the interviewing assistant. Mothers who had lost a child were significantly more silent, weak, timid, satisfied and in lack of ambition compared with mothers who never lost a child. Only one psychological group was significantly associated with being a case: Irrational, illogical, confused, not interested, self-remorsing (group D). The poorest mothers more often gave the interviewer a more frightened, timid and powerless impression than mothers from other socio-economic groups.

Comparison of different models to predict characteristics of cases: To determine if the alternative indicators for socio-economic status had the ability to compete with traditional indicators, we analysed goodness-of-fit of the following models by their ability to explain variation: traditional indicators, significant statements, all psychological groups, psychological group D and individual significant psychological statements. The significant statements were the best model to characterise cases but psychological group D might also be used.

Public health implications

Cases agreed very strongly to the statement that their husband was always interfering with the childcare and never let them decide on their own. In terms of intervention possibilities it is worth noting that cases were more likely to let a child less than 10 years of age look after younger siblings. A case also more often than controls anticipated problems finding a caretaker in case of child illness.

Maternal neglect has been invoked as an important factor in child mortality. However, there was no difference between cases and controls with respect to the question of whether a mother could see death as her child's destiny. The degree of resignation with respect to what can be done in case of severe childhood illness depended strongly on ethnic group. Some important observations were made in terms of disease management: the poorest mothers were less likely to let the child eat and drink at leisure. In case of child illness the poorest mothers also had to rely much more on under age caretakers, got no help from the family, and did not count on an ability to borrow medicine from neighbours.

On the assumption that the impression given to an interviewer predicts caring abilities of the mother, the study suggested the following characteristics of the poorest mothers who lost children: they were more timid and powerless but satisfied with their conditions and saw no apparent need to improve their situation.

External income sources were not reliable and could not serve as socio-economic indicators in the present form. We need to do more qualitative research in this field incorporating other alternative incomes (unofficial fees, family networks, important contacts etc.). The effect of having a relative in Europe was insigni-

ficant. The general impression of the intervie-
wers was that the mothers did not rely on this
support and they never knew when the help
would come. Cases were more often short of
money in the rainy season – a period when petty
traders have difficulties because of transport
problems and less food output from their far-
ming.

Future perspectives

Three statements were so strongly associated
with being a case that they deserve further inve-
stigation: case mother's complained over hus-
bands interfering, had to rely on very young
care-takers and were not confident they could
get necessary help from their family when in
need. Some alternative indicators including per-
sonality issues acted stronger among the poorest
of the poor. The present findings should be inve-
stigated in longitudinal studies of birth cohorts
after refinement of the statements and the psy-
chological characteristics. The great challenge
would be how to change family relations, mater-
nal autonomy, maternal perceptions and self-
confidence in order to improve child health
among the poorest of the poor.

References on child care:
49, 50, 54, 94,127,181,187,190,216

Knowing a medical doctor may save children's lives in low resource settings

Background

During the past 10-15 years, most low-income countries (DC) in sub-Saharan Africa have undergone extensive structural re-adjustments, which have incorporated a considerable cut in government health budgets. The countries have adopted a district and community-based health system with decentralization of resources and decision-making. At the same time vertical primary health care (PHC) programmes have absorbed a relatively large proportion of global funds for health improvements. This has left the referral level in the hospital sector in a financial vacuum with poor performance, low staff morale and low confidence. Equity and access to public health services in low-income countries has become a priority for large donors, e.g. The World Bank. Few studies have examined equity and quality of care in hospital settings linked to community data. Unfortunately there are no studies of the influence of illegal or un-official user fees, corruption or "favouritism" on type of care provided at different levels of public health care. We therefore examined the importance of favouritism for successful care seeking.

Results

We interviewed care takers from the study area who consulted their sick children at the paediatic outpatient clinic at the Hospital Nacional Simão Mendes in Bissau. Information on prior care seeking was obtained together with information on whether the primary care taker was acquainted with a person working in

Predictor	Mortality risk Odds ratio (95%CI)
Maternal acquaintance or familiarity with a medical doctor	
Yes	0.55 (0.33-0.94)
No	1
Mother arrived between 7 p.m. and 7 a.m.	
Yes	1.74 (1.00-3.01)
No	1
Clinical state	
Severely ill	4.81 (2.00-11.6)
Moderately ill	1.22 (0.55-2.69)
Not very ill	1
Child consulted elsewhere > 48 hours < 14 days	
Yes	2.84 (1.72-4.71)
No	1
Mother less than 20 years old	
Yes	1.92 (1.00-3.67)
No	1
Sex of child	
Male	0.45 (0.28-0.73)
Female	1
Child age	
0-30 days	5.58 (2.59- 12.0)
31-364 days	0.87 (0.47-1.60)
1 – 2 years	1
3-4 years	0.42 (0.18-1.00)
5 – 15 years	0.62 (0.30-1.29)

the health sector. At the hospital, 1572 children were registered at their first consultation, 589 (37.5%) were hospitalised immediately and 23 children were hospitalised following repeated consultations within 30 days of the first contact. Within 30 days of the first visit we recorded 129 deaths in the cohort; 104 died in the hospital, 17 were hospitalised but died at home, and 8 died at home without being hospitalised.

In a multivariate analysis including both socio-economic background factors and clinical factors we found acquaintance or familiarity with a physician to be a strong and independent predictor of 30-day mortality (Table). It is noteworthy that the variables mother consulting after seven p.m. and mother less than 20 years of age retained their significance in the multivariate model. These factors are not normally considered to be associated with short-term mortality.

Public health implications

It is well established that socio-economic and other background factors predict mortality. Our finding, that mortality among sick consulting children may be better predicted by unjustified and non-medically grounded variations in the quality of care is surprising. The most important of these non-medically grounded factors was "acquaintance with a medical doctor" which reduced 30-day mortality risk by nearly 50%. It is possible that the acquaintance factor adds further dimensions to the effect of preventive

health care, school education and social class. Mothers seem to bring the right children for consultation judged by the high percentage of consulting children who were hospitalised and it seems unlikely that maternal neglect explains why the child is not hospitalised when it should have been.

If our observations are valid in other settings, a large proportion of child deaths could be avoided by proper training of medical and nurse staff and by setting the goal to reach the lowest observed mortality in each team of nurses or physicians. If we assume we have controlled for all confounders, the 30-day mortality risk would be reduced by 24% if all children had the same health benefits as a mother knowing a medical doctor. An intervention based on our findings in this outpatient clinic is likely to have a pronounced effect on childhood mortality in the study area. To focus exclusively on poverty, health equity and community health can be misleading in the combat against the continuously high childhood mortality in many low-income countries. In some countries, child mortality is strongly related to the hospital sector and "favouritism". It is not only socio-economic status which plays a role for how health care is provided. Improvement of hospital and health worker performance should be given a high priority as these interventions may lower childhood mortality as cost-effectively as many large scale public health interventions.

Future perspectives

Social capital and social networks are important for child survival in low resource settings; the present finding adds further detail to the quality of such "capital". Two perspectives have to be pursued further: one is to investigate how favouritism affects maternal, perinatal and neonatal health. The other is to study how this apparently "quality lifting" factor can be applied as a management tool for quality improvement. The feed-back sessions with health workers at the paediatric ward were promising as the health workers were very eager to be quality rated and they were able to identify the "good" and the "less good" teams before they were presented with the results.

References on quality of care:
49,50,54,94,127,181, 187,190,216

Non-specific effects of vaccines are important

The non-specific effects of measles vaccine have been a main focus of research at the BHP since the very beginning even though it took 15 years to formulate the idea that vaccines may have other than the targeted effects, presumably due to some form of immune stimulation. The high-titre measles vaccine (HTMV) was protective against measles but associated with two fold increased mortality for girls. Hence, such non-specific immune stimulatory effects can be very important for child survival. However, the research community did not pursue these observations, but instead searched for a new and better measles vaccines. However, if a licensed vaccine could have such dramatic effect it can probably happen again unless we understand the specific immunological mechanisms. We have therefore aimed to document the importance of non-specific effects not only for measles vaccine but for all of the routine vaccines.

Ideally one could conduct randomized clinical trial measuring the extent of these non-specific effects beyond the targeted disease prevention of the vaccine. However, that is rarely possible because withholding recommended vaccines would be unethical. We have tried several different approaches to document the importance of non-specific effects as shown in the following chapters.

First, we have measured the effect in observational studies. This is inherently difficult because there are strong selection biases in who gets vaccinated first – see Chapter 14. Still in some observational situations we may approach natu-

ral experiments with limited selection bias as when a new vaccine is introduced (45,65), a vaccine is missing during a certain period (46), or a war interrupts vaccination services (8). We have pursued such natural experiments. Furthermore, we have exploited the fact that different vaccines apparently give very different estimates of their impact on survival. That would not happen if selection biases were the main cause of the estimates.

Second, the HTMV incident suggested a marked sex-differential effect of this vaccine (12). In the pre-vaccination era in West Africa, there was no important sex-difference in post-neonatal child mortality (Figure). Hence, if vaccines have strong sex-differential effects on mortality it suggests that non-specific immune stimulatory effects are important for child survival. As it turns out, all routine vaccines have sex-differential effects suggesting that boys and girls might in fact benefit from different vaccines or different vaccination schedules.

Third, we have also conducted randomised clinical trials to document non-specific effects of vaccines as evidence in several chapters. This has been possible in some situation because the vaccine was not normally used (BCG to low-birth weight children, BCG revaccination), not used in that age group (measles vaccination at 4 months of age), or because several different strains are in common use (strains of measles vaccine). It should also be possible to test variations in current practice which are not the main recommendations but occurring very often; for example, administering BCG and DTP simultaneously or measles vaccine and DTP simultaneously (Chapter 21). Conducting randomised trials, we have found major non-specific effects. However, they have not always been the ones we had expected. The immunological interactions have been more important than initially envisioned. Testing, for example, early measles vaccine, we changed the sequence of vaccinations and that has fundamentally changed the impact on survival because more children received DTP after measles vaccine
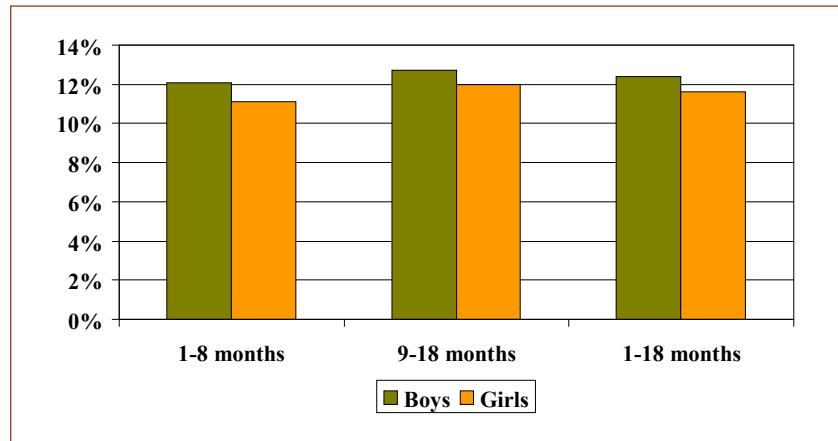
Figure. Annual mortality rates by sex prior to the introduction of vaccines. Bissau, 1978-1980

(Chapter 18). We have also experienced several times that supplementation with micronutrients amplified the immunological effects (Chapter 25).

Randomised trials will be continued but it is becoming clear that it will be increasingly difficult to test the impact on survival because once we intervene and provide services to the community we are also reducing mortality. We need to measure the impact on indicator infections and immunological parameters which are associated with survival and which will indicate whether the immunological profile induced by a vaccine is beneficial or not. This is becoming urgent as more and more new vaccines are being introduced.

Non-specific effects are very important for child survival in low-income countries. From what we know now, live vaccines are beneficial – at least in individuals without immunodeficiency – whereas inactivated vaccines may have negative effects on other than the targeted diseases. Both beneficial and negative effects are strongest for girls. Effects may change fundamentally when vaccines are combined or the sequence inverted. Furthermore, vaccines interact with other forms of immunomodulators like micronutrients and season.

These observations question many assumptions underlying the current program of interventions for children in low-income countries. Taking these observations into consideration in planning the intervention programs may have major impact on child survival.

Survival bias

Background

Most of the observations and hypotheses on non-specific effects (NSE) of vaccines have emerged from observational studies at the Bandim Health Project (BHP). Recently, observational studies from other research groups have appeared to be focusing on the putative negative effect of DTP. The studies from BHP and elsewhere have estimated the impact of DTP to vary between mortality rate ratios of 0.2 (positive NSE) and 3.2 (negative NSE) for DTP-vaccinated compared with DTP-unvaccinated children, see Figure 1. For last five years we have discussed whether methodological differences are involved in this inconsistency (73, 74, 79, 93, 98, 99, 153, 154, 201). One bias has played a central role in this discussion: *survival bias.*

Data collection

Many of the observational studies in which child mortality has been related to vaccination status were conducted in situations where mothers keep vaccination cards on which all vaccinations received are recorded. Typically, a cross-sectional population survey is first conducted by which vaccination cards are examined to record the vaccination status of all children. After an interval of perhaps 3 or 6 months, or longer, a follow-up visit is conducted, and changes in vaccination status are ascertained by examining vaccination cards again. Deaths since the last visit are recorded. Several follow-up visits may be made. Analysis is then conducted to investigate whether there is any association between vaccination status and mortality. A number of potential sources of bias, including survival bias, arise in the interpretation of such observational data.

Survival bias

In many African societies the vaccination card from a child who died will be destroyed. For such children, no record will be collected of any vaccinations received between the date of the last visit when the child was alive and until its death. This will not be the case for children who survive, if their vaccination card is available. The vaccination status of surviving children will thus be more frequently updated between visits simply because they survived until next visit. The follow-up time from the new vaccination to next visit for these surviving children is *immortal* time in the statistical analysis – that is, it would not have been collected had the child died – and will thus bias the survival among vaccinated to the better. This bias has not always been taken into consideration in the statistical analyses. Two main approaches of statistical analysis of this type of data have been used.
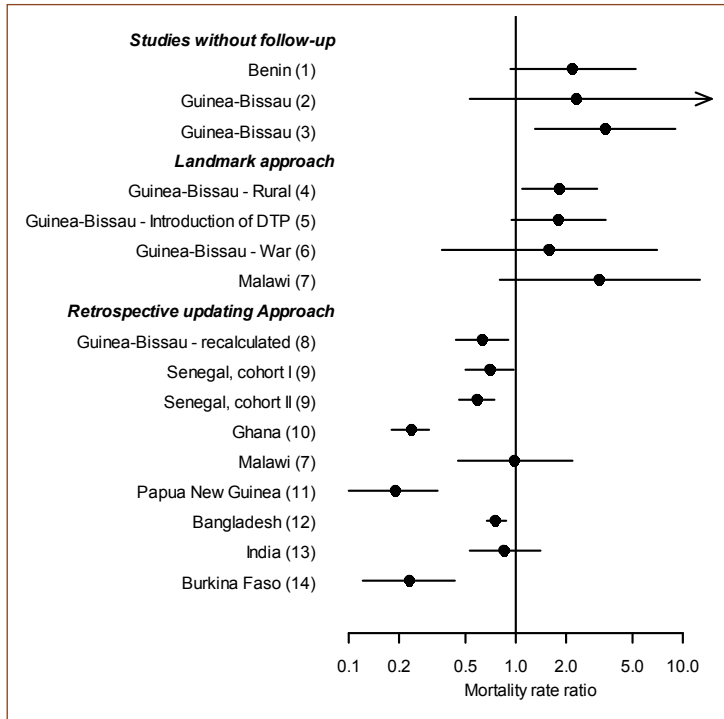
Figure 1. Mortality rate ratios for DTP-vaccinated children compared to DTP-unvaccinated children. The numbers in brackets refer to sources, see (154).

Retrospective updating approach

In the retrospective updating approach vaccination status is a time-varying variable changing from unvaccinated to vaccinated, on the *exact date of vaccination*. This approach will introduce survival bias if information is missing on vaccinations given since latest visit for children who died.

Landmark approach

In the landmark approach vaccination status may also be a time-varying variable but only changes from unvaccinated to vaccinated, on a *date of visit*. In a survival analysis the vaccination status is thus fixed or constant between two visits. Survival bias is thus avoided but an attenuation bias is introduced (see below). The BHP has primarily used the landmark approach.

Since the relative impact of these methodological differences was not clear, we conducted computer simulations using a simple vaccination model with varying assumptions on length between visits, true mortality ratio (MR), vaccination coverage, and number of dead children missing vaccine information between visits ("card destroyed") (153). To assess and compare the impact of the two approaches in a real life example we furthermore, using the retrospective updating approach re-analysed, a cohort from rural Guinea-Bissau which had missing vaccination data depending on the survival of the children (153).

Results from simulations

In Figure 2 it can be seen that for the retrospective updating approach, the estimated effect (MR) was on average lower than the true value, i.e. biased towards zero. The extent of survival bias will depend on the proportion of misclassified (un)vaccinated deaths, and this will depend on the interval between data collection visits and the proportion of children for whom *post-mortem* information is not obtained. In contrast, irrespective of the direction of the true effect, the landmark approach will on average for any combination of simulation parameters lead to conservative estimates of the true effect (bias towards the null hypothesis of a MR=1). The attenuation bias from the landmark approach decreases with shorter intervals and lower vaccination coverage.

Results from re-analysis of BHP data

The data were from the routine collection in rural study areas of BHP. Briefly, children were followed from an initial home visit at 0-6 months of age to a second visit six months later, and only vaccinations given before the initial visit were used to determine vaccination status in the analysis. We only had information on vaccinations given between visits for 3 of the 222 children who died between visits, thus substantial survival bias is present. The Table shows how differently the two approaches allocate follow-up time in the vaccination groups but only the three
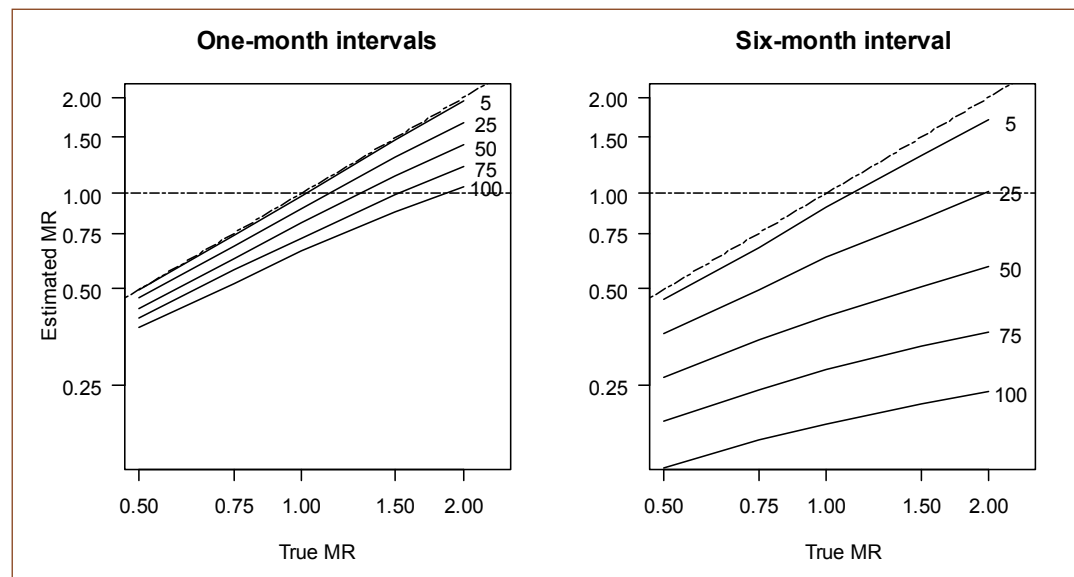
deaths with new vaccine information have changed vaccination status. The consequence of this reallocation is a total change in the mortality ratio between DTP-vaccinated and DTP-unvaccinated children which resembles the divergent effects of DTP described above (Figure 1).

Future Perspectives

The demonstrated potentially high impact of survival bias on the analysis of NSE on survival from observational studies has recently moved the discussion about NSE much forward. In April 2008 an international workshop on NSE of vaccines was held in London from which 3

One-month intervals — **Six-month interval**

Figure 2. Retrospective updating approach. Results from computer simulation study showing effect of interval between visits, mortality ratios (MR), and percentage of dead children whose vaccination status could not be updated. Each full line with number corresponds to percentage of dead children whose vaccination status could not be updated. Dotted lines represent the identity line and MR=1.

Table. Distribution of deaths and person years according to vaccination status and analytical approach (93).

Vaccination status		Landmark			Retrospective updating		
		Deaths	PYRS*	Rate**	Deaths	PYRS	Rate
No BCG	DTP 1 dose	2	9.0	222	2	16.3	123
No BCG	DTP 2 doses	0	4.5	0	0	3.5	0
No BCG	DTP 3 doses	0	1.4	0	0	4.2	0
BCG	No DTP	33	537.6	61	33	334.4	99
BCG	DTP 1 dose	59	595.5	99	60	679.5	88
BCG	DTP 2 doses	21	266.6	79	20	443.4	45
BCG	DTP 3 doses	12	119.6	100	15	425.0	35
Vaccinated		127	1534.2	83	130	1906.3	68
Unvaccinated		95	875.1	109	92	503.0	183
All		222	2409.3	92	222	2409.3	92
Mortality ratios							
DTP 1 vs. DTP-unvaccinated		1.84 (1.10-3.10)			0.68 (0.44-1.04)		
DTP 2 vs. DTP-unvaccinated		1.38 (0.73-2.61)			0.26 (0.15-0.47)		
DTP 3 vs. DTP-unvaccinated					0.16 (0.08-0.32)		

*   Person years of follow-up
**   Mortality rate per 1000 person years.

papers on respective methodology, data collection, and possible randomised clinical trials of NSE of vaccines are to be published.

References on methodological problems:
73, 74, 79, 93, 98, 99, 153, 154, 201

BCG at birth: Non-specific beneficial effects

Background

When the Swedish physician Näslund introduced BCG in the northern part of Sweden (Norrbotten) in the late 1920s – shortly after the discovery of BCG - he was surprised that mortality was 2-3-fold lower among the BCG-vaccinated children than among the unvaccinated children (Figure 1). The effect was difficult to explain as it was strongest during the first year of life whereas TB mostly killed older children. Näslund went as far as suggesting (in French) that "One could be tempted to find the explanation for this much lower mortality among vaccinated children in the idea that BCG provokes a non-specific immunity" (41). Many other physicians at the time noted spectacular effects on child survival when BCG was introduced in the 1920s and 1930s (125). However, the focus was on prevention of TB and Näslund's hypothesis was never formally tested. Still it has remained common knowledge that BCG is a strong immune stimulant in animal studies and laboratory work.

Our analyses of the impact of routine vaccinations on child survival clearly suggested that BCG was associated with a strong beneficial effect. In all the studies comparing several vaccines, BCG and measles vaccine were associated with a beneficial effect whereas DTP had a negative effect (Figure 2). On the assumption that the effect should be strongest among children who had reacted to BCG, we tested whether having a BCG scar or a positive PPD reaction was associated with an improvement in subsequent survival. In all the studies we did indeed find such an association and the effect was much stronger for girls, strengthening the possibility that this was a non-specific immunological effect (7,87,90,133).
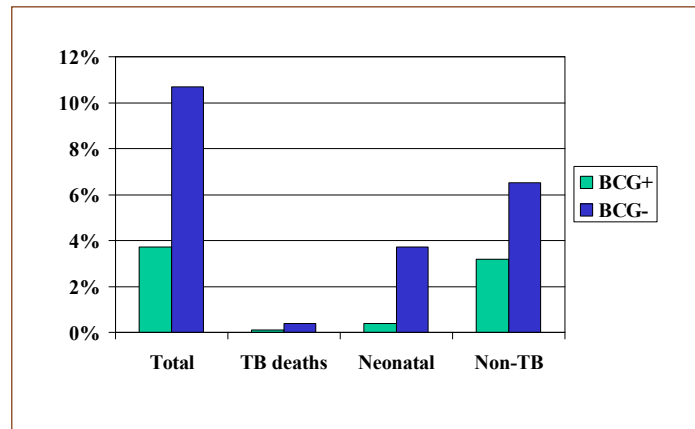
Figure 1. Introduction of BCG in Norrbotten, Sweden, 1927-31: Mortality at 0-4 years of age. 20,000 children

Figure 2. The mortality ratio associated with BCG, DTP and measles vaccine in several community studies from Guinea-Bissau (GB), Benin and Malawi

Still observational studies hold little persuasive power and limited policy implications. We were therefore interested in examining whether BCG had a beneficial non-specific effect in a randomised trial. For logistic reasons, BCG is recommended to be administered at birth in low-income countries and it would be unethical to withhold vaccine from some children. However, many countries, including Guinea-Bissau, have a special policy for low-birth weight (LBW) children who are assumed to be premature and recommended to wait with vaccination until they have reached normal birth weight. In an observational study we had found that BCG was considerably delayed for LBW children and that BCG at birth also to LBW children seemed beneficial (58). We therefore initiated a randomized trial of BCG at birth to LBW children in Bissau.

Results

The majority of births in Bissau are delivered at the maternity of the national hospital in Bissau city. We recruited all LBW children from Bissau city before they were discharged from the hospital. In order to follow these children who mostly were residents outside of the Bandim study area, we had to drive them home to secure proper identification of the residence. LBW children coming to the health centres in the study area for vaccination, after having been born in the home, were also offered enrolment. However, 90% of the LBW children were enrolled at the national hospital.

Between November 2004 and January 2008, we recruited 2,240 LBW children. The median age at BCG vaccination among those randomised to receive BCG at birth was 2 days; among controls

the median age was 49 days, reflecting that many will only get their BCG at 6 weeks of age when they also get DTP and OPV. Follow-up is still ongoing. However, in the first month of life before the subsequent DTP and OPV vaccines were delivered, BCG had a major impact on child survival, reducing mortality by 44% (mortality rate ratio (MRR)=0.56 (0.35-0.89)). This effect was slightly stronger for girls (MRR=0.47 (0.22-0.99)).

Public health implications

There are many implications if this observation can be verified. First, BCG should be administered at birth also to LBW children. Second, maternity wards should vaccinate with BCG before discharging the children. This would not have happened in Bissau unless we had organised and paid for the service as part of the present project. Third, though BCG is recommended at birth for normal-birth-weight children there are often marked delays in BCG vaccination, particularly in rural areas. A bottle of BCG vaccine contains 20 infant doses. It is common practice not to open a bottle for a single child but to require that there should be several children to be vaccinated. Many health centres have therefore only BCG vaccination once a week or once a month. The practice clearly delays BCG vaccination. Fourth, BCG is not a very good vaccine against adult TB, particularly in low-income countries. Numerous groups are therefore trying to develop a new TB vaccine. Replacing BCG with a new TB vaccine could be disastrous if the new vaccine does not have the same beneficial immune stimulatory effects for children (124).

Future perspectives

As Näslund suggested BCG does indeed provoke non-specific immunity. It would be essential to find out precisely what the immunological mechanisms are (163). It is likely that one study is not enough to persuade policy makers, and more studies from other communities would therefore be desirable. We are hoping to develop a network of INDEPTH sites which will be capable of testing the effect of improving BCG delivery in communities with high neonatal mortality.

References to BCG:
7,58,68,87,88,90,115,124, 125,133,163

Oral polio vaccine at birth

Background

Oral polio vaccine (OPV) is currently recommen-
ded at birth as well as in 3 doses together with
diphtheria-tetanus-pertussis (DTP)/Pentavalent
vaccine (DTP+Hepatitis B+Haemophilus
Influenzae type B) at 6, 10, and 14 weeks of age.
This policy to provide OPV at birth was introdu-
ced 20 years ago to increase the coverage for
OPV. OPV at birth may be associated with hig-
her seroconcersion rates. However, the effect of
OPV at birth on the immune response to other
vaccines and on overall child mortality was
never studied.

We recently experienced two periods in 2004
and again in 2007 during which OPV was lack-
ing in our study area in Guinea-Bissau, West
Africa. Hence, some children did not get the
recommended OPV together with BCG at birth.
In 2004, we were following all infants as a part

of a large vitamin A trial; 962 children did not
receive OPV at birth. We studied the effect of not
receiving OPV at birth on the mortality and the
immune response to BCG vaccine given at
birth.

Results

Not receiving OPV with BCG at birth was asso-
ciated with a significantly lower mortality in
boys, but not in girls (Figure). Boys had a 2.5
fold increased mortality if they had received
OPV at birth. We bled a subgroup of the children
at 1½ months of age. Receiving OPV at birth
seemed to have interfered with the immune
response to the simultaneously administered
BCG vaccine; OPV at birth was associated with
significantly dampened ex vivo cytokine respon-
se to PPD (purified protein derivative of M.
tuberculosis). Corroborating this finding, the in

vivo response to PPD (tuberculin skin test) at 2 months of age was significantly reduced among recipients of OPV at birth.

In 2007, we had a similar episode in which 99 children enrolled in a trial of low-birth-weight (LBW) children did not receive OPV at birth. Again, not receiving OPV at birth had sex-differential effects on subsequent mortality.

Public health implications

Based on these observations, receiving OPV at birth may have two negative effects; first, it may increase male mortality, and second, it may interfere with immunity against tuberculosis. In both observational studies, boys not receiving OPV and girls receiving OPV at birth had a 40% lower mortality than in the opposite situation.

OPV may be replaced with inactivated polio vaccine (IPV) in the global strategy to eradicate polio. However, this is unlikely to happen soon as IPV is much more expensive than OPV and polio is still not under control in some low-income countries including Nigeria, Somalia, Namibia, and India. If OPV at birth with BCG does have a harmful effect on boys, OPV at birth should be discontinued for boys immediately

since the marginal contribution of this vaccine to polio immunity and control is limited. It is also important to document whether OPV has a non-specific beneficial effect for on girls before the vaccine is removed. If OPV has a beneficial effect on girls, OPV at birth should be continued for girls or replaced with similar immune modulation.

Future perspectives

OPV at birth is given for logistic reasons, to boost polio immunity. There have been no polio cases in Guinea-Bissau for the last 10 years.

Hence, there is every reason to test in a randomised trial whether not receiving OPV at birth is associated with 1) decreased mortality in boysand 2) increased immunological response to BCG. Such a trial has now been initiated. The trial will contribute importantly to measuringthe importance and sex-differential effects of modulation of the immune system in early life and to understanding the underlying mechanisms. The immunological data are completely novel and highlight the fact that multiple vaccines have co-regulatory effects that need to be understood at a molecular level to allow the vaccine world to design not only better vaccination schedules but also better new vaccines.

References: 223

Figure. Cumulative mortality curves as a function of receiving OPV at birth or not, overall and by sex

Revaccination with BCG

Background

Many observational studies have shown that BCG vaccination is associated with lower mortality in ways that cannot be explained by prevention of tuberculosis. Children who have a BCG scar or a positive tuberculin skin test reaction have better survival, an effect particularly strong for girls. A French-Algerian study from the first part of the 20th century when BCG was used as an oral vaccine reported a 27% reduction in mortality from repeated doses of BCG administered at birth, one, and three years of age. Revaccination with BCG is not recommended by WHO since no protective effect against TB has been demonstrated. However, some countries have used revaccination among young adults.

We therefore conducted a randomised clinical trial to test whether revaccination with intradermal BCG vaccine would improve child survival.

Booster doses of DTP and OPV were administered at 18 months of age in Bissau. We randomised children to BCG revaccination at 19 months of age to reduce interference with booster DTP and planned to include 3000 children. Since animal studies had shown that BCG prevented malaria in mice we also conducted a special investigation to examine whether BCG might reduce the risk of malaria infection in 1½-2 year old children. As part of this study a large number of children were examined for malaria infection and anaemia and treated with iron if they were anaemic.

Results

The study recruited nearly 2900 children between July 2002 and April 2004 at the 3 health centres in the urban study area. The study included only children who did not have large PPD reaction; children with a large reaction were

referred to examination for tuberculosis. Enrolment was stopped prematurely in April 2004 due to a cluster of deaths in the BCG revaccination arm but we continued to follow the children to 2006. As seen in the Figure, mortality increased in the BCG arm at the end of 2003 and beginning of 2004. As a consequence there was a significant change in the mortality rate ratio (MRR) between BCG-revaccinated and control children. As a result there was no overall benefit from BCG revaccination, mortality being slightly higher among revaccinated children. Likewise there was no benefit in terms of protection against malaria infection or hospitalisation for malaria (167). However, there was a sex-differential effect; the overall hospitalisation incidence rate was significantly lower for girls than for boys among BCG revaccinated children (Incidence rate ratio (IRR)=0.66 (0.47-0.93)) whereas there was no sex-difference for controls (225).

We explored possible causes of this unexpected mortality cluster. The sudden increase in mortality coincided with a vitamin A supplementation (VAS) campaign and the treatment of a large number of anaemic children with iron in the end of November 2003 (see Figure). At the same time a measles epidemic was sweeping through the

community. During this short period mortality was nearly three-fold higher among BCG-revaccinated children (Mortality rate ratio (MRR)=2.69 (1.05-6.88)).

Micronutrients received	No vitamin A	Vitamin A+
No Iron	0.34 (0.04-3.29)	8.46 (1.06-67.7)
Iron +	Undefined P=0.070	1.91 (0.35-10.5)
All	1.29 (0.29-5.75)	4.14 (1.17-14.7)

Table 1. Mortality rate ratio (MRR) for BCG+ revaccination versus No BCG revaccination according to micronutrients received

We had monitored who received VAS during the campaign and we had administered iron ourselves. Hence, we could examine whether the increase among BCG-revaccinated children was related to the supplementation with micronutrients (Table 1). The negative effect of BCG revaccination was particularly strong among the large group of children who had received VAS during the campaign. Iron may also have had a negative effect though this was not statistically signifi-

	Mortality rate/100 pyrs (deaths/pyrs)		Mortality rate ratio BCG/no BCG
	BCG revaccination	No BCG revaccination	
	N=1412	1419	
Booster DTP before enrolment	0.4 (5/1393)	1.0 (14/1406)	0.36 (0.13-0.99)
No Booster DTP before enrolment	1.8 (37/2012)	1.0 (21/2022)	1.80 (1.06-3.08)
All	1.2 (42/3405)	1.0 (35/3428)	1.20 (0.77-1.89)

cant. If anything the effect was opposite among children who had received neither VAS nor iron (226).

We also examined whether mortality during the whole trial depended on the vaccination status at enrolment. As seen in Table 2, BCG revaccination was associated with a significant 3-fold reduction in mortality among the children who - as planned - had received booster DTP prior to BCG revaccination. However, 60% of the children had not received all vaccines prior to enrolment and they were told to return to receive the missing doses of DTP, OPV or measles vaccine. Most did return, and among these children BCG revaccination was associated with significantly increased mortality. As a consequence the overall effect of BCG revaccination was slightly negative. The negative effect of VAS among BCG-revaccinated children was only found among children missing vaccines at enrolment and who did receive DTP or measles vaccine or both after enrolment (226).

Public health implications

BCG revaccination did have the hypothesised beneficial effect among the children who as planned had received a booster dose of DTP prior to enrolment in the BCG trial. However, the trial also suggests that BCG vaccination in a situation with several other immune stimuli (VAS, iron, DTP, measles vaccine) may have a negative effect presumably due to some form of overload and misdirection of the immune response in response to infections. BCG revaccination could presumably be used to reduce mortality in settings in which other interventions were controlled.

However, there may be little coordination between intervention programmes being offered to children in low-income countries. It is therefore quite likely that children may receive a delayed BCG vaccination in the age group in which VAS is usually administered. One study from Bangladesh in which a large proportion of the children had received BCG and DTP with measles vaccine after 9 months of age did in fact report a significant two-fold increase in mortality associated with BCG vaccination.

Table 2. Mortality rate and mortality rate ratios by BCG revaccination status, sex and sequence of vaccinations.

BCG

Number at risk: 435   751   1114   1200   1135   1043   912

Control

Number at risk: 424   747   1115   1211   1138   1062   914

Not measles
Measles
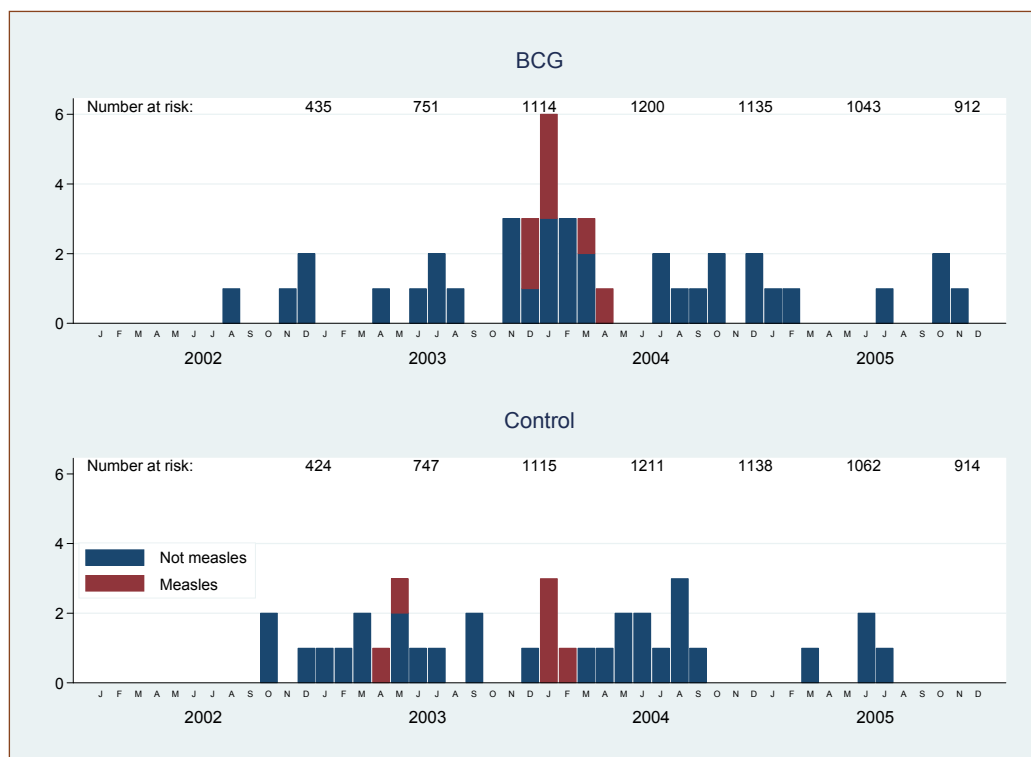
Figure. The number of deaths by month in the BCG revaccinated and the control group from July 2002 to May 2006

Future perspectives: understanding interactions

The study confirmed that non-specific effects of vaccines are important; the differential effects cannot be related to protection against tuberculosis. The study also suggested that non-specific effects may be difficult to control due to unanticipated interactions with a large number of other immune stimuli like vaccines and micro-

nutrients. To get a better understanding of the basis for these interactions will be the next challenge. This and further epidemiological studies will be needed to convince the donors and policy makers that we do need to test the likely interaction between our common interventions in childhood. Though interventions have a beneficial effect when studied in a controlled situation they may in fact have a totally different effect in a real life situation with administration in sequence with many other interventions. Though we believe that our interventions are good and efficient, they may actually have a negative effect under certain circumstances.

References on BCG revaccination: 167,225,226

High-titre measles vaccine: The sequence of vaccinations

Background

The incident of high-titre measles vaccine (HTMV) was the key event in defining the research agenda on non-specific effects of vaccines. HTMV was tested in Guinea-Bissau in the 1980s and found to be protective against measles infection. In 1989, WHO recommended HTMV to be administered at 6 months of age. However, at the same time it became clear that HTMV was associated with increased female mortality in Guinea-Bissau, an observation that was subsequently confirmed in Senegal and Haiti. WHO withdrew the vaccine in 1992.

This incident clearly showed that non-specific events are important. HTMV was fully protective against measles infection. Nonetheless the vaccine was associated with a two-fold higher female mortality but no difference for boys. So not only did vaccines have non-specific effects

these effects were also sex-differential. These effects were major. In the West African meta-analysis, HTMV was associated with a 35% higher mortality between 4 months and 5 years of age.

It has been a challenge to understand how this could happen. If it happens once it could happen again. When the vaccine was withdrawn the common assumption was that we had come too close to the natural disease and that new measles vaccines were needed to protect against measles before 9 months of age. However, that made no sense because why would that be a problem only for girls? When we detected that DTP and other inactivated vaccines were associated with increased female mortality, it offered a completely different explanation of the HTMV incident.

No DTP after HTMV

F/M ratio: 0.96 (0.7-1.3)

DTP/IPV after HTMV

F/M ratio: 1.93(1.3-2.8)

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

Results

In the West African studies, HTMV had been administered 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 West African studies whether this change of sequence was the real cause of increased female mortality. Indeed, excess female mortality was only found among the group of children who did receive DTP/IPV after measles vaccine, the female-male mortality rate ratio (MRR) being 1.93 (Figure). Among the small group of children who did not come back for vaccination at 9 months of age there was no difference, the female-male MRR being 0.96.

There are only two other studies of HTMV from Sudan and Congo which have collected information on mortality after vaccination. We reanalyzed these studies (119). They were also consistent with the new hypothesis. In Sudan girls had also had significantly higher mortality than boys among the HTMV recipients. In Sudan, vaccine information had not been collected after enrolment but vaccination had been registered at enrolment, and it was known that most children had received missing doses of DTP and OPV after enrolment. There was a clear association between the number of doses of DTP missing at enrolment (and presumably given after enrolment) and the female-male MRR after reception of HTMV. In contrast, in Congo the children did not receive DTP after HTMV; most children received HTMV after DTP3 or together with DTP3. The female-male MRR was 0.40 in Congo (119).

In contrast to the common belief that HTMV had a deleterious effect, HTMV per se may have been associated with low mortality. The annual mortality between 6 and 36 months of age was only 1.0% in Congo among children who had received HTMV as the most recent vaccination. In Sudan, HTMV recipients had significantly lower mortality than randomised controls receiving meningococcal vaccine as a "placebo" in the interval before the controls also received measles vaccines (119).

These studies suggested that the sequence of vaccinations was important, the most recent vaccination determining the immunological profile. For girls, it was problematic if the most recent vaccination was DTP and not measles vaccine. Hence, DTP vaccination status at enrolment could determine subsequent mortality in a measles vaccination trial if missing doses of DTP were administered after enrolment. As indicated in the Table, this did turned out to be the case in the three measles vaccine trials from which we had data. After receiving measles vaccination, 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

These data indicate that the real problem was not HTMV but DTP and the change in sequence of vaccination. We removed a good vaccine for
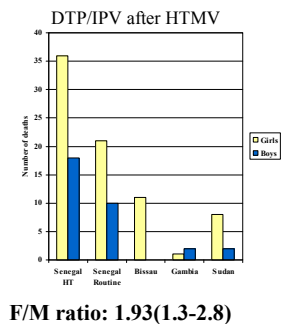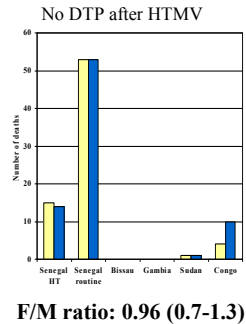
Table. Mortality rate after enrolment in measles vaccination trials in relation to the DTP vaccination status at enrolment

Study	Girls			Boys		
	DTP0-2	DTP3	MR (DTP0-2/DTP3)	DTP0-2	DTP3	MR (DTP0-2/DTP3)
Guinea-Bissau (166)	7.5%	3.8%	1.97 (1.04-3.72)	6.4%	6.0%	1.06 (0.60-1.90)
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			2.10 (1.19-3.72)			1.13 (0.67-1.90)

the wrong reason. Previous studies of vaccines have not taken the interaction and sequence with other vaccines into consideration. However, the data of administering DTP after measles vaccine (Table) or after BCG (Chapter 20) suggest that these effects may be very important for the outcome in vaccination trials.

The reinterpretation of HTMV constitutes the strongest argument for the hypothesis that DTP has negative effects on girls. It is impossible to generate a totally unexpected hypothesis from unconnected studies and find it consistent with all existing data unless a causal biological process is at work. An implication of this hypothesis is that the same may happen again once new measles vaccines are going to be tested in the first months of life.

Future perspectives

These observations also question the current 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 measles vaccine or after measles vaccine. All indications are that such practices are associated with increased mortality (Chapter 21). It would seem necessary to test the practice of administering DTP with measles vaccine or after measles vaccine in randomized clinical trials.

References on HTMV and sequence of vaccinations: 10,12,20,119

Early measles vaccination: Targeted and non-targeted effects

Background

The current policy of providing measles vaccine at 9 months of age in low-income countries was defined by the World Health Organization 25 years ago. The policy was built on studies of seroconversion after measles vaccination at different ages. To interpret the data five assumptions were used: children with antibodies were fully protected; children with no measurable antibodies were fully susceptible; measles in previously vaccinated and unvaccinated children was equally severe; it did not matter whether they got measles in infancy or later; and seeing measles in vaccinated children, so-called "vaccine failures", would lead to lack of confidence in the vaccination program. Based on these assumptions it was decided to vaccinate at 9 months rather than at 7 or 8 months of age. These assumptions were wrong and had the impact on infant survival been assessed in a

randomised trial, it would probably have been better to vaccinate at 6 or 7 months of age. "Vaccine failure" cases have lower case fatality and early vaccination would have moved measles infection to an older age group with lower case fatality. Furthermore, measles vaccination has non-specific beneficial effects which would prevent deaths as soon as measles vaccine was given irrespective of measles infection (1,3,8,84,107). However, the effect on infant survival of measles vaccine at age 9 months compared with measles vaccine at an earlier age was never tested.

There are further reasons to believe that measles vaccination before 9 months of age would reduce mortality more than measles vaccine at age 9 months. First, an increasing proportion of mothers have not had natural measles infection but have been immunised in childhood. Such mothers have lower measles antibody levels and

transfer lower levels to their offspring. Second, many studies have shown that DTP vaccine is associated with higher mortality for girls (12). Hence, it might be beneficial particularly for girls to be vaccinated early with measles vaccine and thereby reduce the time of exposure to DTP as the most recent vaccine (8).

For all these reasons, we have tried different strategies to reduce the age at measles vaccination including high-titre measles vaccination (HTMV) and two-dose schedules at 6 and 9 months of age (12,32). As described in the chapter on HTMV both of these strategies ran into problems with the sequence of vaccinations, due to a negative effect of DTP being administered after measles vaccine. In the most recent trial, we have therefore included children only if they had already received DTP3 and therefore should receive no further DTP after measles vaccination.

Since we have maintained very high measles vaccination coverage in the study area for many years we should have had no measles epidemic. However, coverage has declined in the rest of Bissau and many older children have moved to the study area. An epidemic of more than 2000 measles cases flared up soon after the new measles vaccination trial had started in 2003. This provided an unexpected opportunity for testing the effect of early measles vaccination.

Results

Between August 2003 and May 2004, 1333 children were randomised at the three local health centres in the study area, one third receiving standard Edmonston-Zagreb measles vac-
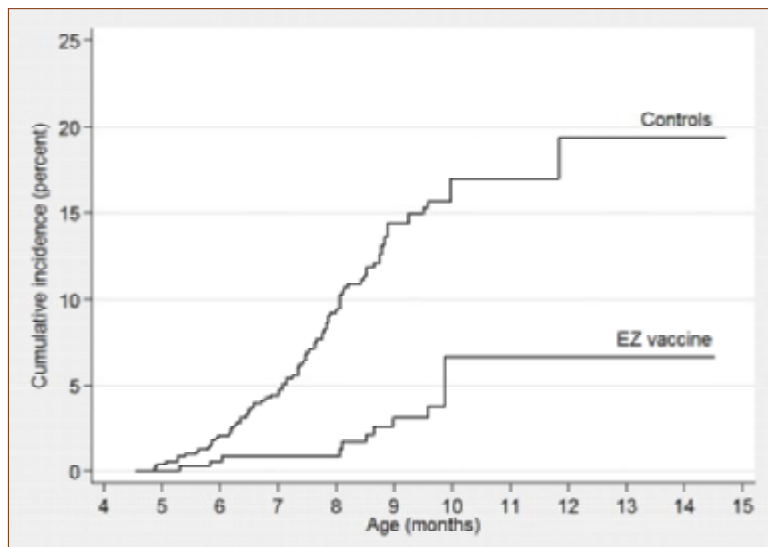
Figure. The cumulative incidence of measles in the early EZ measles vaccine and control groups

cine at 4½ months of age and the rest receiving nothing apart from medical consultation and treatment. All children were invited back at 9 months of age to receive their normal measles vaccination or their second dose of vaccine (214).

Surprisingly only 28% of the children who had measles antibody tested at 4½ months of age had protective antibody levels, the proportion being significantly lower among girls than among boys (RR=0.55 (0.34-0.91)). Consistently with the low level of maternal antibodies, the measles incidence was very high during the epidemic. Before 9 months of age, 19% of the unvaccinated children caught measles. In spite of the fact that we offered clinical treatment to all children including at hospital, the case fatality was still as high as 8%.

Measles vaccination at 4½ months of age turned out to be highly efficacious in the interval between 4½ and 9 months of age in which the children would not normally be protected. The efficacy against serologically confirmed or definite clinical infection was as high as 94% (Figure). Early measles vaccination prevented completely measles hospitalisation and measles death. Though not significant, overall mortality was lower in the early measles vaccination group (MRR=0.18 (0.02-1.36)). Long-term follow-up is still ongoing in the present study so the final result is not yet known. However, the indication is that early measles vaccination may help reduce hospitalisations and non-measles deaths among girls.

Public health implications

In spite of many years of measles vaccination, measles is still potentially a very severe infection in this urban community. The situation has

been aggravated by declining maternal antibody levels. The majority of children are losing maternal antibody levels before 6 months of age. When the current vaccination programme was formulated 25 years ago most children only lost protection between 6 and 9 months of age. Now there is a second group of mothers who has not had natural infection and their offspring may therefore be losing protection already at 3 to 5 months of age. The challenge is therefore to find a policy which may protect both groups of children. One could fear that early measles vaccination would lead to lower antibody levels and therefore a higher risk of measles infection later in life. If that is not the case, the present study suggests that a two-dose strategy at 4 and 9 months might be a good option in countries which have had high measles vaccination coverage for a long time. Already at this stage it seems clear that EZ measles vaccine can be used as early as 4 months of age during outbreaks or in situations with a high concentration of infants.

Future perspectives

If the present beneficial effects on also non-measles deaths of early measles vaccination persist, early measles vaccination should be tested in other populations, particularly for the non-specific beneficial effects. It would be interesting to assess the impact in a situation with higher maternal antibody levels.

Following the problems with HTMV, major donors, including EU, WHO, Gates and NIH, have invested in new measles vaccines which should be able to immunise children early in life. Such new vaccines should be tested for both targeted and non-targeted effects against EZ vaccine which has a proven efficacy in this age range.

References on measles vaccine:
1,3,8,84,97,107,214

Sex-differential and non-specific effects of inactivated vaccines

Background

Several observational studies have suggested that DTP may also have non-specific effects, but in contrast to BCG and measles vaccine the effects of DTP are negative. In medical culture, observational studies have little impact. For these observations to have policy implications, it would be desirable to conduct randomised studies to test a possible negative effect of DTP. However, it is unethical to withhold a vaccine which is already recommended. Hence, the hypothesis that DTP has a negative effect on survival can only be tested indirectly, by making logical deductions and testing these. The more deductions can be shown to be consistent, the more likely it is that the underlying hypothesis reflects a causal process. This process has generated several linked observations which do suggest that DTP has an unwanted effect on girls. It should be noted that most observations have been done in situations with herd immunity to pertussis, and hence a survival benefit from being protected against pertussis has not been evident.

Results

First, in all community studies analysing the impact of several vaccines on survival, BCG and measles vaccine have been associated with a beneficial effect whereas DTP has had a negative effect (Chapter 15).

Second, the effect of DTP has been worse for girls than for boys. This is illustrated by the female-male mortality rate ratio (MRR) in all available studies (Figure 1) (44, 45, 81, 119, 126, 134, 179). This pattern is also different for BCG and measles vaccine which are both associated with lower female mortality. Most studies have focused on mortality but a series of morbidity studies have also indicated that the relative risk

for girls and boys changes depending on the most recent vaccine, incidence being higher for girls than boys while DTP is the most recent vaccine (120,129).

Third, if DTP is in fact associated with increased female mortality one would expect that female mortality is increased over male mortality in the age group in which DTP is the predominant vaccine in communities with high vaccination coverage. Female-male mortality has not previously been analysed in this way but in all the studies we have analysed the pattern has been consistent, as illustrated by the two studies from The Gambia and Malawi (Figure 2). After an initial lower mortality for girls, female mortality increases over male mortality around 3-4 months of age, i.e. the age group in which DTP predominates. Around 9 to 12 months of age, i.e. the age group in which measles vaccine predominates, female mortality declines steeply becoming again lower than male mortality.

Fourth, if these negative effects had to do with the vaccine being "inactivated" rather than with any of the specific antigens of DTP vaccine, one should presumably find similar patterns for hepatitis B vaccine (HBV) and inactivated polio vaccine (IPV). For both vaccines we have in fact shown that they are associated with increased female mortality. For HBV, the female-male mortality ratio in Guinea-Bissau was 2.20 (1.07-4.54) during the period in which HBV was provided to a large number of the children in Bandim (65). Through the 1980s and 1990s, we used IPV as a "control" vaccine in several trials in which meas-les vaccine was provided early at 4-6 months of age. Until the IPV recipients received measles vaccine at 9 months of age, girls had 52% (2-128%) higher mortality than boys (166).

We have obtained and analysed data sets from several other low income countries including The Gambia (134), Senegal (44,201), Ghana, Congo (119), Sudan (119), Malawi (126) and India. All studies have been consistent with the studies from Guinea-Bissau showing sex-differential and non-specific effects of vaccines (Figures 1-2).

Public health implications

Given this consistency and the fact that DTP has been found to have a negative effect for girls when administered after measles vaccine (Chapter 18) and the negative interactions with vitamin A supplementation (Chapter 25), it is difficult to imagine that DTP should not have a negative effect on mortality for girls. DTP has mainly been examined in situations in which herd immunity has been sufficient to prevent deaths from whooping cough. It is therefore not known what would happen if DTP was removed. In the long run, it will be necessary to develop a DTP-type vaccine without negative effects. However, such a vaccine does not exist and we have therefore focused on different strategies to reduce the time of exposure to DTP as the most recent vaccine (Chapters 17 and 19).
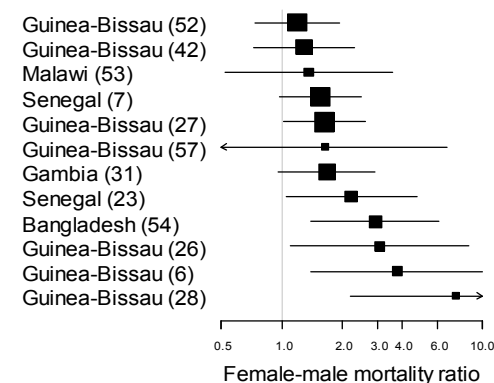
Figure 1. Female-male mortality rate ratios among DTP-vaccinated children

A number of WHO-sponsored studies has questioned our observations. These studies have often had methodological problems with survival bias (153). Furthermore, most studies have examined the effect of DTP when administered simultaneously with BCG whereas we have examined the effect of DTP administered after BCG as currently recommended by WHO (154). The effect of simultaneous BCG and DTP vaccinations is likely to be quite different from sequential administration (201).

Given the current controversy, it is still necessary to conduct further studies of the impact of DTP on overall morbidity and mortality and these should preferably be randomised clinical trials since WHO's Global Advisory Committee on Vaccine Safety has declared that it is unlikely to be persuaded by observational studies.

Future perspectives

We have pursued three slightly different approaches to reduce the time of exposure to DTP as most recent vaccine. As suggested by Figure 2, if the age at measles vaccination was moved forward from 9 months to 4-5 months of age this might reduce female mortality in an age group with a very high mortality. The preliminary results are supportive (Chapter 19). We have also tried to administer BCG revaccination after booster DTP to reduce the negative effect of DTP (Chapter 17). Finally, in a study which is still ongoing, we are randomising children to receive or not receive DTP booster vaccination.

Several other approaches may have to be tested in the future. There is good evidence from observational studies that the effects of combining DTP with either BCG or with measles vaccines are quite different from the effect observed when the vaccines are administered sequentially as recommended by WHO. Since such combined vaccinations are quite common, particularly in rural areas, it would be important to test their effects in randomised trials. It should also be considered to delay the initial DTP vaccination in randomised clinical trials.

References on DTP: 12,26,41,44,45,46,65,73,74,75, 79,81,93,98,99,119,120,126,129,134,149, 150,153,154,161, 166,179,201,217

Figure 2. Age and sex-specific mortality curves from Gambia and Malawi (126,134)
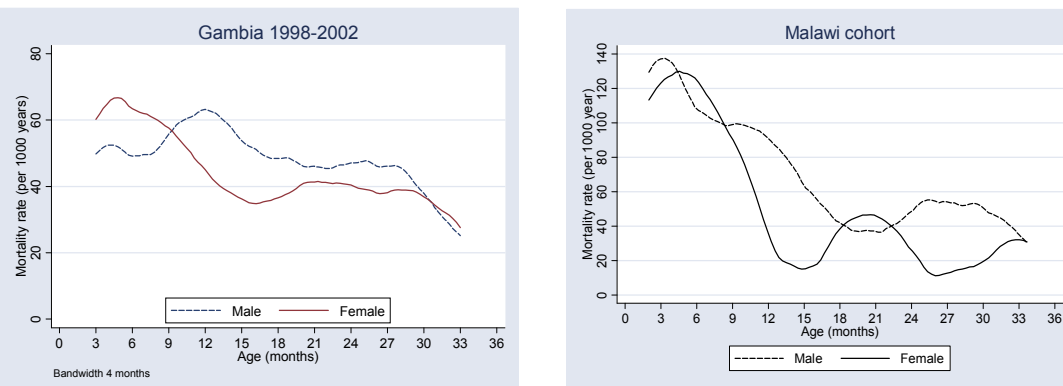
Out-of-sequence vaccinations

Background

The high-titre measles vaccine (HTMV) incident (Chapter 18) made it clear that the sequence of vaccinations was very important for child mortality. This made us look more systematically at the out-of-sequence vaccinations. WHO has for many years recommended a schedule of BCG and OPV at birth and then three doses of DTP and OPV at 6, 10, and 14 weeks of age, and finally measles vaccine at 9 months of age. In some countries the tradition of administering booster doses of DTP and OPV has continued even though this is no longer official WHO policy (Figure). In recent years new vaccines have been added, in particular Hib and HBV often in a penta-valent vaccine with DTP.

In practice most vaccinations may not follow this schedule. Particularly in rural areas there are many reasons why vaccinations get delayed.

If the child is sick or malnourished the mother is likely to postpone vaccination. Many health centres have only a weekly or monthly day for BCG vaccinations. As a result, in the studies from Senegal, Ghana, India, Bangladesh (154), and the Philippines (201) which have reported such data, a majority of the children received BCG together with DTP or after DTP. Likewise, delay in the administration of DTP is very common and for that reason many children receive DTP and measles vaccination simultaneously or DTP after measles vaccination. This tendency has been strengthened by the donor policy of monitoring the performance of the national vaccination programmes through the coverage for DTP3. In rural Bissau 2/3 of the doses of measles vaccine are administered with DTP. We therefore examined available data sets for indication of such changes in the sequence of vaccinations.

Receiving DTP after HTMV or after standard measles vaccine was a strong risk factor for subsequent female mortality. It has not been examined whether the combination of routine vaccinations has an impact on child survival in low-income countries. BCG and measles vaccine are associated with an overall beneficial effect on childhood survival whereas DTP is not. However, these generalisations do not permit us to predict what might happen when these vaccines are given together. We therefore examined the mortality rate and possible sex-differential effects when DTP is given simultaneously with BCG or measles vaccine.

Results

Simultaneous administration of BCG and DTP. We have limited data on BCG and DTP combined but the indication from Bissau (44), Senegal (201) and India is that BCG and DTP combined is better for girls than for boys. For example, in a study of female-male twin pairs from Bissau and Senegal, the female twins had lower mortality as long as combined BCG and DTP were the last vaccinations, the female-male MR being 0.29 (0.02-1.03) (44). The impact on overall mortality of BCG and DTP combined or BCG after DTP may be beneficial.

Simultaneous administration of measles vaccine and DTP. So far we have been able to examine simultaneous administration of DTP and measles vaccine in 5 studies. All studies have found simultaneous DTP and measles vaccinations to be associated with higher mortality than having received measles vaccine as the most recent vaccine (Table). It should be noted that a priori the children who receive measles vaccine and DTP vaccine together may have less compliant mothers and have a higher risk of dying; however, the effect estimates seem too large to be explained merely by selection bias. All studies also suggest that combined measles vaccine and DTP may be worse for boys than for girls. One of these studies from Senegal was a randomised trial and the female-male MRR was 0.58 (0.33-1.03) (12).

The effect of combined DTP and measles vaccine might blur the beneficial effect of measles vaccine in survival analyses looking at the average effects of several vaccines without restricting the analysis to the most recent vaccination. For example, a Bangladesh study reported that the effect of measles vaccine was only 0.93 (0.65-1.34). However, when children with simultaneous DTP/BCG and measles vaccinations were excluded, the estimate of measles vaccine became 0.61 (0.44-0.85). This change implies that the

DTP/BCG and measles-vaccinated children censored in the analysis had strong excess mortality. Hence, this study supports the same trend as the one we observed in our own studies (Table).

Public health implications and future perspectives

There is little tradition for testing the interactions between different interventions. Our data suggest that it makes a difference in which order vaccines have been administered. This possibility has not been taken into consideration in the current immunisation programme which recommends that missing doses of vaccines should be administered whenever there is a possibility.

It would seem necessary to test the effect of these unplanned combinations of both BCG and DTP and of DTP and measles vaccine in randomised trials.

References on out-of-sequence vaccinations: 12,44,119,126,134,150,154, 166,201,217

Country	No. children	Study	MRR (MV+DTP versus MV)
Congo (119)	485	EZ trial at 6 to 36 months	5.38 (1.37-21.2)
Malawi (126)	751	Routine vaccination	5.27 (1.11-25.0)
Guinea-Bissau (150)	403	Hospital case fatality for children aged 6-17 months	1.87 (1.06-3.31)
Guinea-Bissau (217)	201	Campaign with vitamin A in which some children received MV and some MV and DTP; children aged 6-17 months	Undefined; p=0.015
Gambia (134)		10% (4/41) of dead children aged 9-17 mo had received DTP=MV compared with 2% (43/2539) in community	5.59 (2.10-14.8)

Table. Mortality ratio for children receiving measles vaccine (MV) and DTP simultaneously compared with having received MV alone as the most recent vaccine
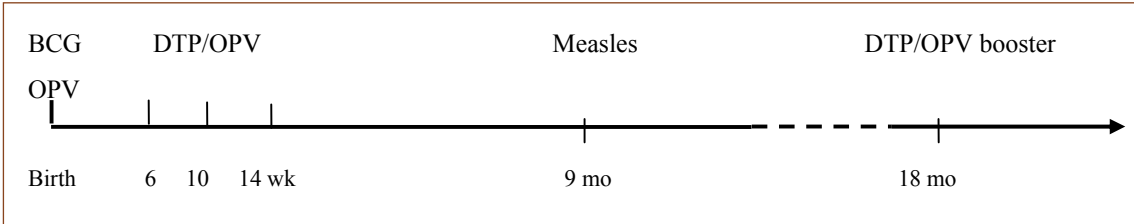
Figure: Vaccination schedule in Guinea-Bissau

Non-specific effects of smallpox vaccine in Guinea-Bissau

Background

Smallpox vaccination was introduced in 1800 and was associated with marked reductions in mortality in the industrialising countries. The last case of smallpox occurred in 1977 and in 1980 the World Health Organization recommended stopping smallpox vaccinations. No assessment was made of the health impact of discontinuing vaccination. Smallpox vaccination is associated with a strong immune stimulation and could have important non-specific effects as observed for other live vaccines such as BCG and measles vaccines.

In order to study a possible non-specific effect of smallpox vaccine, we needed access to vaccination status before 1980. In Guinea-Bissau this was possible through vaccination scar surveys. In several studies we investigate a possible non-specific effect of smallpox vaccine, some 30 years after its administration – an idea that may be over-stretching the imagination of many readers. There are, however, a number of case-control studies from high-income countries suggesting that smallpox vaccination protected against diverse chronic conditions and cancers. In a European study of people with malignant melanoma, it improved survival to have been smallpox vaccinated several years prior to diagnosis.

Results

Evaluation of smallpox vaccine scars as proxy for smallpox vaccination

We located most of the registration books of the vaccination programme in Bissau for the period 1964–1980. Sixty-nine persons with documented vaccination and still living in the study area had been visited at home to examine presence and size of smallpox vaccine scars in connection

with one of our surveys. Ninety percent (62/69) had had a smallpox vaccine scar identified, the median diameter being 16.5mm. The seven adults without a smallpox vaccine scar were revisited and five had a small scar that had not been detected. Among 1,076 teenagers who were too young to have received smallpox vaccine and whose scars were therefore were due to BCG, the median diameter was 5mm (135). Smallpox scar was thus considered a good proxy for smallpox vaccination with at least 90% sensitivity. Since it was not possible to clearly distinguish between large BCG and small smallpox vaccination scars, results were also presented as any scar (smallpox or BCG vaccination) versus no scar in addition to smallpox scar versus no scar.

Adult mortality

From January 1998 to January 1999, with pauses during periods of war, field workers assessed vaccination scars for 1893 adults above 25 years of age in Bandim 1 and 2. Only individuals above 25 years of age were eligible for inclusion, since smallpox vaccination in Bissau ceased in 1980. Survival was assessed in a survey conducted 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 ratio (MR) adjusted for age was 0.60 (0.40–0.91) during the non-war periods (Figure 1). The effect of smallpox vaccination may have been stronger for women (MR = 0.51) than men. The effect of smallpox vaccine appeared to be similar among individuals with a BCG
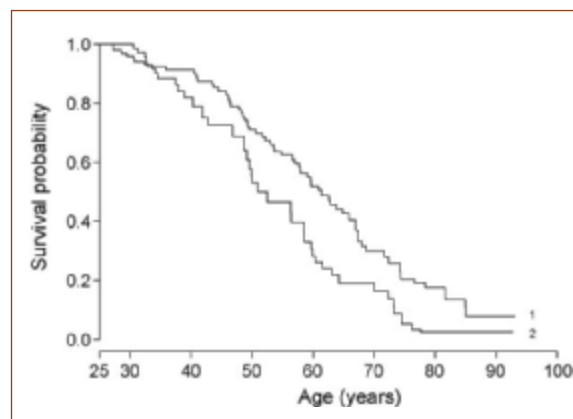
scar, and the effect of having any type of scar on mortality was 0.61 (0.41–0.89)(135).

It was possible to test our findings from the Bissau scar survey within a case-control study of HIV-2 infection in Caio, rural Guinea-Bissau. In 2003, 367 individuals born before 1974 (and therefore possibly smallpox-vaccinated) were examined for vaccine scars by a physician. Of these, 141 were only HIV-2-infected, 23 were only HIV-1-infected, 29 were dually infected, and 174 were uninfected. Survival was assessed in 2006 and over the three years of follow-up, 13% (47/367) had died. Individuals with a smallpox vaccine scar had lower mortality than individuals without any scar. The MR was 0.22 (0.08–0.61) adjusting for age, sex, village and HIV status. The MR for women was 0.19 (0.06–0.57) and 0.40 (0.04–3.74) for men. The tendency was the same comparing individuals with any scar to individuals without any scar, the MR being 0.25 (0.10–0.62). The association with better

survival was found for both HIV-negative and HIV-2 infected individuals (151).

HIV-infection

When analysing the Bissau scar-survey, there was an unexpected association between small-pox vaccination scars and HIV-2 infection. Persons with a smallpox scar were more likely to be HIV-2-infected compared with individuals with no vaccination scar, the prevalence ratio (PR) being 2.45 (1.06–5.65). We were able to confirm this finding within the Caio study; the prevalence of HIV-2 infection was 52% (131/251) among people with a smallpox vaccination scar but only 34% (39/116) among those without. The HIV-2 prevalence ratio for individuals with a smallpox vaccination scar was 2.08 (1.14–3.78) compared with individuals without any scar. The epidemiology of HIV-2 is consistent with a period of more intense transmission in the 1950s or 1960s (183). The smallpox vaccination campaigns could have contributed to the transmission of blood-born infections like HIV-2, presumably due to insufficient sterilization of the knife or needle used for vaccination.

Since HIV-1 was not present in Guinea-Bissau in the 1970s, vaccination with smallpox has not contributed to the spread of HIV-1. However, considering the marked effects of smallpox vaccination scars on mortality, we turned our attention to a possible association of smallpox vaccination with HIV-1. The most recent survey of HIV-infection in Bandim was conducted in 394 randomly selected houses between 2005 and 2006. The study was designed to examine the prevalence of HIV and included individuals aged 15 years or older living; 2082 individuals born before 1974 were included and had a blood-sample collected. Among those whose HIV-1 status was determined, we also managed to determine smallpox vaccination scar status in 1472 of these individuals. There was a statistically significantly reduced risk of having HIV-1 among women with a smallpox scar compared with women without a scar. A similar tendency was observed for men (Table 1).

This is the first time smallpox scar status has been found to be associated with a lower risk of having HIV-1. The hypothesis was generated by our previous findings of reduced mortality and morbidity among smallpox vaccinated individuals as well as findings on non-specific effects of other live vaccines. The underlying mechanism is thought to be an immunological general response to the vaccines that is not only specific to the targeted disease but also modifies the capacity of the immune-system more generally.

Public health implications

With the growing evidence for non-specific effects of vaccines, examining such effects of smallpox vaccine seems relevant considering its potency as an immune stimulator. We may gain important insight into the long-term non-speci-

Males (N=598)			Females (N=874)		
Scar status	% HIV-1 pos. (N/Ntotal)	OR (95 % CI)	Scar status	% HIV-1 pos. (N/Ntotal)	OR (95 % CI)
No scar	5.7 (7/122)	1	No scar	8.1 (17/209)	1
smallpox scar	3.4 (14/417)	0.53 (0.20-1.40)	smallpox scar	4.6 (26/560)	0.44 (0.22-0.90)
BCG or other scar	0.0 (0/59)	-	BCG or other scar	8.6 (9/105)	0.66 (0.27-1.62)

Table 1. Percent HIV-1 positives and OR of having HIV-1 controlled for relevant explanatory variables (birth year, ethnicity, number of children and fieldworker). (Preliminary results).

fic effects of live vaccines. Due to the unacceptably high rate of side-effects, it is however not likely that smallpox vaccine will be reintroduced in low-income countries.

Future perspectives

The studies in Bissau have generated large cohorts of individuals who have been assessed for smallpox vaccine and BCG scarring. These cohorts will be followed to study association with other health outcomes as, for example, cholera infection during the current epidemic in Bissau. The data from Bissau also suggested a very strong association with arm-circumference, smallpox vaccinated individuals having a much larger circumference. Larger arm-circumference is a strong predictor of subsequent survival. This association as well as immunological stu-

dies of the associations between smallpox vaccine and various infectious and chronic diseases are warranted. Modified vaccinia Ankara (MVA) is used in experiments as a vector for other antigens and it would be interesting to study its possible non-specific effects.

References on smallpox vaccination: 135,151

Non-specific effects of small-pox vaccine and BCG: Taking the observations to Denmark

Background

Smallpox vaccination (vaccinia) was discontinued in the late 1970s. In several European countries, compulsory BCG vaccination practise was discontinued at the same time. A number of case-control studies from high-income countries suggest that smallpox vaccination protect against diverse chronic conditions and cancers. In a European study of people with malignant melanoma, smallpox vaccination reduced the risk of developing malignant melanoma and smallpox-vaccination improved survival among malignant melanoma patients who had been small-pox-vaccinated several years prior to diagnosis. Considering these observations and our findings from Guinea-Bissau, we wanted to study whether smallpox vaccine and BCG also had non-specific effects in a European setting. In Denmark, we could obtain information about smallpox and BCG vaccination before 1980 as well as status of other vaccines through inspection of the school-health records.

Results

Information on smallpox vaccination was obtained from the Copenhagen School Health Records Registry (CSHRR).Persons born 1965 to 1976 were studied, since selection to vaccinated and unvaccinated would be more random during a time when a vaccination practise was ceasing than when vaccination was compulsory and a small group refused or had contraindications against vaccination (Figure). As expected, BCG coverage declined in a similar way as smallpox vaccination, just a few years later.

Atopy, allergic rhinitis and asthma From an ecological perspective, the termination of small-pox vaccination in high-income countries coinci-
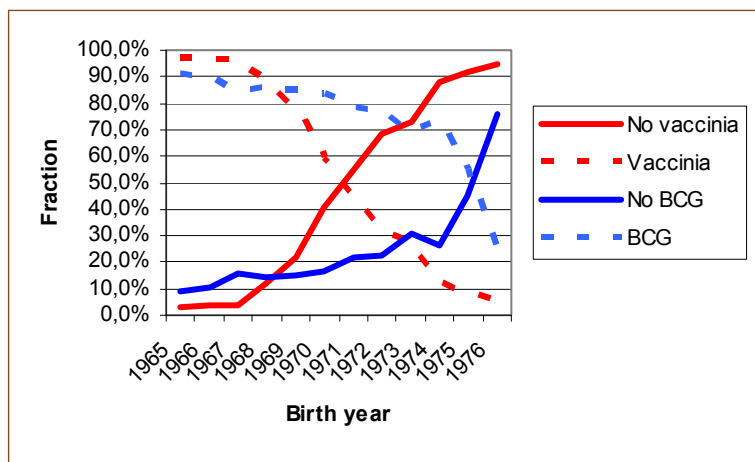
Infectious disease hospitalisations
Through linking of the CSHRR to the Danish registry of hospitalisations we were able to determine infectious disease hospitalisation (N=765) for the 2039 individuals for whom we had determined vaccination status. Preliminary analysis shows that BCG is associated with a lower risk of all-cause infectious disease hospitalization among women and a tendency towards smallpox-vaccinated subjects having a lower risk of all-cause infectious disease hospitalisation than subjects not vaccinated with these vaccines (Table). Smallpox-vaccinated subjects were less likely to have skin infections and BCG-vaccinated subjects less likely to be hospitalised for sexually transmitted infections (STI) than unvaccinated individuals. These observations are being pursued with studies of specific STIs including HIV-1. The preliminary indications are that BCG protects women against HIV-1 infection (hazard ratio (HR) 0.30 (0.12-0.77)) whereas the effect for smallpox vaccine was smaller (HR=0.81 (0.24-2.73)).

ded with an increased incidence of asthma. We examined the occurrence of atopy, allergic rhinitis, and asthma among Danish women within a national birth cohort study. Among the 1960 women for whom sera were available, 552 (28%) were classified as atopic; among the 1927 women with information on allergic rhinitis and asthma, 263 (14%) had allergic rhinitis, and 165 (9%) were cases of asthma. Overall, smallpox vaccination was not associated with risk of atopy or allergic rhinitis compared to unvaccinated women. However, smallpox vaccination was associated with an OR of asthma of 0.55 (0.30 to 1.00) adjusting for birth cohort, sibship size, age of the women's mother at birth, and social class. Hence, women who had received smallpox vaccination were less likely to have asthma, an association previously not described (9).

Future perspectives

The work on smallpox and BCG vaccination in a Danish context is in the early phase. The cohort of individuals with information on vaccination status in the 1970s will be enlarged and linkage with disease registers will be pursued to detect other associations between vaccination status and health.

References on smallpox vaccination: 9

Table. All infectious disease hospitalisation and the subgroup of skin infections and sexually transmitted infections in relation to smallpox and BCG vaccination status. Preliminary analysis in a Cox regression model with repeated events, controlling for sex and the other vaccine

Number of hospitalizations		All infections	Skin infections	Sexually trans-mitted inf.
		(765/2039)	(100/2039)	(77/2039)
Smallpox vaccination				
Women:	Non-smallpox	1(ref)	1(ref)	1(ref)
	Smallpox	0.85 (0.64-1.12)	0.51 (0.21-1.20)	1.10 (0.56-2.17)
Men:	Non-smallpox	1(ref)	1(ref)	1(ref)
	Smallpox	0.78 (0.58-1.06)	0.45 (0.20-1.04)	1.33 (0.42-4.27)
Total	Non-smallpox	1(ref)	1(ref)	1(ref)
	Smallpox	0.82 (0.65-1.02)	0.48 (0.25-0.94)	1.16 (0.62-2.19)
BCG vaccination				
Women:	Non-BCG	1(ref)	1(ref)	1(ref)
	BCG	0.76 (0.59-0.99)	0.93 (0.41-2.09)	0.35 (0.19-0.67)
Men:	Non-BCG	1(ref)	1(ref)	1(ref)
	BCG	1.06 (0.78-1.44)	1.36 (0.61-3.01)	0.38 (0.12-1.18)
Total	Non BCG	1(ref)	1(ref)	1(ref)
	BCG	0.87 (0.71-1.07)	1.11 (0.65-1.92)	0.36 (0.21-0.63)

Breastfeeding and improved child health:
Effect or confounding

Background

Not only is breastfeeding the ideal nutrition for infants; in environments with a high pressure of infectious diseases, breastfeeding is essential for a reduction in child morbidity and mortality. However, only few randomised studies have investigated the practical implications of breast-feeding promotion, and most data originate from observational studies. Introduction of complementary food leading to complete cessation of breastfeeding is a gradual process affected by many biological, behavioural and cultural factors. Thus, data from observational studies always bear a considerable risk of being influenced by unknown confounders. In settings with excessive child mortality and a strong association between breastfeeding and mortality, it is important to know if and how improved breastfeeding practices can reduce infant and child mortality.

Results

In a randomised study including 1,721 children the intervention group received individual health education encouraging the mothers to postpone introduction of water and weaning food until the child had reached the age of 4-6 months (189). Both water and weaning is introduced early in this community. However, for both water and weaning food the introduction was significantly delayed in the intervention group. Despite mothers being responsive to the intervention by postponing water and weaning food, we did not find an improvement in child health during the 6 months of follow-up. There was no difference in diarrhoea morbidity or hospitalisation between the intervention group and the control group. Children aged 4-6 months of age, those in the intervention group had a significantly lower weight compared with the control group and though not statistically signi-

ficant, mortality was slightly higher in the intervention group, the hazard ratio (HR) being 1.86 (0.79 - 4.39). However, the sample size was too small to detect a difference in mortality.

In an observational study following 1,724 children (2), we investigated the impact of the mother's reason for weaning on subsequent mortality. Following termination of breastfeeding, 66 children died before 36 months of age. Sixty-two % were weaned because they were "healthy". For 237 children weaned due to a new pregnancy the mortality ratio was 3.25 (1.45-7.30). Median length of spacing between an index child and a new sibling was 28 months irrespective of whether the index child survived or died before three years of age. The majority of the deaths occurred before the birth of the new sibling. Thus, confounding due to the mothers reasons for weaning may play a major role in the results from observational breastfeeding studies.

Public health implications

Promotion of breastfeeding has theoretically a great potential for reducing in morbidity and mortality in low-income countries. However, in real life it is questionable whether large scale promotion of exclusive breastfeeding will lead to any improvement in infant health in countries with a tradition for a long breastfeeding period. It would demand a great effort to change current behaviour patterns while the effect on mortality seems small. There is little reason to discourage good local practices unless there are strong data justifying such a change.

Future perspectives

It should be investigated if more appropriate approaches might help to avoid premature (< 1 year) stop of breastfeeding, for example, maintaining breastfeeding during illness or hospitalisation, or supply secure birth control methods to avoid short intervals between pregnancies. Our studies do not include infants under the age of 7 days. The fact that infant mortality is very high in the first 7 days of life makes it an important issue to investigate.

References on breastfeeding: 2, 24, 147, 189

Vitamin A and vaccines

Background

High-dose vitamin A supplementation (VAS) and vaccines are among the most important tools to reduce child mortality in low-income countries. To increase VAS coverage the World Health Organization (WHO) has recommended the integration of VAS with the Expanded Programme on Immunization (EPI). Two main strategies have been pursued. First, it is recommended to provide VAS at routine vaccination contacts after 6 months of age. Second, VAS can be provided at national immunisation days or vaccination campaigns (Table 1).

The WHO VAS policy was introduced after a number of randomised trials in the mid/late eighties and early nineties had shown that high-dose VAS for children between 6 months and 5 years of age reduces overall mortality by impressing 23-30 %. The effect was ascribed to the prevention and treatment of vitamin A deficiency. None of the trials had linked VAS with vaccinations or studied the effect of VAS according to vaccination status. The implementation of the EPI was still in its youth, and vaccination coverage was low. Hence, the current WHO policy of providing VAS with vaccines has never been tested in randomised trials. In other words, one of the major policies to reduce child mortality has never been evaluated for its overall effect on child mortality.

In fact the effect of VAS may depend on the type of vaccine with which it is given. In 2003, we published the hypothesis that VAS amplifies the non-specific effect of vaccines, being beneficial when administered with the live BCG and measles vaccines, but potentially harmful when given with the inactivated DTP vaccine (26). Since its formulation, we have aimed to test this hypothesis in observational and randomised trials.

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Results

To date we have been the only group to conduct studies with the specific aim to explore vitamin A-vaccine interactions in terms of mortality and continuously compare the evidence for our hypothesis against the evidence for the "prevention-of-deficiency"-hypothesis. Since it would be unethical to randomise children to most vaccines, we have had to be pragmatic when designing the trials. Hence, our studies have taken many different forms.

A smaller dose may be even better than a high dose

One of the observations which were contradictory according to the "prevention-of deficiency" hypothesis was made in a WHO multicenter trial. Almost 10,000 children were randomised to 25,000 IU vitamin A or placebo with the three DTP vaccines. The children who received vitamin A would receive 25,000 IU with measles vaccine at 9 months of age whereas those who had received placebo would receive 100,000 IU with measles vaccine. According to the survival curves, mortality was slightly higher among VAS compared with placebo recipients during the first 6 months of life. However, the curves crossed and mortality from 9 to 11 months of age when follow-up ended was significantly higher in the group that had received 100,000 IU with measles vaccine than in the group that had received 25,000 IU. If VAS worked only by preventing vitamin A deficiency this was an implausi-

ble finding. However, if VAS interacted with vaccines and their non-specific effects, a smaller dose of vitamin A might be better than a large dose. Hence, when a national campaign providing oral polio vaccine (OPV) and VAS to children aged 6 months-5 years was due in Guinea-Bissau in November 2002 we randomised children to the WHO-recommended dose of vitamin A or half that dose. We hypothesised that the smaller dose would be even more beneficial than the recommended dose. In brief, as hypothesised we found a tendency for a better effect of a smaller dose (109). This was due to a strongly significant beneficial effect in girls. The beneficial effect of the low dose tended to be most apparent in girls who had DTP as their most recent vaccine prior to the campaign. The finding was in line with the finding from the WHO multicenter trial – and hence both studies were incompatible with the "prevention-of-deficiency" hypothesis. The results suggest that VAS exerts its effects on mortality by other mechanisms than merely prevention and treatment of vitamin A deficiency.

VAS with BCG at birth In 2002, we initiated a large randomised trial of VAS given with BCG vaccine at birth. The trial was born from the observation that two previous trials of VAS at birth, both conducted in Asia, had found significant beneficial effects on mortality. The trial is described in more details in the chapter on neonatal vitamin A supplementation. In brief, VAS given with BCG at birth tended to be beneficial

as long as BCG vaccine was the most recent vaccine. However, the results suggested that VAS interacted negatively with subsequent DTP vaccine in girls, resulting in increased overall mortality, and increased risk of diarrhoea and measles as well as impaired vitamin A status in girls (173, 179, 205, 209).

VAS with missing vaccines In 2003, Guinea-Bissau had national immunisation days in November, providing VAS and missing vaccines to all children above 6 months of age who came to the health posts. We registered all participating children along with their treatment. Hence, we were able to test the hypothesis that VAS would be beneficial when given with the live measles vaccine, but negative when given with DTP vaccine. This proved to be the case (217). The effect of VAS differed significantly depending on the type of vaccine with which it was given (Figure 1). Receiving VAS with DTP compared with receiving only VAS was associated with significantly increased mortality. Furthermore, receiving VAS with DTP compared with not receiving neither VAS nor DTP (non-participants) was also associated with significantly increased mortality, though non-participants in such campaigns normally have higher mortality than participants. Numbers were small and it was an observational study. In particular we cannot exclude that children, who were missing DTP vaccines, had a higher risk of dying a priori compared with children who were missing measles vaccine or who did not miss any

vaccines, though control for background factors did not change the conclusions. Nonetheless the results provided support for our main hypothesis. Combining VAS with measles vaccine seemed more beneficial than combining VAS with DTP. In this study there were no sex differences, the combination of VAS and DTP seemed equally bad for boys and girls.

A reanalysis of one of the original VAS trials The latest opportunity to test our hypothesis came when we were allowed to reanalyse data from one of the original vitamin A trials. The trial, conducted in rural Ghana from 1989 to 1991, enrolled 6-90-month-old children and randomised them to VAS or placebo every 4 months for a period of 2 years. The trial was undertaken in the period when coverage with routine vaccinations was low and many children had no vaccination card. In the beginning of the trial vaccination status was assessed. The original team had not analysed data by vaccination status. The trial had shown a 19% significant mortality reduction after VAS. However, when we reanalysed the data we found that VAS only had a beneficial effect in children without a vaccination card, the mortality reduction being significant 36% in these children, but only a non-significant 5% reduction in children with a health card. This differential effect was particularly pronounced in girls. Among children with a card the effect of VAS differed significantly in boys and girls. This

was due to a significantly negative effect of VAS in girls who had received 0 to 2 doses of DTP at enrolment and were likely to receive DTP during follow-up. The reanalysis supports that VAS interacts with vaccines and the effect differs between the two sexes, in particular that the combination of VAS and DTP may have negative effects on girls.

Discussion
The initial formulation of the hypothesis of vitamin A-vaccine interactions has led to a series of studies of different designs. We conducted an observational study during the vitamin A campaign, randomised trials with VAS given with BCG at birth, and two different doses of VAS, and we reanalysed data from an old vitamin A trial from the perspective of vaccination status.

The results did not support the existing interpretation that VAS acts by preventing vitamin A deficiency, since a smaller dose seemed more beneficial than a larger dose in girls. The results on the other hand supported the hypothesis that VAS and vaccines interact. First, the effect of VAS given with DTP was different from the effect of VAS given with measles vaccine, and children who received VAS with DTP had hig-

her mortality than children who had received VAS alone or who did not receive anything. Second, VAS given with BCG at birth interacted with subsequent DTP vaccines in girls. Third, the effect of VAS depended on vaccination status, being beneficial in boys, but harmful in girls who received DTP during follow-up.

Public health implications

If VAS and vaccines interact to produce at times beneficial but at time harmful effects, it would be of outmost importance for future programmes.

Future perspectives

Testing VAS vaccine interactions is particularly important since the circumstances under which the original VAS trials were conducted have changed; vaccination coverage is increasing, particularly for DTP vaccine, since the national vaccination programmes are now being evaluated based on the coverage for DTP3. The final proof of VAS-vaccine interactions would have to come from randomised trials. Since it is unethi-

Table 1. WHO's vitamin A policy

Summary table of target groups and potential immunization contact

Target group	Immunization contact	Vitamin A dose
All **mothers**, irrespective of their mode of infant feeding, up to six weeks post-partum if they have not received vitamin A supplementation after delivery	BCG, OPV-0 or DTP-1 contact up to six weeks	200 000 IU
Infants 9-11 months of age	Measles vaccine contact	100 000 IU
Children 12 months and older		200 000 IU
Children 1-4 years old	Booster doses*	200 000 IU
	Special campaigns*	
	Delayed primary immunization doses*	

* Avoid giving a dose too soon after a previous dose of vitamin A supplement — the *minimum* interval between doses is 1 month (exceptionally, the interval may be reduced in cases of clinical VAD).

Figure 1.

VAS campaign, Guinea-Bissau 2003
Missing routine vaccines provided at the same time
Children aged 6-17 months

VAS+DTP versus VAS only: adjusted MR=3.4 (1.4-8.6)
VAS+DTP versus VAS+MV: P<0.001

cal to withhold recommended vaccines, such trials would have to be designed in special ways, for instance testing the effect of VAS or placebo with DTP versus the effect of VAS or placebo with measles vaccine, or by testing the effect in situations in which a vaccine is normally postponed, e.g. testing VAS versus placebo in low-birth weight children who are randomised to receive the recommended delayed BCG vaccine versus in children who are randomised to receive an early BCG vaccine.

References on vitamin A supplementation and vaccines: 26,91,109,121,149,173,179,184,209, 217
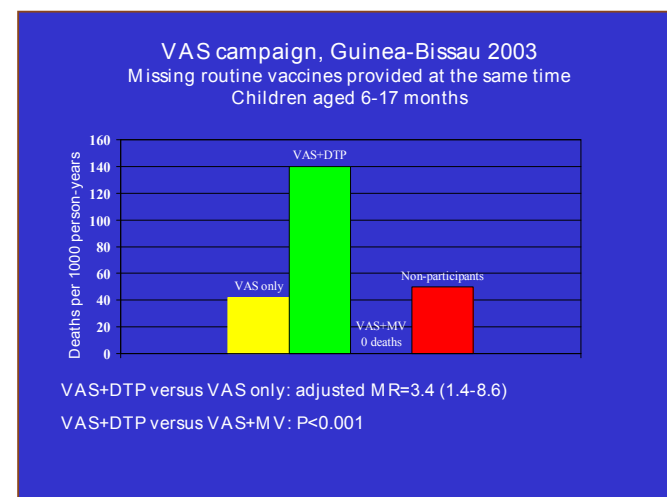
Neonatal vitamin A supplementation

Background

In 2002, two trials from Asia had both found a significantly beneficial effect of VAS at birth. We hypothesised that VAS interacted with routine childhood vaccinations, being beneficial when given with the live BCG vaccine at birth or live measles vaccine at 6-9 months of age, but not when given with inactivated diphtheria-tetanus-pertussis vaccine (DTP) at 1-5 months of age (26). We aimed to investigate the effect of high-dose VAS given with BCG vaccine at birth in an African setting with high infant mortality. Since BCG vaccine is postponed for LBW infants weighing below 2500 g, we only enrolled infants weighing > 2500 g. The primary study hypothesis was that VAS would be associated with at least a 30% reduction in mortality during the first year of life. We expected the effect to be most pronounced within the first 4 months of life like it had been in the two previous trials.

Furthermore, evidence for sex-differential effects of VAS accumulating during the trial made us hypothesise that VAS would be particularly beneficial for boys.

Results

VAS, BCG, and overall mortality. Unexpectedly we found no overall effect of VAS on overall mortality (209) (Figure 1). The effect, however, was not the same in boys and girls (Figure 2). Boys if anything tended to have a beneficial effect of vitamin A throughout the first year of life. In girls the effect became negative after the first months of life (209). The finding made us speculate that perhaps vitamin A given with BCG at birth had interacted negatively with subsequent DTP vaccines in girls. A post hoc analysis revealed that indeed there was a tendency for a beneficial effect of VAS as long as BCG vaccine

was the last vaccine to be received, but once DTP vaccine was received there was a significantly negative effect of having received VAS at birth in girls.

VAS, BCG, and morbidity. Within the trial we conducted a subgroup study of the effect on rotavirus infection and diarrhoea of VAS with BCG at birth (205). Unexpectedly we also experienced a measles epidemic which gave us the chance to study the effect on measles incidence. Receiving VAS at birth was associated with an increased risk of rotavirus infection and rotavirus diarrhoea below 6 months of age in both sexes (205)(Chapter 3). There was no effect in older children. At the same time VAS at birth was associated with a decreased risk of non-rotavirus diarrhoea in boys below 6 months of age, but an increased risk in girls 6 months or older. Significant sex-differential effects, with a tendency for a negative effect on girls of vitamin A at birth, were also seen for measles infection. Hence, the morbidity findings supported the existence of sex-differential effects of VAS at birth. Furthermore, the diarrhoea subgroup study was conducted among 1-8- month-old children who almost exclusively had DTP vaccine as their most recent vaccine during the study period, and the negative effect of VAS on measles infection was seen among girls who had received DTP but not measles vaccine. Hence,

the finding also supported the hypothesis that the negative effect on girls could be due to a negative interaction between VAS and subsequent DTP vaccination in girls.

VAS, BCG, and vitamin A status. As a part of the trial we studied the effect of VAS on vitamin A status at 6 weeks of age and 4 months of age (179). Overall vitamin A status improved during this period. However, there was a significant inverse relationship between increase in vitamin A status and number of DTP vaccinations received in girls, which was particularly evident among VAS recipients. The finding underscored

the possibility of a negative interaction between VAS and subsequent DTP vaccinations in girls.

Discussion. Five trials of neonatal VAS have now been published. Three trials from South Asia showed beneficial effects on mortality of neonatal VAS. Two trials from Africa found no overall beneficial effect, the estimates going in the other direction. Differences in vitamin A status do not seem to explain the divergent results. We have proposed that the divergent results may be explained by differences in vaccination intensity in the five trials (210). In our trial from Guinea-Bissau, all children received BCG at the same time as VAS or placebo (209). Having received VAS tended to be beneficial as long as BCG was the last vaccine to be received. However, once children received DTP vaccine, mortality in girls who had received VAS at birth was significantly 2-fold higher compared with girls who had received placebo at birth. Hence, in our experience neonatal VAS has a beneficial effect as long as BCG is the last vaccine but may have a negative effect on girls once they receive DTP. As a consequence the survival curves of vitamin A and placebo recipients should cross over once they start receiving DTP around two months of age if the coverage for DTP is high. This pattern is seen both in Guinea-Bissau and in the other African trial from Zimbabwe.

Such vitamin A-vaccine interactions could help explain the variation in trial results. Vaccination intensity was high in Guinea-Bissau and probably also in Zimbabwe as judged by national coverage data. This was not the case in the trials from Asia. Furthermore, all the Asian studies were characterised by a high neonatal mortality, but low mortality in the months in which a negative interaction between vitamin A at birth and DTP vaccine would matter. Hence, existing data are compatible with the hypothesis that early DTP vaccination might interfere with the beneficial effect of neonatal VAS in girls.

Public health implications

A heated debate regarding a global or regional recommendation of neonatal VAS is ongoing (210, 215). A policy of providing VAS in South Asia has many advocates. If our hypothesis is correct and neonatal VAS is made a general policy in South Asia, the intervention may cease to be beneficial or even become detrimental as the DTP coverage increases and more children are vaccinated early in life, especially in populations in which mortality is not limited to the first months of life. However, there will be no way of knowing because it is considered unethical to conduct further trials once an intervention has become policy.

It will be up to the WHO to weigh the evidence for and against a neonatal VAS policy in South

Asia. So far there is limited scientific evidence for the interpretation that neonatal VAS is most beneficial in areas with highest degree of VAD and baseline mortality. We need better explanations for the contradictory results before we make subgroup policies.

References on vitamin A supplementation at birth: 26,140,173,174,179,184,205,209,210,215
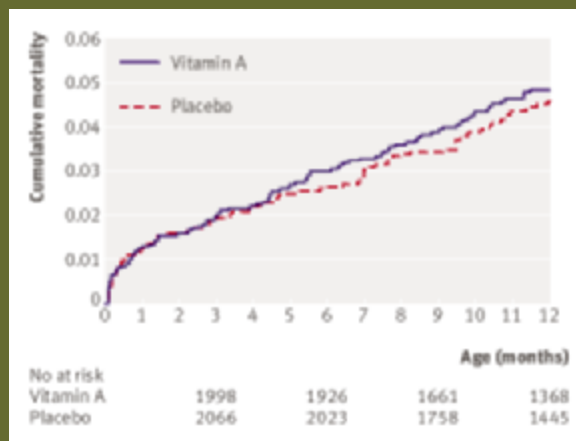
Figure 1. The effect of vitamin A supplementation at birth on overall mortality during the first year of life
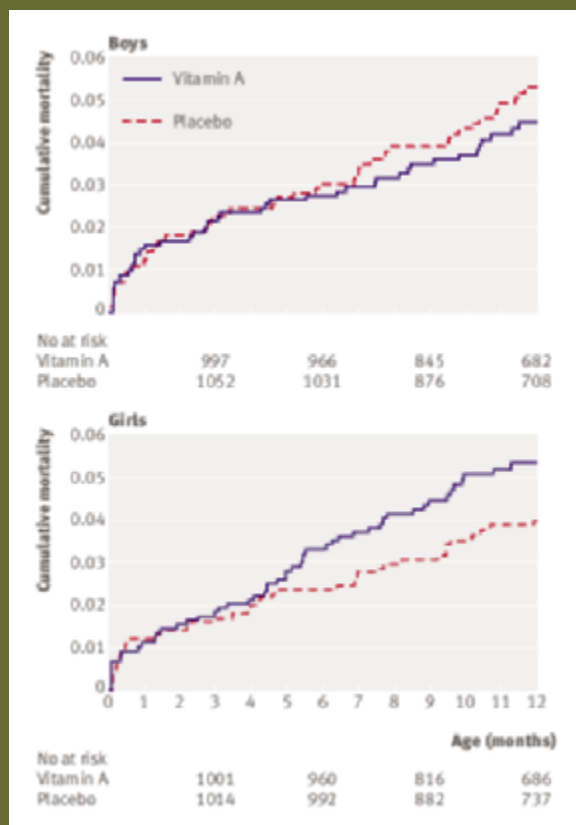
Figure 2. The effect of vitamin A supplementation at birth on overall mortality during the first year of life by sex

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Redosing with vitamin A

Background

Vitamin A supplementation (VAS) continues to be one of the most important health interventions for children in low-income countries. WHO recommends supplementing children between 6 months and 5 years of age with 4 to 6-month-intervals. While studying the hypothesis that VAS and vaccines interact, we made observations which suggest that the effect of VAS may depend on prior dosing.

Results

Half versus recommended dose of VAS.
In recent years, children aged 6 months to 5 years have received VAS during annual campaigns in Guinea-Bissau. In 2002 and 2004 VAS was provided together with oral polio vaccine (OPV) at National Immunisation Days (NIDs). We studied the effect of different doses of VAS on overall mortality. Children in the study area were randomized to receive the dose recommended by WHO or half this dose together with OPV and followed for mortality.

In the study from 2002 we found a significant beneficial effect of the lower dose compared with the WHO-recommended dose in girls (109). After 6 months of follow-up the mortality rate ratio (MRR) was 0.19 (0.06-0.66). However, this was not the case for boys. Overall mortality was lower among trial participants than among non-participants, and there was no indication that the high dose was associated with increased mortality; a smaller dose just seemed even more beneficial in girls.

Repeating the study in 2004 did not confirm these results. After 6 months and 12 months of follow-up there was no difference between the doses for either boys or girls. However, excluding the children who had previously received

VAS, the analysis produced results similar to the 2002 results (Table). Since NIDs with VAS were introduced in 2001, and maybe more importantly no children had received VAS at birth at that time, the children from the 2002 study were less likely to have received VAS previously than the children from the 2004 study. Hence, a possible beneficial effect of having received a priming dose of VAS on the subsequent response to a high dose of VAS would be less pronounced. This observation made us speculate that prior dosing with VAS may influence the effect of subsequent doses.

VAS or placebo at birth, followed by VAS at 12 months of age.
In the group of children participating in the randomised study of VAS with BCG at birth we intended to provide 100,000 IU of vitamin A (FU-VAS) to all children when they reached 12 months of age, irrespective of previous allocation. The effect of VAS at birth had not been beneficial during the first year of life (Chapter 26). However, among children who received FU-VAS after 12 months of age, it was strongly beneficial in the second and third year of life to have received VAS at birth compared with placebo at birth, the mortality rate ratio (MRR) being 0.53 (0.30-0.91). This difference was confined to girls who had a significant difference in mortality rate between 12 and 36 months of age depending on the randomisation group at birth (p=0.03) whereas there were no differences for boys (Figure).

Discussion.
The two studies differed considerably in design. However, they both indicate that VAS may have a priming effect on the effect of subsequent VAS on mortality, in particular for girls.

Public health implications

If VAS has a priming effect on the effect of subsequent VAS, implementing a policy may not achieve the full benefit in the short term and may depend on prior policies. The sex differences suggest that the overall impact of VAS could be optimized by having different recommendations for boys and girls.

Future perspectives

The current vitamin A policy makes it difficult to study the effect of re-dosing with VAS in randomised trials since depriving some children of a potentially beneficial vitamin A supplement would not be considered ethical. However, though we cannot conduct randomised trials, we can continue to pursue the observations using data from already conducted studies to evaluate the effect of repeated doses. The aim is to find the most beneficial dosing regime which will optimise the effect of VAS on overall mortality for both boys and girls.

References: 109, 209
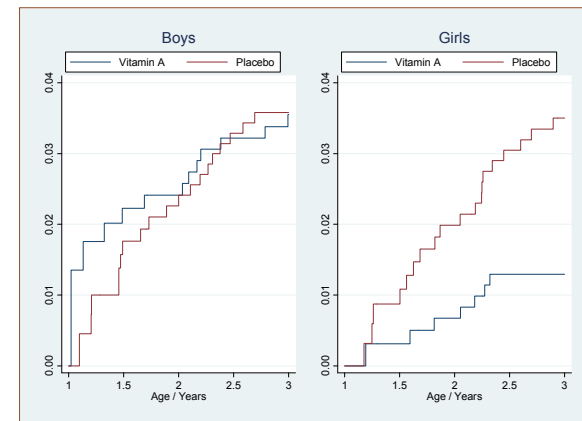
Figure. Effect of VAS / Placebo at birth on mortality after reception of FU-VAS

	Mortality rate ratios (95% CI) for a low versus a high dose		
Trial year	**Boys**	**Girls**	**All**
2002	1.98 (0.74-5.29)	0.19 (0.06-0.66)	0.69 (0.36-1.35)
2004	0.91 (0.26-3.13)	1.41 (0.57-3.45)	1.20 (0.58-2.47)
2004:No previous VAS	0.48 (0.09-2.63)	0.72 (0.23-2.26)	0.61 (0.24-1.58)
2004:Previous VAS	2.55 (0.27-25)	6.28 (0.77-51)	4.46 (0.97-20)

Table. The effect on mortality of receiving a low dose of vitamin A versus the WHO recommended dose

Immunological studies: Vitamin A and non-specific effects of vaccines

Background

A number of immunological studies have been initiated in order to address the mechanisms behind the findings of non-specific effects of vaccines and potential interactions with vitamin A supplementation (VAS). Some have focused on exploiting the often unique possibilities lying within the framework of the randomised studies conducted in Guinea-Bissau. Other studies have looked toward simplified systems such as dendritic cell cultures or animal studies. As we are able to measure an exponentially increasing number of immunological markers it is important not only to explore the effects of VAS and vaccines on various immunological markers, but also to establish the associations between such markers and subsequent mortality and morbidity. Hence, the work can be divided into two parts; first, to identify immunological markers, which are affected by VAS and vaccines, second, to establish a correlation between immunological markers and mortality and morbidity.

Results

Exploring the immunological effects of VAS and vaccines
Human studies: Whole blood stimulations.
A methodological mainstay of the immunological approaches performed at the National Laboratory in Guinea-Bissau is *ex vivo* whole blood stimulations. The assay is designed to test different parts of the cellular immune system lending information of both innate and adaptive immune functions (Table 1). Currently the whole blood assay is used in the evaluation of the immunological effects of the following interventions: VAS and BCG at birth, early measles vaccine, VAS in combination with measles or DTP vaccinations, DTP booster vaccination, and OPV at birth

The cytokine response to the stimulations is measured by means of Luminex technique. The method allows simultaneous quantification of numerous cytokines on a very small volume of sample material. This is a major advantage when working with small children as a large volume of blood is neither available nor desirable.

Faced with the challenges of performing immunological studies in Guinea-Bissau we have first-hand experienced the value of critically reviewing the methods applied. In a study of the immunological effects of early measles vaccination, the goal was to obtain venous samples at 4.5 months of age and 6 weeks later. At the time of sampling this was, however, only possible for about half of the children. In the remaining children capillary blood samples were obtained instead. We tested if method of sampling influenced the cytokine levels. This turned out to be the case. The venous blood samples displayed lower production of TNF-α and Interleukin (IL)-10 than those obtained through capillary blood sampling (176).

The background for BHP's work on the immunology of VAS is the hypothesis that VAS has direct effects on the immune system and that it amplifies the non-specific effects of vaccines with which VAS is given (22). As a consequence

sex is taken into consideration in all analyses. An example of this is a study investigating the effect of VAS on the immune response to BCG vaccine when given simultaneously at birth. In the study approximately 2700 infants receiving BCG were randomised to either VAS or placebo. Skin reactions to PPD (purified protein derivative of M. Tuberculosis) were evaluated at 2 and 6 months of age. At 2 months of age the proportion of responders was lower among boys who received VAS than among boys in the placebo group. Capillary blood samples were collected from a subgroup of infants at 6 weeks of age. These samples were utilized to quantify the ex vivo response to PPD by means of whole blood stimulations. With regards to interferon-γ, a key marker of cellular immune response to TB, more boys who had received VAS were responsive than in the placebo group. No effect of VAS was seen among girls in either of the tests (184).

Dendritic cells cultures.
Dendritic cell cultures have been used to examine the immunological effects of BCG vaccine. Artificially matured dendritic cells were co-cultured in the presence of live BCG and their subsequent ability to stimulate naïve T cell development was measured. No Th1 or Th2 polarisation was observed but the BCG co-culture led to an increase in IL-10 producing dendritic cells which again primed naive T cells to develop into IL-10 producing T cells (Figure). This cell-type is associated with the down-regulation of immune responses. The results suggest that BCG vaccination might result in the development of IL-10 producing dendritic cells as well as IL-10 produ-

cing T-cells that could contribute to restricting overt inflammation in infants exposed to pathogens and thus lead to lower infant mortality (163).

Animal models.

Animal models are well-suited to explore VAS/vaccine-interactions, in particular VAS/DTP interactions, which may be harmful for girls and unethical to conduct in humans. In a collaborative pilot study with a group at Stanford University, we investigated the effects of combined VAS/DTP-treatment on the outcome of influenza infection in mice. The study was not conclusive but produced interesting results which compelled us to go forward with another experimental model of infection. The model chosen was a well-established murine model of cerebral malaria and the studies were performed in collaboration with Department of Clinical Microbiology, University of Copenhagen. We found no effects on the main outcome, development of cerebral malaria, but repeated experiments showed that mice receiving the combination of VAS and DTP had higher levels of parasites in the blood.

Searching for immunological markers of mortality and morbidity

Several immunological markers of subsequent mortality and morbidity have been established. Thymus size, having a BCG scar and a positive delayed type hypersensitivity response to PPD have previously been identified as markers of better survival, and this association was verified in recent studies (133,211). We aim to pool the data from the whole blood stimulation studies and link them to survival to identify cytokine markers of mortality or better survival as well.

Future perspectives

The addition of immunological studies has the potential to substantiate some of the controversial findings by offering biologically plausible explanations. The studies have to a large extent been possible due to collaborations with other research groups, and we will continue to initiate and explore these possibilities through future studies. An example of this is within the field of lymphocyte homing. Dendritic cells from the intestinal mucosa have the ability to "instruct" lymphocytes to preferentially migrate to the gut. It appears that retinoic acid, a vitamin A metabolite produced by these dendritic cells, acts as the signal that induces the observed gut-specificity/preference. We are currently addressing this topic in collaboration with researchers at Lund University. This study will hopefully fill a current gap between in vitro and animal studies and the situation of children in Guinea-Bissau receiving VAS.

The magnitude and nature of data "produced" by modern immunological techniques when applied to large populations in immuno-epidemiological studies pose a number of analytical challenges. Repeated measurements of the same individual are performed and multiple parame-

ters are measured simultaneously creating large amounts of information. The parameters measured are often reflecting interacting processes but the extent of these interactions is less than straightforward and may not be similar across the groups investigated. Often distributions are not normal and have a large proportion of undetectable samples. These challenges combined with the sheer number of observations require more advanced statistical methods than often applied to exploit the full potential of the obtained information. In collaboration with Leiden University Medical Center, we are currently exploring and developing statistical methods which can deal with data from immunoepidemiological studies.

References on immunology:
26, 133, 163, 176, 184, 211

Figure 4. IL-10 production by naive T cells exposed to DC matured in the presence of LPS only (mDC), DC matured in the presence of LPS and IFN-γ (IFN-γ-DC), DC matured in the presence of LPS and SEA (SEA-DC), and DC matured in the presence of LPS and increasing concentrations of BCG from 2.5×10^3 to 2.5×10^5 BCG (BCG-DC). The production of IL-10 by T cells induced by DCs matured in the presence of IFN-γ, SEA or BCG was calculated relative to IL-10 production in T cells exposed to mDC (DCs matured in the presence of LPS only) which was set to 100% in each experiment. The data show the mean and SEM (error bars) of six separate experiments.

Stimulant	Function tested
LPS	General recognition of gram-negative bacteria (innate – TLR4)
Pam3-cys	General recognition of gram-positive bacteria (innate – TLR2)
Poly I:C	General recognition of virus (innate – TLR3)
PHA	Unspecific T-cell activation
TT	Recall of tetanus toxoid part of DTP vaccine (adaptive)
PPD	Recall of BCG vaccine given at birth (adaptive)
OPV	Recall of polio vaccine (adaptive)

Table. Stimulants used in ex vivo whole blood stimulations

Re-introducing sex-differential treatment of children

Though it is well-accepted that boys and girls have different susceptibility to diseases in childhood there has been very little interest in investigating whether boys and girls benefit from the same health interventions. Gender has been an issue when it came to ensuring that boys did not receive preferential treatment, i.e. boys and girls should receive the same health intervention at the same time. It has not been considered that we may in fact be treating boys and girls differently giving them different survival probabilities, when we offer them the same health intervention at the same time.

We have consistently found that the major interventions to reduce morbidity and mortality in low-income countries have sex-differential effects. BCG and measles vaccine reduce overall mortality, and this is most pronounced in girls. DTP is associated with increased female mortality compared with male mortality (Figure).

OPV at birth may be associated with increased male mortality. Vitamin A supplementation (VAS) benefits boys more than girls.

In high-income countries there is an enormous focus on individualised prevention and treatment, including the prospect to target the intervention towards subgroups with different genetic profiles based on rapid genetic tests. It will take many years before that kind of genetic screening will be feasible in low-income countries. However, there is one genetic test which can be done without any remedies and at no cost: to determine the sex of a child. Based on our research, sex may be a very good determinant of the optimal health intervention programme.

In 10 years' time, we expect these ideas to be more generally accepted and many will be working to develop policies optimised for both

boys and girls. We have started gradually to examine whether major interventions should differ for boys and girls. Thus, we are testing whether MV given at 4½ months of age may reduce the negative effect of DTP for girls and whether girls might benefit from not receiving a booster dose of DTP but only OPV. We are also testing whether low- birth-weight infants may benefit from a sex-differential treatment and the potential sex-differential effect of administering VAS with routine vaccinations as recommended by WHO.

References on sex-differential treatment: 139,140,177

Figure. Female-male mortality rate ratios among measles and DTP vaccinated children in all available studies
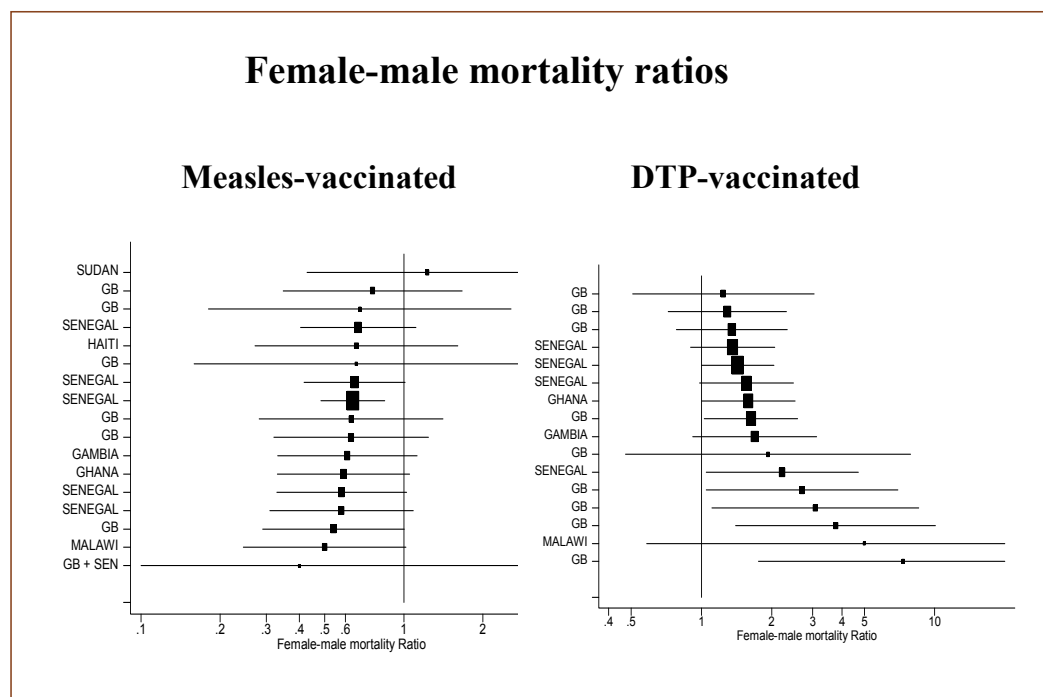
Maternal mortality: reducing severe postpartum haemorrhage

Background

Previous cohort studies conducted by the mobile team of Bandim health Project (BHP) revealed a maternal mortality ratio of 822 per 100,000 live births. The most important cause of maternal death was postpartum haemorrhage (PPH). The primary cause of PPH is generally known to be uterine atony accounting for 70 percent of cases.

Several drugs are known to reduce PPH by facilitating contractions of the uterus. Ergot derivatives, oxytocin and injectable prostaglandins are standard treatment for the condition in all well equipped delivery units all over the world. However, these drugs require cool storage to remain effective. Moreover, most uterotonics must be administered as injections, which requires sterile utensils and training in safe administration, prerequisites unavailable for most women delivering in low-income countries.

Misoprostol, a prostaglandin E1 analogous, is heat stable and can be administered orally, rectally or sublingually. A WHO-sponsored multicentre study have found misoprostol to be less effective for prophylaxis than injections of 10 IU i.v./i.m. oxytocin, but failed to address the possible benefit of misoprostol to the large number of women who give birth outside a health facility with refrigerators.

All previous randomised studies of misoprostol for prevention of PPH have examined the oral and rectal route of administration. As pharmacokinetic studies have indicated that the sublingual route of administration secures the highest peak concentration and the best bioavailability we planned to test if routine administration of sublingual misoprostol could reduce the inci-

Results

Using a more accurate method of measuring quantities of blood lost after delivery, a blood loss of more than 1000 ml was found in 17% of women in the placebo group, but only in 11% in the misoprostol group (relative risk (RR) = 0.66 (0.45 to 0.98)). Severe PPH of more than 1500 ml was found in 8% of control women, but only in 2% of misoprostol receivers (RR=0.28 (0.12 to 0.64)).The mean blood loss was significantly lower in the misoprostol group (555 ml (standard deviation(SD): 355 ml)) compared with the control group (655 ml (SD: 523 ml)) (P<0.01). Significantly more women in the misoprostol group experienced shivering and pyrexia, but the discomfort eased off a few hours after ingestion.

Public health implications

In Bandim 31% of pregnant women were found anaemic, making them vulnerable to the blood loss occurring during a delivery. Maternal mortality is found to be 4-fold higher in severely anaemic women compared with non-anaemic controls. In rural Guinea-Bissau, 75 % of women give birth at home and worldwide only about 50 % give birth in health facilities. Therefore, strategies must be identified to increase safety of deliveries not attended by skilled birth attendants. According to our studies it might be safe and useful to distribute misoprostol among traditional birth attendants, to teach safe use of the drug

dence of PPH in a resource-poor, primary health care setting. For this purpose we randomised 661 women, who gave birth at Bandim Health Centre between March 2003 and August 2004, to prophylaxis with sublingual misoprostol 600µg (N=330) or placebo (N=331).

and other forms of prevention of PPH and to raise awareness of potential complications among the pregnant women. The mothers and their helpers should be informed that shivering and mild fever can be expected and continuous bleeding after misoprostol and uterine massage requires prompt attendance by the health care system.

Future perspectives

Worldwide there is at strong need to investigate simple measures, which can be applied universally at a community level to reduce the burden of postpartum haemorrhage. It is well docu-mented that active management of the third stage of labor (administration of an uterotonic agent, early cord clamping and uterine massage) reduces blood loss after delivery, but it is still unclear how these components interact and if misoprostol could substitute oxytocin or methergin in the active management strategy. There is also little empirical research to evaluate the effectiveness of continued uterine massage. BHP holds a unique position to study the use of miso-prostol and other simple and inexpensive procedures outside tertiary health facilities.

References on maternal mortality and postpartum haemorrhage: 35,39,114,137

Table 1. Blood loss and change in haemoglobin

	Misoprostol n=330	Control n=331	RR (95% CI)
Measured blood loss (median [IQR])	475ml [319-736]	512ml [315-774]	P = 0.15*
No of pt. with Blood loss > 500 ml (n [%])	150 [45%]	170 [51%]	0.89 (0.76-1.04)
No of pt. with Blood loss > 1000 ml (n [%])	37 [11%]	56 [17%]	0.66 (0.45-0.98)
No of pt. with Blood loss > 1500 ml (n [%])	7 [2%]	25 [8%]	0.28 (0.12-0.64)
Haemoglobin decrease (median [IQR])	0.2mmol/l [-0.3-0.9]	0.4mmol/l [-0.2-1.1]	P = 0.08*
No of pt. with 10% fall in hgb level (n [%])	105 [32%]	115 [35%]	0.92 (0.74-1.14)

* Wilcoxon rank-sum test

Low birth weight delivery and maternal mortality

Background

Maternal mortality (MM) is a leading cause of death among women of reproductive age and a burden on health systems in the low-income countries. However, the knowledge on how to prevent MM is limited. Many of the most affected countries do not have good health statistics and few risk factors have been identified. We studied the association between giving birth to a low-birth-weight (LBW) infant and maternal mortality.

Results

A total of 5230 mothers giving birth at the National Hospital of Guinea-Bissau and whose children participated in a randomised trial were followed for mortality during the first 90 days after delivery. Mortality from delivery to 90 days postpartum was higher among mothers of LBW infants compared with mothers of normal-birth-weight infants (NBW), the adjusted mortality rate ratio being 12.7 (3.6-44). The disparity between mothers of LBW and mothers of NBW infants was particularly apparent after the first week postpartum. Mothers of LBW infants continued to have a high mortality rate during the 90 days (Figure).

Public heath implications and future perspectives

In low-income countries the burden of MM is high and consequences are widespread for families and the society. It is well-known that motherless children have a significantly higher mortality, hence preventing a maternal death often also implies saving her offspring's life. To single out the mothers at highest risk would help con-

centrate the sparse resources on those most in need. Experiences from other countries support the belief that the high incidence of MM can be reversed with concerted efforts. Methods to define risk groups must be uncomplicated, not using any advanced equipment, to be functional in low-income countries. Based on our results, the distinction between mothers of LBW infants and mothers of NBW infants is an important contribution to the understanding of MM. An LBW delivery is a serious symptom that should lead to increased awareness and to programmes for monitoring and improving the health of mothers who deliver an LBW infant.
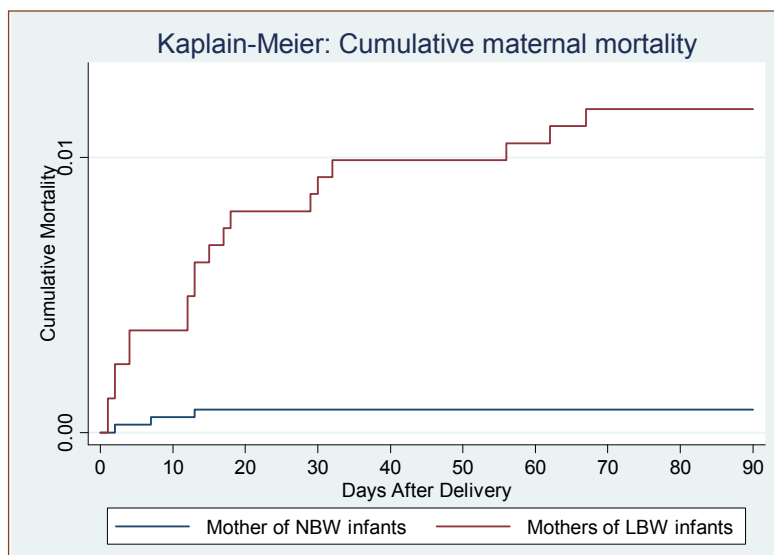
Figure. Cumulative mortality among mothers of LBW infants and mothers of NBW infants

Retroviral epidemics in Guinea-Bissau:
HIV-1, HIV-2, and HTLV

Background

Studies of HIV in Guinea-Bissau began in 1987 estimating an HIV-2 prevalence of 9% in adults above 14 years in urban Bissau. No HIV-1 infection was found at that time. The first case of HIV-1 infection was identified in 1989, when one case of dual infection of HIV-1+HIV-2 was detected.

Over the last 20 years the two viruses have shown a different pattern of spread in the country; the prevalence of HIV-2 seems to be stabilising or decreasing, while HIV-1 prevalence has been increasing gradually [208]. In contrast to neighbouring countries, Senegal and Guinea-Conakry, with a low HIV-1 prevalence (<1 and 1.5%, respectively), Guinea-Bissau has a prevalence comparable to the levels of the most affected countries in the sub-region (Côte d'Ivoire, Nigeria and Mali) with an HIV-1 prevalence higher than 4%.

Results

Two long-term epidemiological studies of retrovirus infections have been conducted in Guinea-Bissau since the late 1980s and a new study with a clinical approach was initiated in 2007.

Serological surveys of HIV infection in the urban study area of BHP.
Three adjacent urban districts in the capital, Bandim 1, Bandim 2 and Belém, have been followed with epidemiological sero-surveys of HIV infection since 1987. The cohort comprises individuals over 15 years of age living in randomly selected houses in these three districts. Children were included in the first survey in 1987, but due to the very low prevalence it was decided to restrict the following sero-surveys to adults. Over the years, the same methodology has been maintained, covering a 10% sample of houses in the three districts. The number of

houses included in the study has therefore been gradually enlarged from 100 to 399 with 2548 participants tested in the last sero-survey in 2006 [208].

HIV-1. The overall prevalence of HIV-1 in this population has increased from 2.3% (54/2301) in 1996 to 4.6% (118/2548) in 2006 (Table 1). Single HIV-1 infections increased three-fold, whereas the prevalence of dual infections declined (prevalence ratio 0.46 (0.23–0.93)).

HIV-2 infection has shown the opposite trend. The overall HIV-2 prevalence among adults has declined from 8.9% in 1987 to 7.4% in 1996 and 4.4% in 2006 (Table 2). The prevalence is declining in both sexes whereas this decline was initially only in men. The HIV-2 incidence rate between 1996 and 2006 was half the incidence in the preceding 10-year period. Hence, the decrease in the HIV-2 risk is genuine and not due merely to mortality or migration from a previously established cohort. Nevertheless, it should be noted that the prevalence of HIV-2 is not declining among older people. This may indicate a cohort effect, previously infected young adults coming of age due to the longer survival of HIV-2-infected individuals. However, older women continue to become infected. Hence, HIV-2 may continue as an independent infection among the oldest for some time, especially in women. An increased susceptibility to HIV and human T cell lymphotropic virus (HTLV) infections in older women possibly due to changes in vaginal mucosal immunity has previously been suggested to be the cause of this pattern.

HTLV. Like HIV-2, HTLV has also declined. The prevalence of HTLV was 3,6% in 1996, 2.2% for men and 4.6% for women. In the latest sero-survey, the prevalence had declined to 2.3%, 1.5% for men and 2.9% for women.

Since HIV-1 is increasing, the declines in HIV-2 and HTLV are unlikely to be due to the introduction of safe sexual practices. It seems more likely that they are both relatively inefficient sexually transmitted infections. They may both have been dependent on blood transfusions to maintain the previous high levels found around 1990. Once transfusions became controlled for HIV infection they would both have declined as the two infections were often linked.

Sero-surveys in a rural population. Sero-surveys among adults were also performed in 1989, 1996-1998 and in 2006 in a rural community in Caió, in the northwestern part of Guinea-Bissau by the Medical Research Council (The Gambia) in collaboration with BHP. These surveys indicate that the HIV-2 prevalence had remained stable at around 7.8%, whilst the HIV-1 prevalence has increased (2.7%). Studies from both Bissau and Caio have found an association between having a vaccinia scar and being HIV-2 infected, suggesting that HIV-2 may have been spread initially with the smallpox vaccination eradication campaigns conducted in the 1960s [135,151].

The West African Retrovirus and Acquired Immune Deficiency cohort study (The WARAID cohort study). In 2007 the PSB started a new project to create a clinical database and a plasma/blood repository biobank with HIV-infected individuals from Bissau and with an extensive follow-up in all patients living in the BHP study Area. This study is used for gaining insights in the overall effect of introducing ART in a treatment naïve population. The main objectives are to focus on the host/viral determinants of failure of first line ARV treatment in this unique population with concomitant infections of 3 retroviruses (HIV-1, HIV-2 & HTLV) and to study the effect of multiple infections on immune response and ART outcome. PSB has established close relationships with the National HIV Programme helping to develop new forms for the HIV care, the national codification system for the patients and opening the HIV clinic at the National Simão Mendes Hospital. This clinic has become the major HIV centre in the country following around 1000 patients and the training centre for doctors and nurses willing to work with HIV in their country.

Since May 2008 the PSB is supporting the National HIV Programme in implementing a mother-to-child transmission (MTCT) prevention strategy at the maternity of the Simão Mendes Hospital. HIV testing and counseling is offered to all the women giving birth in the facility. This is an important entry-point for women to interventions to prevent MTCT, including ARV prophylaxis for the infant, and other HIV-related treatment and care services. The follow-up of the HIV-exposed children is carried out in collaboration with the NGO Ceu e Terras in Bissau.

Other studies. In 2007 the PSB carried out a prevalence study of sexual transmitted infections in 190 commercial sex workers in five regions of the country; 20% were HIV-1 infected and 7% had only HIV-2. Bissau was the region with the highest HIV prevalence (67%) among these women.

Public health implications and future perspectives

Since the beginning of the epidemic, the BHP has been actively involved in understanding the epidemics of retroviruses. The studies have generated valuable information for the National HIV Programme in order to plan public heath interventions. The HIV epidemic in Guinea-

Bissau is still evolving and further studies are needed to monitor the trends in these infections.

Given that antiretroviral treatment (ART) has recently been implemented in the country monitoring the clinical response to the treatment and the viral resistance patterns is essential. These studies will contribute to develop appropriate guidelines for implementing ART regimens in populations where HIV-2 is endemic or when multiple infections with retroviruses are common. It will also be a challenge to develop collaboration between the national programmes so that patients benefit from both HIV and TB treatment. In the future BHP may also get involved in testing new vaccines against both infections.

References on retrovirus infections: 4,19, 23, 38, 56, 100, 101,135,151,182,183,191,208,212

Table 1. HIV-1 prevalence in 1996 and 2006 with dual infections included in the Bissau urban community study (208)

Sex	1996 %	2006 %	2006 vs 1996 Risk ratio (95% CI)
Men	2.1	3.6	1.7 (1.0-2.8)
Women	2.5	5.3	2.1 (1.4-3.2)
Overall	2.3	4.6	1.9 (1.4-2.6)
Single HIV-1	1.4	4.2	2.9 (1.9-4.3)
Dual infection	0.9	0.5	0.5 (0.2-0.9)

Table 2. HIV-2 prevalence in 1996 and 2006 with dual infection included in the Bissau urban community study (208)

Sex	1996 %	2006 %	2006 vs 1996 Risk ratio (95% CI)
Men	5.4	2.7	0.5 (0.3-0.8)
Women	9.0	5.5	0.6 (0.5-0.8)
Overall	7.4	4.4	0.6 (0.5-0.7)
Single HIV-2	6.4	3.9	0.6 (0.5-0.8)
Dual infection	0.9	0.5	0.5 (0.2-0.9)

HIV-1, HIV-2, AND HTLV-I: DUAL INFECTIONS

Background

The three human retroviruses, HIV-1, HIV-2, and HTLV-I circulate in the general populations both in urban and rural areas of Guinea-Bissau. While HIV-1 is responsible for the global HIV pandemic, HIV-2 is limited in its spread and mainly confined to West Africa. Guinea-Bissau has constituted the epidemiological focus of HIV-2, with the world's highest prevalence. HTLV-I is also highly prevalent in the area. The major form of transmission for all three viruses in Guinea-Bissau is by heterosexual contacts. Hence different combinations of dual and even triple infections with these viruses occur. In 1989 the first dual HIV-1/HIV-2 infection was identified (0.2%) (Table 1). In the 1996 screening, the prevalence of dual HIV-1/HIV-2 infections had increased to 1.0% (N=22/2301). In Caio, a rural area of the country, the prevalence of dual HIV-2/HTLV-I infection was 1.0%, the great majority of them being in women.

Both HIV and HTLV-I replicate through reverse transcription and both viruses infect CD4+ T-cells with different pathogenic outcome. HIV-2 is more closely related by nucleotide sequence to simian immunodeficiency virus of sooty mangabey origin (SIVsm) than to HIV-1. The time to onset of symptomatic HIV-2 infection is estimated to be at least 10 years longer than for HIV-1 infection, and HIV-2 is less transmissible. This lower pathogenic feature of HIV-2 makes it a suitable model for comparative studies of HIV-1 pathogenesis. In contrast, 95% of infected individuals remain asymptomatic carriers of HTLV-I.

Results

Dual infections and the epidemiological features
In 1998-2000 a screening of HIV and HTLV-I in adults above 35 years of age was performed in Bandim (4). One of the main objectives with the

study was to examine the epidemiological dynamics of retroviral infections around 45 years of age, since previous studies from the area had demonstrated an increased prevalence of retroviral infections particularly in women above 45 years. The prevalence of dual HIV-1/HIV-2 was 0.6%. For dual HIV-1/HTLV-I it was 0.2% and for HIV-2/HTLV-I it was 2.2%. The evolution of the prevalence of various combinations of dual infections is shown in Table 1. The observed prevalence of dual infections was generally higher than expected in women, while this pattern was not observed for men. Adjusting for age group, the female-to-male odds ratio (OR) for any combination of dual retroviral infection was 7.8 (3.1-19.9).

This pattern could not be explained by behavioural factors so we proceeded with mortality analyses to assess whether differential HIV and HTLV associated mortality could explain the differences in dual infections for men and women. However, the higher prevalence of dual retroviral infections in women could not be explained by sex-differential mortality patterns. The objective was also to assess the mortality patterns of various combinations of dual retroviral infections as little is known on the subject. As observed in previous studies, HIV-1- associated mortality was higher than HIV-2 associated mortality (Table 2). The mortality rate ratio (MRR) for HIV-1/HIV-2 dual infections was 5.9 (95%CI 2.4, 14.3), resembling the MRR found for HIV-1. No significant differences were found in mortality between HIV-2 single, HTLV-1 single and HIV-2/HTLV-1 dual infections (191).

Table 1. Prevalence (%) of dual retroviral infections 1987-2006

Year of screening	HIV-1/HIV-2	HIV-2/HTLV-1	HIV-1/HTLV-I	Age
1987	0	-*	-*	>15 years of age
1989	0.2			>15 years of age
1990¤	-*	2.3	0	>50 years of age
1992	0.6			>15 years of age
1996	1.0	1.0	0.1	>15 years of age
1998	0.6	2.2	0.2	>35 years of age
2006	0.5	0.4	0.2	>15 years of age

* Not examined

Table 1. Prevalence (%) of dual retroviral infections 1987-2006

	MRR* (95% CI)
HIV-1	4.9§ (2.3, 10.4)
HIV-2	1.8§ (1.5, 2.3)
HIV-1/2 dual	5.9§ (2.4, 14.3)
HTLV-I all	1.7# (1.1, 2.7)
HTLV I positive/ HIV- negative	2.3& (1.3, 3.8)
HTLV-I/HIV-2 dual infections	1.7 &(0.7, 4.3)

* MRR= Mortality rate ratio; § Comparing with HIV-negative; # Comparing with HTLV-I-negative; & Comparing with HIV and HTLV-I-negative

Comparison of HIV-1, HIV-2 and HIV-1/HIV-2 dual infections. From the latest screening performed in Bandim 2004-2006 (208), 56 dually and 5 triply infected individuals were identified and enrolled along with 73 singly infected and 34 uninfected controls in an immunological case-control study. Plasma from HIV singly or dually infected individuals were tested for their ability to neutralise HIV isolates of different subgroups in a checkerboard fashion. Preliminary results from this heterologous neutralisation test show that the neutralisation ability of plasma from dually HIV-1/HIV-2 infected may differ as compared with plasma from singly HIV-1-infected. Plasma from dually HIV-1/HIV-2 infected displayed significantly lower neutralising capacity against HIV-1 isolates than plasma from singly infected HIV-1-infected; no such difference could be observed comparing neutralising capacity against HIV-2 isolates for plasma from dually HIV-1/HIV-2-infected and HIV-2 singly infected individuals. Further analyses are ongoing.

Public health implications

The pattern of the HIV-epidemic in Guinea-Bissau differs from that of South and East Africa as the epidemic started with the less virulent HIV-2. This population is also afflicted by a third retrovirus, HTLV-I, which can lead to disease. We have seen from the mortality data that HTLV-I infection is independently associated with higher mortality. Why HTLV-I-infected die to a higher extent than non-HTLV-I-infected remains to be clarified. Studies are underway

that hopefully will elucidate this. The country has recently started with anti-retroviral treatment (ART) on a national basis to asses the effects of ART on a population level in a context with three different retroviruses.

There is still no vaccine available against HIV. It is not completely known what type and quantity of immune responses should be induced by vaccination to protect against HIV/AIDS. It is therefore of special interest to study immunity such as neutralising antibodies in a population where several human retroviruses HIV-1, HIV-2 and HTLV-I coexist. In particular, HIV-2 infections that have a lower pathogenic outcome than HIV-1 may provide important information on the type of antibody response needed.

Future perspectives

Continuation of the epidemiological studies along with studies on the biology and immunology of the virus are essential for the future monitoring of the HIV epidemic. We will assess possible implications of neutralisation and innate immunity studies from a vaccine perspective. Furthermore, we will study the effects of HTLV-I single and dual HIV/HTLV-I infections on an individual and public health level, analysing which diseases are associated with HTLV-1 in this community. This will include assessment of auto-antibodies in HTLV-I infected individuals and relating HTLV proviral load to HIV and clinical symptoms.

References on dual infections: 4, 191, 2008

Tuberculosis studies:
Clinical tuberculosis studies

Background

The set-up for enrolling TB patients in clinical studies was strengthened in 2003 in order to conduct randomised trials. The previously collected data were used to develop a clinical score for characterisation of severity of TB disease and to assess outcome in trials. A systematic collection of blood samples from TB patients and healthy controls from the study area was used to describe vitamin D status and to study genetic risk factors. Finally this set-up was used to conduct the first major randomised trial of the effect of vitamin D supplementation for TB patients.

In the pre-antibiotic era, vitamin D was used for treatment of tuberculosis (TB). In recent years researchers have reported evidence for an important role of vitamin D in the immune response towards TB and increased risk of TB in individuals with vitamin D deficiency. We therefore aimed to assess whether vitamin D levels in the blood and vitamin D receptor polymorphisms were associated with TB risk in this high-burden setting, and whether supplementation would affect disease outcome (172).

Results

We developed a clinical severity score for TB based on the WHO manual for TB and HIV and applied the score to an existing dataset of 698 TB patients in the study area (180). The TBscore showed a high degree of sensitivity to change and we found that 93% of the patients had high scores at the beginning of treatment and 79% had a low score at the end of treatment. Being in the highest severity class predicted subsequent mortality with high accuracy.

Vitamin D status was examined in 362 TB patients and 494 healthy adults (185). We found female sex, old age, certain ethnic groups and Moslem faith to be risk factors for low vitamin D status, whereas having no formal schooling was protective. Controls with vitamin D deficiency were also at higher risk of having latent TB infection determined as a positive Mantoux test > 10mm. Controlling for background factors, we found overall lower 25 hydroxy-vitamin D (25(OH)D3) concentrations among TB patients, but severe vitamin D deficiency (25(OH)D3 < 25nmol/l) was surprisingly rare among TB patients, although this was seen in 5% of healthy controls. The data suggest a role of vitamin D in TB but it may just be a symptom.

In a genetic case control study, we investigated the role of DC-SIGN (CD209), long pentraxin 3 (PTX3) and vitamin D receptor (VDR) gene single nucleotide polymorphisms (SNPs) in susceptibility to pulmonary tuberculosis (TB) in 321 TB cases and 347 healthy controls from the study area(175). We found that two polymorphisms, one in DC-SIGN and one in VDR, were associated in a non-additive model with disease risk when analysed in combination with ethnicity (P=0.03 for DC-SIGN and P=0.003 for VDR). In addition, PTX3 haplotype frequencies significantly differed in cases compared to controls and a protective effect was found in association with a specific haplotype (OR 0.78 (0.63–0.98)). Our findings support previous data showing that VDR and DC-SIGN modulate the risk of TB in West Africans (66,199) and suggest that variation within DC-SIGN and PTX3 also affects the disease outcome.

Finally, we included 365 TB patients in a randomised double-blind placebo-controlled trial (218). Patients were given 100,000 IU cholecalciferol/placebo together with directly-observed antituberculous treatment. Cholecalciferol/placebo was repeated after 5 and 8 months of treatment. No serious adverse effects were reported, mild hypercalcemia was a rare event and present in both treatment arms. The clinical score showed a similar decrease in severity among vitamin D and placebo recipients (Figure 1). There was no difference in weight gain or time to sputum conversion. Overall mortality was 54/365 (15%) at one-year follow up and did not differ significantly among the two groups (Figure 2) but a subgroup analysis stratified for HIV infection raised the possibility that vitamin D treated HIV-1 infected TB patients had higher mortality.

Public health implications

We have developed the first clinical score for assessing severity of TB disease which can be used in low-resource settings. With this tool a physician with a stethoscope, a scale and a measuring tape may be able to have important prognostic information on which patients should achieve increased attention or hospitalisation. When grouped in severity classes the signs and symptoms in the TBscore construct a robust index usable in settings where advanced laboratory measurements are not available.

The vitamin D studies did not confirm the hypothesis that vitamin D plays an important role in development or treatment of TB, but associations were found which merits further investigation.

Future perspectives

We are currently further assessing the TBscore for inter-observer variation, and the TBscore may be used in trials to select high-risk patients including smear negative TB patients. The set up for clinical TB trials is an important resource which may be used to answer urgent research questions about the effect of micronutrient/diet supplementation of TB patients (206) and the efficacy of promising TB vaccine candidates.

References on TB: 25,28,51,66,103,105,144,160,164,165,171,172,175,180,185,199,206,218

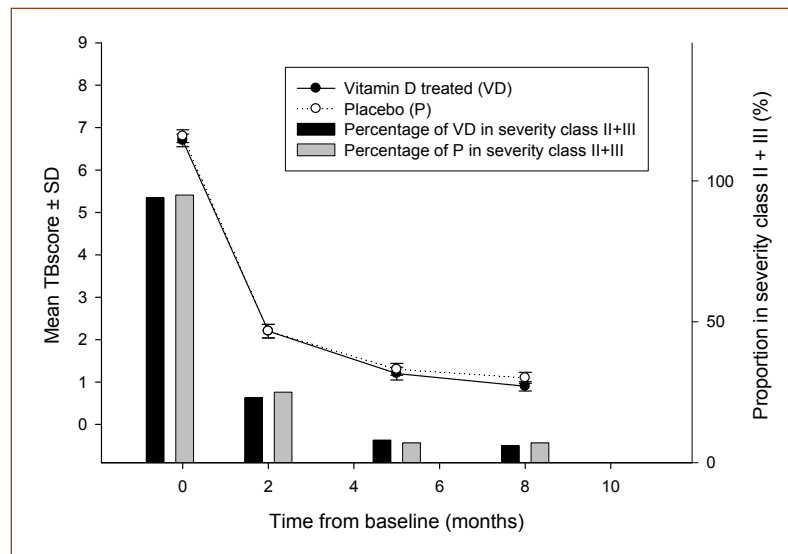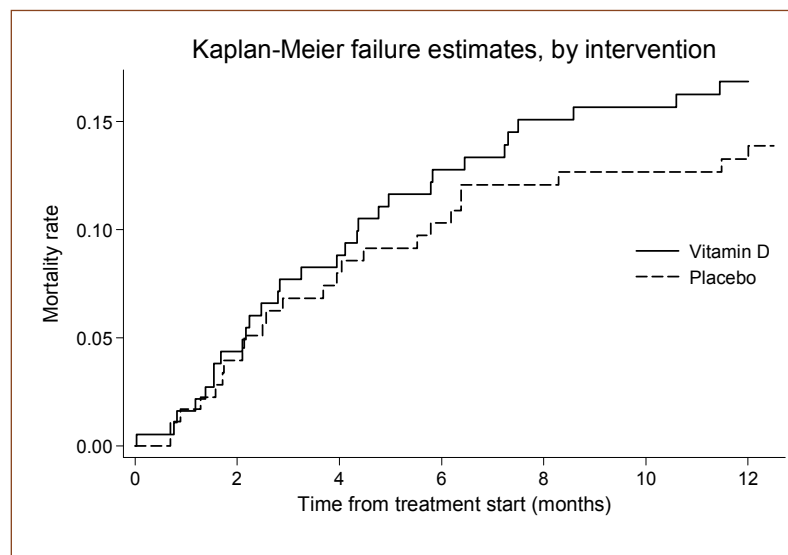**Figure 1.** I TBscore at different time points for the two treatment arms

Figure 2. Kaplan-Meier plot of cumulative mortality for the two treatment groups

TB-negative individuals: Using SuPAR to identify individuals with high mortality risk

suPARnostic ELISA

Background

Tuberculosis (TB) continues to affect the lives of millions of people worldwide. In Guinea-Bissau, TB is a common cause of morbidity and mortality. In a community study the incidence TB was estimated to be 471/100,000 person-years. Of those who come to the hospitals with symptoms of TB, only one in four is diagnosed with active TB based on sputum smear and X-ray. The individuals who are negative in sputum and X-ray are called "assumed TB-negative" (aTBneg). Little is known about this group because follow-up is not routinely conducted by National Tuberculosis Programmes in sub-Sahara Africa. We have previously shown that plasma levels of soluble urokinase receptor (suPAR) were eleva-ted in patients with active TB, carried prognostic value during the treatment period and that suPAR levels decreased in patients that responded to therapy. Since the group of assumed TB negative individuals is very large, we aimed to determine 1) mortality levels among these individuals and 2) whether suPAR can be used to determine post-consultation mortality.

Results

Mortality among aTBneg
Baseline characteristics and mortality during 3-month follow-up according to suPAR quartiles are shown in Table 1. Interestingly, there are more women than men in this group whereas there are more men than women among patients diagnosed with TB. The total mortality rate among individuals that were aTBneg was 21 per 100 person-year-observations (PYO) compared

with 0.03 per PYO among 4983 age-matched controls from the study area. Thus, mortality was 7-fold higher among aTBneg patients.

HIV and mortality

Both HIV-1 and HIV-2 was common among aTBneg individuals with 185 HIV-1-positive and 85 HIV-2- positive out of the 947 aTBneg individuals tested. The mortality rate was much higher for HIV infected subjects compared to HIV-negative subjects, especially HIV-1 compared with HIV-negative subjects. Higher suPAR levels were found among HIV-1 compared with HIV-2 infected and HIV negative individuals.

suPAR as a predictor of mortality

suPAR was included as a linear predictor in a survival analysis that included HIV status and gender. Mortality increased with suPAR levels regardless of HIV status as shown in the Figure 1. A 1ng/ml increase in suPAR was associated with a 46% (95% CI 34-59%) increase in the mortality rate.

Public health implications

Our study suggests that the National Tuberculosis Control Programme should develop guidelines for the management of aTBneg individuals to reduce the high mortality of this group. The problem is to identify individuals in need of further testing among this large group of patients. We found that the suPARnostic® assay can identify individuals with high post-consul-tation risk of mortality. An increased suPAR level is likely to reflect an elevated inflammatory and ongoing progressive disease state. Hence, an increased suPAR level should lead to further diagnostic testing to identify the cause of increased risk of mortality. This could include testing for HIV, further TB diagnostic testing with culture or PCR methods, sepsis, malaria, bacterial pneumonia, bronchiectasis, bronquial carcinoma, pneumocystis carinii, lung abscess, empyema and chronic obstructive pulmonary diseases.

Future perspectives

Our data provides a solid ground for future development of guidelines for the management of individuals that are TB-negative in order to reduce the high mortality of this group. Further cost-effective studies are needed to determine how suPAR can be included in a clinical decision tree for the management of aTBneg individuals. Thus, we hope in future to conduct a randomised study including suPAR as a decision marker in one arm of the trial.

References on TB negative individuals and suPAR: 229

Characteristic	1st suPAR Quartile (0.9–2.6)		2nd suPAR Quartile (2.6–3.3)		3rd suPAR Quartile (3.3–4.4)		4th suPAR Quartile (4.4–45)		Total Range (0.9-45)		P-value
No. of subjects	239		240		239		240		958		
Median age years	32	(17-66)	38	(18-70)	44	(19-71)	41	(21-69)	38	(18-69)	<0.01
Male	113	(47%)	91	(38%)	113	(47%)	100	(42%)	417	(44%)	0.11
Female	126	(53%)	149	(62%)	126	(53%)	140	(58%)	541	(56%)	
HIV-1*	24	(10%)	42	(18%)	36	(15%)	80	(33%)	182	(19%)	<0.01
HIV-2	9	(4%)	20	(8%)	25	(10%)	31	(13%)	85	(9%)	
HIV negatives	203	(85%)	177	(74%)	174	(73%)	124	(52%)	678	(71%)	
No HIV status	3	(1%)	1	(0%)	4	(2%)	5	(2%)	13	(1%)	
Died	2	(1%)	3	(1%)	3	(1%)	38	(16%)	46	(5%)	<0.01

Table 1 Characteristics of aTBneg individuals according to quartiles of suPAR in ng/ml

Figure. Log-mortality rates are plotted against plasma suPAR concentrations according to HIV status. Mortality increased with increasing suPAR independent of HIV status.
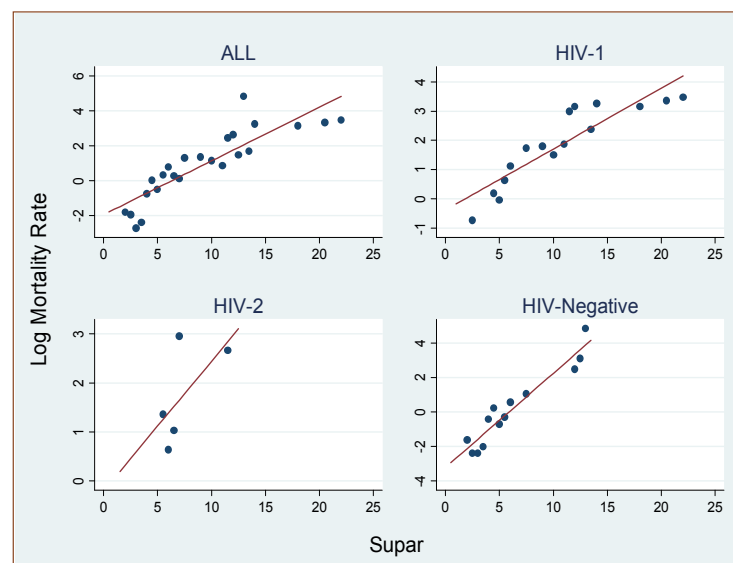
Global Health perspective: Too many chefs in Africa

Child mortality fell rapidly in the 1980s concomitantly to the introduction of childhood measles vaccination and maternal tetanus vaccinations but this momentum was lost in the beginning of the 1990s and the fall in child mortality diminished. With vaccination coverage of less than 50% in some low-income countries it has not been possible to decrease child mortality through this intervention. Very little has happened in terms of mortality from pneumonia among children in low-income countries and a fall in neonatal mortality has been completely absent.

Millennium goals

By 2000 the UN agreed on eight development goals for year 2015: *The Millennium Development Goals.* Three of these goals are directly aimed at health issues: reduction in child mortality, improved maternal health and reduction in prevalence of several diseases such as HIV and malaria. The high ambitions expressed by the goals have strengthened several large-scale global health initiatives such as *Global Fund to fight AIDS, TB and Malaria, Roll Back Malaria, and The Global Alliance for Vaccines and Immunization.*

Foresight in a small mind

There are available and well documented interventions that could prevent 6 of the 10 million deaths among children under five that occur every year. Likewise available knowledge is often deliberately not used to tackle certain health problems: increasing birth spacing to 36 months or more can reduce under-five mortality by 35%. In 2004, ten years after the introduction of impregnated bed nets only 5% of under-five-year-old children slept under a bed net in malaria areas. It took the WHO further 3 years to publicly apologize for their resistance to approve and distribute impregnated bed nets which Margaret Chan did at the Global Forum for

health research in Beijing 2007. For the past 5 years many countries in Africa have experienced a rapid and substantial decline in malaria prevalence most likely due to the distribution of bed nets. Political, religious and methodological research issues impede the process to reach at least some of the goals in some low-income countries. The goals are meant as a moral incentive and have been developed over more than a decade. Their fulfilment requires a long term commitment with overall solutions and an obligation to establish the foundation for the necessary continuous funding. The goals have put a focus on capacity building and poverty reduction as crucial for the process.

Uncertain numbers
The central parameter for child health in the 2015 goals is the reduction in under-five mortality and among the most important sources to monitor this are the repeated demographic health surveys (DHS). However, it has been argued that the number of children participating in the surveys is too small to capture the changes in child mortality that is expected by 2015. With the uncertainty of mortality figures from the DHS surveys it will not be possible to detect a fall in mortality with five year intervals.

Yet, uncertain data like these will form the basis for donations to the health sector, and low-income countries that are not able to demonstrate a reduction in child mortality will experience that donations are withheld or are blocked completely. Nevertheless, longitudinal demo-graphic health surveillance research sites in low-income countries follow child populations that are large enough to capture even smaller changes in child mortality.

On the contrary, donors have excluded long term involvement with rapid diminishing resources for development aid, demands for short term goals and lacking support of valid mortality data. In an editorial in The Lancet 2004 it was concluded that biomedical research had failed to tackle the massive health problems in DC.

Increasing worries
Many low-income countries do not stand a chance of achieving the 2015 goals before 2015. The most worrying is that the countries farthest from the goals are those with the biggest likelihood of not achieving them. Many agree that the largest hindrance for achievement of the millennium goals is the health care system itself and the World Bank has pointed out that poverty and child mortality levels are increasing in some African countries. Donors are only just realising that an important bottleneck in health development is the weak and fragmented health care systems in low-income countries that have lost the confidence of the population and are unable to deliver even the simplest services with a reasonable level of quality and coverage. Hospitals in low-income countries suffered during the boost into public health campaigns in the early 1980s and now have to fight a vicious circle of low quality of care, lack of motivation and

mistrust of the population. Donors on the other hand prioritise disease oriented vertical programmes with no intention of building capacity in the existing health care system. WHO's *High Level Forum on Achieving the Health Millennium Goals* has pointed out that a major challenge is to increase donor cooperation.

The bitterest pill of them all: we don't know enough

The *Bellagio*-group on child mortality showed in a 2003 paper in The Lancet that there is a need for investments in how to translate rational knowledge into action but nothing has happened since then. Research in pneumonia and diarrhoea among children, two diseases that are responsible for 50% of child deaths in low-income countries, only constitute 1% of research funds in childhood diseases in low-income countries.

In spite of the fact that interventions promoting simple hand washing reduce diarrhoea incidence at virtually no cost, large-scale water supply and sewerage projects are still stealing scanty funding for low-income countries. We lack knowledge on why some good health information spreads rapidly while other (too simple?) information is left unused. Less than 3% of the funding from the National Institute of Health (NIH) and the Gates Foundation go to research in the dissemination of interventions. The past four years not a single US dollar has been given to better coverage of proven effective childhood vaccines like the measles vaccine. There are,

however, large areas of Africa where the coverage of this vaccine is less than 1%. By increasing coverage of existing interventions to 99% we would be able to reduce under-five child mortality by 30-50%.

Unexpected observations

At the epidemiological level it is not uncommon to see that child mortality varies by up to 50% within geographically small areas and homogenously poor populations. At the individual level it is surprising that some poor mothers can bring all of their children through childhood without losing any of them, while her equally poor neighbour has lost more than half of her 8 births. In Guinea-Bissau in the period 1998-2002, 7% of mothers who lost a child were responsible for 34% of all child deaths in the same period. Some mothers lose more children, while others never lose a child. Death is not a random event and is highly determined by socio-economic conditions, but there are other factors that can weaken or re-enforce the effect of poverty. Two such factors would be social capacity and favouritism and if these factors are not taken into account when interventions are created we run the risk that the intervention does not reach those in most need of it: the poorest population groups. Health research is also about accepting the validity of unexpected observations. Such observations often arise within longitudinal studies of population groups over longer time spans (typically more than 10-15 years). Among others the Danish-Guinean Bandim Health Project in Guinea-Bissau has come up with such results.

A poor choice

Poverty and poor health condition fix people in a helpless situation: even if one intervention prevents a child from dying of malaria, the next year the child could die from measles because there are no vaccination campaigns in the poorest and most rural areas. Discrimination by sex, ethnic belonging and religion further boost the vulnerability of the poor.

The poorest children in Indonesia have a four times higher risk of dying before they reach five years of age compared with children of the richest families in the same country. It is estimated that in 2000 99% of all under-five child deaths in the world took place in poor areas. The World Bank Reaching the Poor programme last year published a case report from 12 low-income countries on health and disease in which they demonstrated that the richest 20% of these populations receive more of public health budgets than the poorest 20%.

Some are deader than others

The poor are badly off, but other vulnerable groups like illiterates, rural populations, anemic or malnourished children, low birth weight children and very young mothers associated with poverty are also independent risk factors.

There are fundamental problems in the health care system in low-income countries and these problems have consequences for all population groups regardless of social status although with varying impact. The child mortality problem in low-income countries is not solved by exclusive focus on the ultra poor. Their less poor fellow citizens are also exposed to low quality of care and violation of simple human rights in access to preventive and curative care. The richest population groups in Guinea-Bissau still have a child mortality 30 times higher than an average Scandinavian level. Quality of preventive health care and care in low-income countries is elementarily different from what should be expected from professional well trained health care workers. If we fail to acknowledge the significance of this we are definitely facing serious problems in reaching the 2015 goals.

No laboratory model

In today's Western medicine it is impossible to introduce a pink child plaster without placebo controlled double blind studies, but in Africa it is possible to turn entire health care systems upside down without the least bit of evidence or scientific background. Child vaccines produced and tested under the past reality of European and American epidemics were introduced without further testing in low-income countries with completely different patterns of disease transmission and morbidity burden with high incidences of diarrhoea and malaria as dummies in the game. Only now after 20-25 years of use the first studies on long term effects of child immunizations in DC have demonstrated that some of the vaccines in a best case scenario are useless and in a worst case scenario are detrimental to health. User fees were tested in small hierarchal Muslim societies and thereafter introduced on a large scale as a principle all over Africa. These fees have, like the decentralisation process, com-

pletely paralyzed the entire health system and only increased inequity in access to health care.

Shoot first – then ask

This absence of a scientific public health basis in low-income countries directly opposes a knowledge-based development in the field and the responsibility for this rests on the shoulders of donor organisations, WHO, NGOs and national governments. The will and power to coordinate knowledge-based development and constant learning by experience is simply not present. Decision makers should realize that every new health programme introduced kills one or more existing health programme or activity because resource allocation to health in DC is extremely limited.

One year they prioritise primary health care with free medicine, next year it is malaria prevention that is popular, and then it is control of diarrhoeal diseases with oral rehydration that is important or a cholera epidemic calls for desperate health measures, then it is polio eradication campaigns because the vaccines were donated, vitamin-A in campaigns, everybody needs impregnated bed nets, two-drug malaria treatment last year and now HIV treatment. The health care sector is forced to focus on the priorities of the donor organisations this year, while there is no national incentive to try and monitor efficacy and long-term consequences of these radical yearly changes. As a result we see a falling coverage of measles vaccination because health workers get the idea that this activity is no longer an important health activity.

Too many chefs in Africa

There are too many chefs in Africa: NGO's, WHO, UNICEF, UNDP, UNFPA, The World Bank, vertical disease programmes, relief organisations, religious health programmes and hospitals, unregulated private clinics and national health authorities.

The national authorities have no available professional counsellors to help prioritise and coordinate the many offers from donors in relation to national health plans and future needs. The result is a fragmented and anarchistic health sector where simple diseases and trivial infections turn into a catastrophic social event that forces entire families to spend most of their savings and time on a substandard health product.

A Kyoto agreement on health

The WHO is weakened economically and in terms of influence. We need a strong international professional health institution to serve as the advocate for users of health care and which is capable of generating better knowledge on how to use sound evidence. The international board of health should create and govern a Kyoto agreement on health rights ensuring the foundation on which we can reach the 2015 health goals.

(This paper is a shortened and slightly revised version of a paper that was originally published in the Danish Medical Bulletin, 2007; 54(1):52-54. Reprinted with permission)

BANDIM HEALTH PROJECT: PUBLICATIONS

2003

1. Garly ML, Aaby P. The challenge of improving the efficacy of measles vaccine. Acta Trop 2003;85:1-17

2. Jakobsen MS, Sodemann S, Mølbak K, Alvarenga IJ, Nielsen J, Aaby P. Termination of breastfeeding after 12 months of age due to a new pregnancy and other causes is associated with increased mortality in Guinea-Bissau. Int J Epidemiol 2003;32:92-6

3. Aaby P, Bhuyia A, Nahar L, Knudsen K, Francisco A, Strong M. The survival benefit of measles immunisation may not be explained entirely by the prevention of measles disease: Community study from rural Bangladesh. Int J Epidemiol 2003;32: 106-115

4. Holmgren B, da Silva Z, Larsen O, Vastrup P, Andersson S, Aaby P. Dual infections of HIV-1, HIV-2, and HTLV-I are more common in older women than men in Guinea-Bissau. AIDS 2003;17:241-53

5. Marret-Muir W, Scott FT, Aaby P, John J, Matondo P, Chaudhry QL, Siqueira M, Poulsen A, Yaminishi K, Breuer J. Genetic Variation of Varicella-Zoster Virus: Evidence for Geographical Separation of Strains. J Med Vir 2003; 70:S42-S47.

6. Stensballe LG, Devasundaram JK, Simoes EAF. Respiratory syncytical virus epidemics: the ups and downs of a seasonal virus. Pediatr Inf Dis J 2003; 22:S21-32

7. Garly ML, Martins CL, Balé C, Baldé MA, Hedegaard KL, Gustafson P, Lisse IM, Whittle HC, Aaby P. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa: A non-specific beneficial effect of BCG? Vaccine 2003;21:2782-90

8. Aaby P, Garly ML, Balé C, Martins C, Jensen H, Lisse IM, Whittle H. Survival of previously measles-vaccinated and measles-unvaccinated children in an emergency situation: an unplanned study. PIDJ 2003;22:798-805

9. Bager P, Westergaard T, Rostgaard K, Nielsen NM, Melbye M, Aaby P. Smallpox vaccination and risk of atopy and asthma. J Allergy Clin Immunol 2003;111:1227-31

10. Aaby P, Jensen H, Simondon F, Whittle H. High-titre measles vaccination before 9 months of age and increased female mortality: Do we have an explanation? Seminars in Pediatric Infectious Diseases 2003;14: 220-32

11. Kofoed PE, Lopes F, Aaby P, Hedegaard K Rombo L. Can

mothers be trusted to give malaria treatment to their children at home? Acta Tropica 2003;86:67-70

12. Aaby P,Jensen H, Samb B, Cisse B, Sodeman M, Jakobsen M, Poulsen A, Rodrigues A, Lisse IM, Simondon F, Whittle H. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. Lancet 2003;361: 2183-88

13. Veirum JE. The impact of monitoring case management on mortality during and after hospitalization among children admitted to the pediatric ward, Hospital Simao Mendes, Guinea-Bissau. University of Copenhagen, 2003 [PhD]

14. Steinsland H, Valentiner-Branth P, Grewal HMS, Gaastra W, Mølbak K, Sommerfelt H. Development and evaluation of genotypic assays for the detection and characterization of enterotoxigenic Escherischia coli. Diagn Microbiol Infect Dis 2003;45:97-105.

15. Nielsen NM, Rostgaard K, Juel K, Askgaard D, Aaby P. Long-term mortality after poliomyelitis. Epidemiology 2003;14:355-60

16. Steinsland H, Valentiner-Branth P, Gjessing HK, Aaby P, Mølbak K, Sommerfelt H. Protection from natural infections with enterotoxigenic Esherichia coli: longitudinal study. Lancet 2003; 362: 286-91.

17. Westergaard T, Begtrup K, Rostgaard K, Krause TG, Benn CS, Melbye M. Reproductive history and allergic rhinitis among 31,145 Danish Women. Clin Exp Allergy 2003; 33:301-5.

18. Valentiner-Branth P, Steinsland H, Fischer TK, Perch M, Scheutz F, Dias F, Aaby P, Mølbak K, Sommerfelt H. Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens in the first 2 years of life. J Clin Microbiol 2003; 41: 4238-45

19. Schim van der Loeff M. HIV-2 in West Africa. Epidemiological studies. University of Amsterdam 2003,[Thesis]

20. Aaby P, Jensen H, Whittle H. High-titre measles vaccine and female mortality. Lancet 2003;362: 1765

21. Fischer TK, Gentsch JR. Rotavirus typing methods and algorithms. Rev Med Virol 2003;13:1-13

22. Fischer TK. Epidemiological studies of rotavirus infection in Guinea-Bissau, West Africa. Dan Med Bull 2003;50:103-17

23. Lagarde E, Schim M, Enel C, Holmgren B, Dray-Spira R, Pison G, Piau JP, Delaunay V, Mboup S, Ndoye I, Coeuret-Pellicer M, Whittle H, Aaby P. Mobility and the spread of human immunodeficiency virus into rural areas of West Africa. Int J Epidemiol 2003;32: 744-52

24. Jakobsen MS, Sodemann M, Nylén G, Balé C, Nielsen J, Lisse IM, Aaby P. Breastfeeding status as a predictor of mortality among refugee children in an emergency in Guinea-Bissau. Int Hlth Trop Med 2003;8:992-6

25. Campbell SJ, Sabeti P, Fielding K, Sillah J, Bah B, Gustafson P, Manneh K, Lisse I, Sirugo G, Bellamy R, Bennett S, Aaby P, McAdam KPWJ, Bah-Sow OLienhardt C, Hill AVS. Variants of the CD40 ligand gene are not associated with increased susceptibility to tuberculosis in West Africa. Immunogenetics 2003:55:502-7

26. Benn CS, Bale C, Sommerfelt H, Friis H, Aaby P. Hypothesis: Vitamin A supplementation and childhood mortality: amplification of the non-specific effects of vaccines? Int J Epidemiol 2003:32: 822-8

27. Benn CS. Early life exposures and risk of allergy. Epidemiological studies based on Danish birth cohorts. University of Copenhagen, 2003 [PhD thesis]

28. Gustafson P. Epidemiological aspects of tuberculosis in Guinea-Bissau, West Africa. Lund University, 2003 (Med. Dr. Thesis)

29. Jespersen SB. In search of recognition: a study of war veterans in Guinea-Bissau Institute of Anthropology, University of Copenhagen, Copenhagen 2002 [MSc thesis]

30. Flanding M: Experiences of war. Institute of Anthropology, University of Copenhagen, Copenhagen 2003 [MSc thesis]

31. Vigh H. Navigating terrains of war: youth and soldiering in Guinea Bissau. Institute of Anthropology, University of Copenhagen, Copenhagen 2003 [PhD thesis]

32. Sodemann M, Aaby P. Bandim Health Project 1978-2003: Improving child health? Århus: Bandim Health Project, 2003

33. Benn CS, Benfeldt E, Andersen PK, Olesen AB, Melbye M, Björkstén B. Atopic Dermatitis in Young Children: Diagnostic criteria for Use in Epidemiological Studies Based on Telephone Interviews. Acta Derm Venereol 2003; 83: 347-350

34. Bager P, Melbye M, Rostgaard K, Benn CS, Westergaard T. Mode of delivery and risk of allergic rhinitis and asthma. J Allergy Clin Immunol 2003; 111: 51-6

35. Høj L, da Silva D, Hedegaard K, Sandström A, Aaby P. Maternal mortality: Only 42 days? Br J Obstet Gynaecol 2003;110:995-1000

36. Steinsland H. The epidemiology of enterotoxigenic Escherichia coli infection and diarrhea in early childhood. Bergen, Bergen University 2003 [PhD thesis]

37. Fischer TK, Page AN, Griffin DD, Eugen-Olsen J, Pedersen AG, Valentiner-Branth P, Mølbak K, Sommerfelt H, Nielsen NM. Characterization of incompletely typed rotavirus strains from Guinea-Bissau: identification of G8 and G9 types and a high frequency of mixed infections. Virology 2003;311:125-33

38. Ariyoshi K, Berry N, Cham F, Jaffar S, Schim van der Loeff M, Jobe O, N´Gom PT, Larsen O, Andersson S, Aaby P, Whittle H. Quantification of Human T-Lymphotropic Virus Type (HTLV-1) proviral load in rural West Africa population: no enhancement of Human Immunodeficiency Virus Type-2 pathogenesis, but HTLV-1 proviral load relates to mortality. JID 2003;188:1648-51

39. Ronsmans C, Etard JF, Walraven G, Høj L, Dumont A, Bernis Ld, Kodio B. Maternal mortality and access to obstetric services. TMIH 2003;8:940-8

2004

40. Kofoed PE, Rodrigues A, Co F, Hedegaard K, Rombo L, Aaby P. Which children come to the health centre for treatment of malaria. Acta Tropica 2004;90:17-22

41. Aaby P. Vet vi vad vi gör med vacciner? Läkartidningen 2004;101:294-7

42. Kæstel P. Micronutrient supplementation and other predictors of birth size and perinatal mortality in Guinea-Bissau. The Royal Veterinary and Agricultural University, Denmark, 2004 [PhD Thesis]

43. Kofoed PE. Poulsen A, Co F, Hedegaard K, Aaby P, Rombo L. No benefit from combining chloroquine with artesunate for three days for treatment of Plasmodium falciparum in Guinea-Bissau. Trans Roy Soc Trop Med Hyg 2003; 97:429-33

44. Aaby P, Jensen H, Rodrigues A, Garly ML, Benn CS, Lisse IM, Simondon F. Divergent female-male mortality ratios associated with different routine vaccinations among female-male twin pairs. Int J Epidemiol 2004;33:367-73

45. Aaby P, Jensen H, Gomes J, Fernandes M, Lisse IM. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. Int J Epidemiol 2004;33:374-80

46. Aaby P, Rodrigues A, Biai S, Martins C, Veirum JE, Benn CS, Jensen H. Oral polio vaccination and low case fatality at the paediatric ward in Bissau, Guinea-Bissau. Vaccine 2004;22:3014-7

47. Simondon KB, Elguero E, Marra A, Diallo A, Aaby P, Simondon F. Season of birth is not associated with risk of

early adult death in rural Senegal. Int J Epidemiol 2004;33:130-6

48. Nielsen NM, Rostgaard K, Askgaard D, Skinhøj O, Aaby P. Life-long morbidity among Danes with poliomyelitis. Arch Phys Med Rehabil 2004;85:385-91

49. Masmas T, Jensen H, da Silva D, Co A, Høj L, Sandström A, Aaby A. Survival among motherless children in rural and urban areas in Guinea-Bissau. Acta Paediatr 2004;93:99-105

50. Masmas TN, Jensen H, da Silva D, Høj L, Sandström A, Aaby P. The social situation of motherless children in rural and urban areas of Guinea-Bissau. Soc Sci Med 2004;59:1231-9

51. Gustafson P, Gomes VF, Vieira CS, Rabna P, Seng R, Johansson P, Sandström A, Norberg R, Lisse IM, Samb B, Aaby P, Nauclér A. Tuberculosis in Bissau: Incidence and risk actors in an urban community in sub-Saharan Africa. Int J Epidemiol 2004;33:163-72

52. Aaby P. Being wrong in the right direction? Lancet 2004;364:984

53. Kofoed PE, Alifrangis M, Poulsen A, Rodrigues A, Gjedde SB, Rønn A, Rombo L. Genetic markers of resistance to pyrimethamine and sulphonamides in Plasmodium falciparum parasites compared to the resistance patterns in isolates of E. coli from the same children in Guinea-Bissau. Trop Med Int Hlth 2004;9:171-7

54. Sodemann M, Veirum J, Biai S, Nielsen J, Bale C, Jakobsen MS, Gustafson P, Aaby P. Reduced case fatality among hospitalized children during a war in Guinea-Bissau: a lesson in equity. Acta Pædiatr 2004;93:959-64

55. Benn CS, Melbye M, Wohlfahrt J, Björkstén B, Aaby P. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during the first 18 months of life. BMJ 2004;1223-7

56. Lizeng Q, Nilsson C, Sourial S, Andersson S, Larsen O, Aaby P, Ehnlund M, Björling E. Potent neutralizing serum immunoglobulin A (igA) in human immunodefiency virus type-2-exposed IgG-seronegative individuals. J Virol 2004;78:7016-22

57. Benn CS, Wohlfahrt J, Aaby P, Westergaard T, Benfeldt E, Michaelsen KF, Björkstén B, Melbye M. Breastfeeding and Risk of Atopic Dermatitis, by Parental History of Allergy, During The First 18 Months of Life. Am J Epidemiol 2004;160:217-23

58. Roth A, Jensen H, Garly ML, Djana Q, Martins CL, Sodemann M, Rodrigues A, Aaby P. Should low birth weight infants receive Bacille Calmette-Guérin vaccination at birth? Community study from Guinea-Bissau. PIDJ 2004;23:544-50

59. Benn CS, Böttcher MF, Pedersen BV, Filtau SM, Duchén K. Mammary epithelial paracellular permeability in atopic and non-atopic mothers versus childhood atopy. Pediatr Allergy Immunol 2004;15:123-6

60. Stensballe L, Poulsen A, Nante E, Jensen IP, Kofoed PE, Jensen H, Aaby P. Mothers may transmit RSV infection more easily or severely to sons than daughters: Community study from Guinea-Bissau. Scan J Inf Dis 2004;36:291-5

61. Nielsen J, Valentiner-Branth P, Martins C, Cabral F, Aaby P. Malnourished children and supplementary feeding during war emergency in Guinea-Bissau in 1998-99. Am J Clin Nut 2004; 80:1036-42

62. Steinsland H, Valentiner-Branth P, Aaby P, Mølbak K, Sommerfelt H. Clonal relatedness of enterotoxigenic Escherichia coli strains isolated from a cohort of young children in Guinea-Bissau. JCM 2004;42:3100-7

63. Newport MJ, Goetghebuer T, Weiss HA, The MRC Gambia Twin Study Group, Whittle H, Siegrist C-A, Marchant A. Genetic regulation of immune responses to vaccines in early life. Genes and Immunity 2004:1-8

64. Jeppesen DL, Hasselbalch H, Lisse IM, Ersbøll AK, Engelmann MDM. T-lymphocyte subsets, thymic size and breastfeeding in infancy. Pediatr Allergy Immunol

2004;13:127-32

65. Garly ML, Jensen H, Martins CL, Balé C, Balde MA, Lisse IM, Aaby P Hepatitis B vaccination associated with higher female than male mortality in Guinea-Bissau: An observational study. Pediatr Infect Dis J 2004;23:1086-92

66. Bornmann L, Campbell SJ, Fielding K, Bah B, Sillah J, Gustafson P, Manneh K, Lisse I, Allen A, Sirugo G, Sylla A, Aaby P, McAdam KPWJ, Bah-Sow O, Bennett S, Lienhardt C, Hill AVS. Vitamin D receptor polymorphisms and susceptibility to tuberculosis in West Africa: A case-control and family study. JID 2004;190:1631-41

67. Perch M, Kofoed PE, Fischer TK, Co F, Rombo L, Aaby P, Eugen-Olsen J. Serum levels of soluble urokinase plasminogen activator receptor is associated with parasitaemia in children with acute Plasmodium falciparum malaria infection. Parasite Immunol 2004;26:207-11

68. Adam Roth: Specific and non-specific effects of BCG. University of Copenhagen, 2004 (PhD-thesis)

69. Jensen H, Brookmeyer R, Aaby P, Andersen PK. Shared Frailty Model for Left-Truncated Multivariate Survival Data. Research Report 2, 2004, Department of Biostatistics, University of Copenhagen, Denmark.

70. Dræbel TA. Public health response in a complex political emergency and its aftermath: the role of national health professionals in conflict and health system change in Guinea-Bissau. University of Copenhagen, 2004 (PhD-thesis)

71. Steinsland H, Valentiner-Branth P, Gjessing HK, Aaby P, Sommerfelt H, Mølbak K. Beskyttelse mod infektioner med enterotoksigene Escherichia coli. Ugeskr Læg 2004;166:4051-3

72. Linneberg A, Petersen J, Gronbaek M, Benn CS. Alcohol during pregnancy and atopic dermatitis in the offspring. Clin Exp Allergy. 2004 Nov;34(11):1678-83.

73. Aaby P, Benn CS, Lisse IM, Jensen H. Replicating findings from Guinea-Bissau? BMJ, Rapid responses, November 25, 2004

74. Jensen H, Benn CS, Lisse IM, Aaby P. Non-specific beneficial effects of vaccinations in Burkina Faso: Survival bias? BMJ, Rapid responses, November 25, 2004

75. Aaby P, Benn CS, Lisse IM, Jensen H. Sex-differential "non-effects" of vaccination. BMJ, Rapid responses, December 21, 2004

76. Poulsen A, Kæstel P, Kofoed PE. Rabies. A case of supposed exposure. Ugeskr Læger 2004;166:2676-7

2005

77. Nielsen J, Jensen H, Andersen PK. Creating a reference to a complex emergency situation using time series methods: War in Guinea-Bissau 1998-99. J Applied Stat 2005;32:75-86

78. Nielsen J. Mortality patterns and interventions during the war in Guinea-Bissau 1998-99. Statistical methods and inference in complex emergency situations. University of Copenhagen, 2005 (PhD-thesis)

79. Aaby P, Jensen H. Commentary: Contrary findings from Guinea-Bissau and Papua New Guinea. Int J Epidemiol 2005;34:149-151

80. Lisse IM, Qureshi K, Poulsen A, Kofoed PE, Nielsen J, Vestergaard BF, Aaby P. T-lymphocyte subsets and eosinophil counts in acute and convalescent chickenpox infection: A household study in Guinea-Bissau. J Infection 2005;50:125-9

81. Veirum JE, Sodemann M, Biai S, Jakobsen M, Garly ML, Hedegaard K, Jensen H, Aaby P. Routine vaccinations associated with divergent effects on female and male mortality at the paediatric ward in Bissau, Guinea-Bissau. Vaccine 2005;23:1197-1204

82. Aaby P, Hedegaard K, Sodemann M, Nhante E, Veirum JE, Jakobsen M, Lisse IM, Jensen H, Sandström A. Childhood mortality after oral polio immunisation campaign in

Guinea-Bissau. Vaccine 2005;23:1746-1751

83.   Aaby P, Rodrigues A, Biai S, Martins C, Veirum JE, Benn CB, Jensen H. Vaccines and unexpected observations: flaws or cause for concern? Vaccine 2005;23:2405-6

84.   Aaby P, Jensen H. Do measles vaccines have non-specific beneficial effects on mortality. Bull WHO 2005;83:238

85.   Poulsen A, Cabral F, Nielsen J, Roth A, Lisse IM, Vestergaard BF, Aaby P. Varicella Zoster in Guinea-Bissau: Intensity of exposure and severity of infection. PIDJ 2005;24:102-7

86.   Whittle H, Aaby P. Measles. Weatherall DJ, Ledingham JGG, Warrell DA. Oxford Textbook of Medicine, 4th edition. Oxford: Oxford University Press 2003

87.   Roth A, Gustafson P, Nhaga A, Djana Q, Poulsen A, Garly ML, Jensen H, Sodemann M, Rodriques A, Aaby P. BCG vaccination scar associated with better childhood survival in Guinea-Bissau. Int J Epidemiol 2005;34:540-7

88.   Stensballe LG, Nante E, Jensen IP, Kofoed P-E, Poulsen A, Jensen H, Newport M, Marchant A, Aaby P. Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls. Community based case control study. Vaccine 2005;23:1251-7

89.   Poulsen A, Cabral F, Nielsen J, Roth A, Lisse I, Aaby P. Growth, morbidity and mortality after chickenpox infection in young children in Guinea-Bissau. J Infect 2005; 51:307-313.

90.   Roth A, Sodemann M, Jensen H, Poulsen A, Gustafson P, Gomes J, Djana Q, Jakobsen M, Garly ML, Rodriques A, Aaby P. Vaccination technique, PPD reaction and BCG scarring in a cohort of children born in Guinea Bissau 2000 – 2002. Vaccine 2005;23:3991-8

91.   Nielsen J, Benn CB, Bale C, Martins C, Aaby P. Effect of a vitamin A intervention during a war-emergency in Guinea-Bissau 1998-99. Acta Tropica 2005;93:275-82

92.   Stensballe LG. Risk factors for severe respiratory syncytial virus infection. Epidemiologic studies in a low- and a high-income setting. University of Copenhagen, 2005 (PhD-thesis)

93.   Jensen H, Benn CS, Nielsen J, Lisse IM, Rodrigues A, Aaby P. Diphtheria-tetanus-pertussis vaccination in low income countries: improved child survival or survival bias? BMJ Rapid Responses February 16, 2005; http://bmj.bmjjournals.com/cgi/eletters/329/7478/1297

94.   Sodemann M, Rodrigues A, Nielsen J, Aaby P. Maternel vulnerability and socio-economic inequalities in child mortality in West Africa: an explorative study. Indepth Network: Measuring Health Equity in Small Areas. London: Ashgate 2005; 87-108

95.   Benn CS, Melbye M, Wohlfahrt J, Björkstén B, Aaby P. Kohortestudie af effekten af søskende og infektionssygdomme på udvikling af atopisk dermatitis. Ugeskrift for Læger, 2005, 167: 1754-7

96.   Nielsen NM, Eugen-Olsen J, Aaby P, Mølbak K, Rodrigues A, Fischer TK. Characterisation of rotavirus strains among hospitalised and non-hospitalised children in Guinea-Bissau, 2002. A high frequency of mixed infections with serotype G8. J Clin Virol 2005;34:13-21

97.   Broutin H, Mantilla-Beniers NB, Simondon F, Aaby P, Grenfell BT, Guégan JF, Rohani P. Epidemiological impact of vaccination on the dynamics of two childhood diseaees in rural Senegal. Microb Infect 2005;7:593-9

98.   Aaby P, Jensen H, Benn CS, Lisse IM. Survival bias may explain findings. BMJ 2005;330;844-5

99.   Jensen H, Benn CS, Aaby P. DTP in low income countries: improved child survival or survival bias? BMJ 2005;330:845-6

100.   Andersson S, Larsen O, da Silva Z, Linder H, Norrgren H, Dias F, Thorstensson R, Aaby P, Biberfeld G. HIV-2-specific T-lymphocyte proliferative responses in HIV-2-

infected and in HIV-2-exposed but uninfected individuals in Guinea-Bissau. Clin Exp Immunol 2005;139:483-9

101. Jaffar S, Schim van der Loeff M, Eugen-Olsen J, Vincent T, Sarje-Njie R, Ngom PT, Meyer AM, Berry N, Aaby P, Whittle H. Immunological predictors of survival in HIV type 2-infected rural villagers in Guinea-Bissau. Aids Res Hum Retrovir 2005;21:560-4

102. Benn CS, Michaelsen KF. Does the effect of breast-feeding on atopic dermatitis depend on family history of allergy? J Pediatr 2005;147:18-9

103. Knudsen TB, Gustafson P, Kronborg G, Kristiansen TB, Moestrup SK, Nielsen JO, Gomes V, Aaby P, Lisse I, Møller HJ, Eugen-Olsen J. Predictive value of soluble hemoglobin scavenger receptor CD163 serum levels for survival in tuberculosis patients. Clin Microb Infect 2005;11:730-5

104. Kæstel P, Michaelsen KF, Aaby P, Friis H. Effects of prenatal multimicronutrient supplements on birth weight and perinatal mortality: a randomised, controlled trial in Guinea-Bissau. Eur J Clin Nut 2005; 59:1081-9

105. Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, Warndorff D, Palayew M, Lisse I, Donkor D, Diallo S, Manneh K, Adegbola R, Aaby P, Bah-Sow O, Bennett S, McAdam KP. Investigation of the risk factors for tuberculosis: a case-control study in three counties in West Africa: Int J Epid 2005;34:914-23

106. Stensballe LG, Kristensen K, Nielsen J, Aaby P. Diagnosis coding in The Danish National Patient Registry for respiratory syncytial virus infections. Scan J Inf Dis 2005;37:747-52

107. Kabir Z, Aaby P. Non-Specific Effect of Measles Vaccination on All-cause Child Mortality: Revisited. Indian Pediatrics 2005;42:541-4

108. Nielsen J, Jensen H, Andersen PK, Aaby P. Mortality patterns during a war in Guinea-Bissau 1998-99: changes in risk factors? Int J Epidemiol 2005;epub

109. Benn CS, Martins C, Rodrigues A, Lisse IM, Aaby P. Randomised study of the impact of different doses of vitamin A on childhood morbidity and mortality. BMJ 2005;331:1428-32

110. Westergaard T, Rostgaard K, Wohlfart J, Andersen PK, Aaby P, Melbye M. Sibship characteristics and risk of allergic rhinitis and asthma. Am J Epidemiol 2005;162:125-32

111. Westergaard T, Rostgaard K, Wohlfart J, Andersen PK, Aaby P, Melbye M. Response to "Sibship effects and a call for a comparative disease approach". Am J Epidemiol 2005;162:139

112. Steenhard NR. Concurrent infection with intestinal worms and bacteria in man and domestic animals. The Royal Veterinary and Agricultural University, Copenhagen 2005 (PhD-Thesis)

113. Leutscher PD, Pedersen M, Raharisolo C, Jensen JS, Hoffmann S, Lisse I, Ostrowki SR, Reimert CM, Mauclere P, Ullum H. Increased prevalence of leukocytes and elevated cytokine levels in semen from Schistosoma haematobium-infected individuals. J Infec Dis 2005; 191:1639-47

114. Hoj L, Cardoso P, Nielsen BB, Hvidman L, Nielsen J, Aaby P. Effect of sublingual misoprostol on severe postpartum haemorrhage in a primary health centre in Guinea-Bissau: randomised double blind clinical trial. BMJ 2005;331:723-

115. Roth A, Nielsen J. A non-beneficial effect of BCG on non-tuberculous childhood mortality? Vaccine 2005;epub;Vaccine 2007;25:12-13

116. Olesen AB, Andersen G, Jeppesen DL, Benn CS, Juul S, Thestrup-Pedersen K. Thymus is enlarged in children with current atopic dermatitis. A cross-sectional study. Acta Derm Verereol 2005;85:240-3

117. Poulsen A. Determinants of severity of acute viral disease and their long-term consequences. University of Copenhagen, 2005 [PhD thesis]

2006

118. Linneberg A, Simonsen J, Petersen J, Stensballe L, Benn CS. Differential effects of risk factors on infant wheeze and atopic dermatitis emphasize a different etiology. J Allergy Clin Immunol 2006; 117:184-9.

119. Aaby P, Ibrahim S, Libman M, Jensen H. The sequence of vaccinations and increased female mortality after high-titre measles vaccine: Trials from rural Sudan and Kinshasa. Vaccine 2006;24:2764-71

120. Valentiner-Branth P, Nielsen J, Steinsland H, Perch M, Fischer T, Sommerfeldt H, Mølbak K, Aaby P. Community cohort study of cryptosporidium parvum infections: Sex-differential incidences associated with BCG and diphtheria-tetanus-pertussis vaccinations. Vaccine 2006;24:ePub;Vaccine 2007;25:2733-41

121. Benn CS, Diness BR, Fisker AB, Christoffersen D, Friis H. Højdosis A-vitamin-tilskud sænker børnedødeligheden i lavindkomstlande – kan det blive bedre? Ugeskrift for Læger 2006;168:2442-5

122. Kamper-Jørgensen M, Wohlfahrt J, Simonsen J, Thrane N, Benn CS, Temporal trend in paediatric infections in Denmark. Arch Dis Childh 2006;91:401-4

123. Masmas TN, Garly ML, Lisse IM, Rodriques A, Petersen PT, Birgens H. Inherited hemoglobin disorders in Guinea-Bissau, West Africa: a population study. Hemoglobin 2006;30:355-64

124. Roth AE, Stensballe LG, Garly ML, Aaby P. Beneficial non-targeted effects of BCG – ethical implications for the coming introduction of new TB-vaccines. Tuberculosis (Edinb.) 2006; 86(6):397-403

125. Roth AE, Garly ML, Jensen H, Nielsen J, Aaby P. Bacillus Calmette-Guérin vaccination and infant mortality. Expert Rev Vaccines 2006;5:277-93

126. Aaby P, Vessari H, Nielsen J, Maleta K, Benn CS, Jensen H, Ashorn P. Sex-differential effects of routine immunizations and child survival in rural Malawi. PIDJ 2006;25:721-727

127. Sodemann M, Biai S, Jakobsen MS, Aaby P. Knowing a medical doctor is associated with reduced mortality among sick children consulting at paediatric ward in Guinea-Bissau, West Africa. TMIH 2006;11:1868-1877

128. Aaby P. Sundhedsforskning i et af verdens fattigste lande. Novo Nordisk Fondens Årskrift 2005, p 34-37

129. Rodrigues A, Fischer TK, Valentiner-Branth P, Nielsen J, Steinsland H, Perch M, Mølbak K, Aaby P. Community cohort study of rotavirus infection and other enteropathogens: Are routine vaccinations associated with sex-differential incidence rates? Vaccine 2006;24:4737-47

130. Nielsen NM, Rostgaard K, Hjalgrim H, Askgaard D, Skinhøj P, Aaby P. Psychiatric hospitalizations in a cohort of Danish polio patients. Am J Epidemiol 2006;ePub (doi: 10.1093/AJE/KWK003)

131. Poulsen A, Stensballe LG, Nielsen J, Benn CB, Balde A, Roth A, Lisse IM, Aaby P. Long-term consequences of lower respiratory tract infection in early childhood in Guinea-Bissau. PIDJ:25:1025-31

132. Garly ML, Balé C, Martins CL, Whittle HC, Nielsen J; Lisse IM, Aaby P. Prophylactic antibiotics to prevent pneumonia and other complications after measles: community based randomised double blind placebo controlled trial in Guinea-Bissau. BMJ 2006;333:1245

133. Roth A, Sodemann M, Jensen H, Poulsen A, Gustafson P, Wejse C, Gomes J, Djana Q, Jakobsen M, Garly ML, Rodriques A, Aaby P. Tuberculin reaction, Bacile Calmette-Guérin scar, and lower female mortality. Epidemiology 2006: 17(5):562-8.

134. Aaby P, Jensen H, Walraven G. Age-specific changes in female-male mortality ratio related to the pattern of vaccinations: An observational study from rural Gambia. Vaccine 2006; 24:4701-08

135. Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, Sodemann M, Holmgren B, Stabell Benn C, Garly ML, Lisse IM, Jensen H. Vaccinia scars associated with better survival for adults. An observational study from Guinea-Bissau. Vaccine 2006;24:5718-25

136. Kofoed PE. Treatment of ucomplicated malaria in Guinea-Bissau. Karolinska Institutet, Stockholm, 2006 [Doctoral thesis]

137. Nielsen BB, Hoj L, Hvidman LE, Nielsen J, Cardoso P, Aaby P. Reducet post partum blødning efter sublingual misoprostol: a randomiseret dobbeltblindt klinisk studie I et udviklingsland. Ugeskr Laeger. 2006;168:1341-3

138. Kofoed PE, Ursing J, Poulsen A, Rodrigues A, Bergquist Y, Aaby P, Rombo L. Different doses of amodiaquine and chloroquine for treatment of uncomplicated malaria in children in Guinea-Bissau: implications for future treatment recommendations. Trans Roy Soc Trop Med Hyg 2006;ePub; 2007;101:231-8

139. Benn CS, Aaby P. Kønsforskelle i respons på præventive sundhedsprogrammer. Måske behandler vi piger og drenge forskelligt, når vi behandler dem ens? Ugeskr Laeger 2006; 168:3045-6

140. Benn CS, Fisker AB, Diness BR, Aaby P. Neonatal vitamin A supplementation: Sex-differential effects on mortality? JID 2006;194:721

141. Stensballe LG, Kristensen K, Simoes EAF, Jensen H, Nielsen J, Benn CS, Aaby P. Atopic disposition, wheezing, and subsequent respiratory syncytial virus hospitalisation in Danish children younger than 18 months: A nested case-control study. Pediatrics 2006;118:e1360-8

142. Kamper-Jørgensen M, Wohlfahrt J, Simonsen J, Grønbæk M, Benn CS. Population-based study of the impact of childcare attendance on hospitalisations for acute respiratory infections. Pediatrics 2006;118:1439-46

143. Aaby P. International Sundhed derhjemme: At tage erfaringerne med hjem. Ugeskr Laeger 2006:168:3015-17

144. Tosh K, Campbell SJ, Fielding K, Sillah J, Bah B, Gustafson P, Manneh K, Lisse I, Sirugo G, Bennett S, Aaby P, McAdam KP, Bah-Sow O, Lienhardt C, Kramnik I, Hill AV. Variants in the SP110 gene are associated with genetic susceptibility to tuberculosis in West Africa. Proc Natl Acad Sci U S A. 2006; 103(27):10364-8.

145. Kofoed, P. E., Rodrigues, A., Aaby, P. and L. Rombo. Continued efficacy of sulfadoxine-pyrimethamine as second line treatment for malaria in children in Guinea-Bissau. Acta Tropica 2006;epub; 2007;01:231-238

146. Ursing J, Kofoed PE, Rombo L, Gil JP. No pfmdr1 amplification in samples from Guinea-Bissau and Liberia collected between 1981 and 2004. JID 2006;194:718-9

147. Qureshi K, Mølbak K, Sandström A, Kofoed PE, Rodrigues A, Dias F, Aaby P, Svennerholm AM. Breast milk reduces the risk of illness in children of mothers with cholera. Observations from an epidemic of cholera in Guinea-Bissau. PIDJ 2006;25:1163-6

148. Sodemann M. Der er for mange kokke i Afrika. Ugeskr Laeger 2006;168:2999-3001

149. Benn CS, Fisker AB, Jørgensen MJ, Aaby P. Why worry: Vitamin A with DTP vaccine? Vaccine 2006; ePub; 2007;25(5):777-779

150. Aaby P, Biai S, Veirum JE, Sodemann M, Lisse I, Garly ML, Ravn H, Benn CS, Rodrigues A. DTP with or after measles vaccination is associated with increased in-hospital mortality in Guinea-Bissau. Vaccine 2006;ePub;2007;25:1265-1269

151. Jensen ML, Dave S, van der Loeff MS, da Costa C, Vincent T, Leligdowicz A, Benn CS, Roth A, Jensen H, Lisse IM, Whittle H, Aaby P. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. PLoS One 2006;1:e101

152. Fisker AB. Let us protect the children not the policies.

Unplanned observations are essential for improving current policies. In Global Forum for Health Research: Young voices in Research for Health, 2006: 35-38

2007

153. Jensen H, Benn CS, Nielsen J, Lisse IM, Rodrigues A, Andersen PK, Aaby P. Survival bias in observational studies of the impact of routine vaccinations on childhood survival. TMIH 2007;12:5-14

154. Aaby P, Benn CS, Nielsen J, Lisse IM, Rodrigues A, Jensen H. DTP vaccination and child survival in observational studies with incomplete vaccination data. TMIH 2007;12:15-24

155. Aaby P. Is susceptibility to severe infection in low-income countries inherited or acquired? J Intern Med 2007;261:112-122

156. Sodemann M. Too Many Chefs in Africa. Dan Med Bull 2007;54:1-3

157. Garly ML, Balé C, Martins CL, Whittle HC, Nielsen J; Lisse IM, Aaby P. Measles in developing countries: Vitamin A and prophylactic antibiotics. BMJ 2007;rapid responses, 28 Jan 2007

158. Garly ML, Nielsen J; Aaby P. The measure of effect is not transferable. BMJ 2007;rapid responses, 30 Jan 2007

159. Ursing J, Rodrigues A, Kofoed PE. Response to Laufer. NEJM 2007;356:868

160. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY, Frodsham AJ, Walley AJ, Kyrieleis O, Khan A, Aucan C, Segal S, Moore CE, Knox K, Campbell SJ, Lienhardt C, Scott A, Aaby P, Sow OY, Grignani RT, Sillah J, Sirugo G, Peshu N, Williams TN, Maitland K, Davies RJ, Kwiatkowski DP, Day NP, Yala D, Crook DW, Marsh K, Berkley JA, O'neill LA, Hill AV. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet 2007;39:523-8

161. Valentiner-Branth P, Nielsen J, Steinsland H, Perch M, Fischer T, Sommerfeldt H, Mølbak K, Aaby P. Community study of cryptosporidium infection: Sex-differential incidences associated with BCG and diphtheria-tetanus-pertussis vaccine. Vaccine 2007;25:2733-41

162. Garly ML. The choice of antibiotic for prophylaxis. BMJ 2007;rapid responses, 20 Mar 2007

163. Larsen JM, Benn CB, Fillie Y, Kleij D, Aaby P, Yazdanbakhsh M. BCG stimulated dendritic cells induce an IL-10 producing T-cell population with no T helper 1 or T helper 2 bias in vitro. Immunology 2007;121:276-82

164. Gustafson P, Gomes VF, Vieira CS, Samb B, Naucler A, Aaby P, Lisse I. Clinical predictors for death in HIV-positive and HIV-negative tuberculosis patients in Guinea-Bissau. Infection 2007;35:69-80

165. Gustafson P, Lisse I, Gomes VF, Vieira CS, Lienhardt C, Nauclér A, Jensen H, Aaby P. Risk factors for positive tuberculin skin test in Guinea-Bissau. Epidemiology 2007;18:340-347

166. Aaby P, Garly ML, Jensen H, Martins C, Balé C, Benn CS, Rodrigues A, Lisse IM. Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines. Observations from vaccination trials in Guinea-Bissau. Pediatr Infect Dis J 2007;26:247-52.

167. Rodrigues A, Schellenberg JA, Roth A, Benn CS, Aaby P, Greenwood B. Revaccination with Bacillus Calmette-Guerin (BCG) vaccine does not reduce morbidity from malaria in African children. TMIH 2007; 12: 224-9

168. Rodrigues A, Carvalho M, Monteiro S, Mikkelsen CS, Aaby P, Mølbak K, Fischer TK. Hospital surveillance of rotavirus infection and nosocomial transmission of rotavirus disease among children in Guinea-Bissau. Pediatr Infect Dis J. 2007-26:233-7

169. Kamper-Jørgensen M, Wohlfahrt J, Simonsen JB, Benn CS.

The Childcare Database: a valuable register linkage. Scand J Public Health 2007;35:323-9

170. Orozova-Bekkevold IM, Jensen H, Stensballe L, Olsen J. Maternal vaccination and preterm birth: using data mining as a screening tool. Pharm World Sci 2007;epub DOI10.1007/S11096-006-9077-8

171. Ruhwald M, Bjerregaard-Andersen M, Rabna P, Kofoed K, Eugen-Olsen J, Ravn P. CXCL10/IP-10 release is induced by incubation of whole blood from tuberculosis patients with ESAT-6,CFP10 and TB7.7. Microbes Infect 2007;9:806-12

172. Wejse C. The role of vitamin D in the pathogenesis and treatment of tuberculosis. University of Århus, 2007 (PhD Thesis)

173. Diness BR. High-dose vitamin A supplementation with Bacille Calmette-Guérin vaccine at births. University of Copenhagen, 2007 (PhD Thesis)

174. Nante JE, Diness BR, Ravn H, Roth A, Aaby P, Benn CS. No adverse events after simultaneous administration of 50,000 IU vitamin A and Bacille Calmette-Guerin vaccination to normal-birth-weight newborns in Guinea-Bissau. Europ J Clin Nutr 2007;epub; 2008:62:842-8

175. Olesen R, Wejse C, Velez D, Bisseye C, Sodemann M, AabyP, Rabna P, Worwui A, Chapman H, Diatta M, Adegbola RA, Hill P, Østergaard L, Williams S, Sirugo G. DC-SIGN (CD209), Pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. Genes Immunity 2007; doi: 10.1038/sj. gene.6364410.

176. Eriksson M, Sartono E, Martins CL, Balé C, Garly ML, Whittle H, Aaby P, Pedersen BK, Yazdanbakhsh M, Erikstrup C, Benn CB. A comparison of ex vivo cytokine productions in venous and capillary blood. Clin Exp Immunol 2007;150:469-76

177. Benn CS, Aaby P. Sex-differential responses to preventive health interventions. Maybe we treat boys and girls differently when we treat them equally? Dan Med Bull 2007;54:153-4

178. Aaby P. International health at home: learning from low-income countries. Dan Med Bull 2007;54:147-9

179. Fisker AB, Lisse IM, Aaby P, Erhardt JG, Rodrigues A, Bibby BM, Benn CS. Effect of neonatal vitamin A supplementation with BCG vaccine on vitamin A status at 6 wk and 4 mo of age. Am J Clin Nut 2007;86:1032-9

180. Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL, Sodemann M. TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. Scand J Infect Dis 2008;40:111-20.

181. Veirum JE, Biai S, Jakobsen M, Sandström A, Hedegaard K, Kofoed PE, Aaby P, Sodemann M Persisting high hospital and community childhood mortality in an urban setting in Guinea-Bissau. Acta Paediatr. 2007;96:526-30

182. Leligdowicz A, Yindom LM, Onyango C, Sarge-Njie R, Alabi A, Cotten M, Vincent T, da Costa C, Aaby P, Jaye A, Dong T, McMichael A, Whittle H, Rowland-Jones S Robust Gag-specific T cell responses characterize viremia control in HIV-2 infection. J Clin Invest 2007;117:3067-74

183. Schmidt WP, Schim van der Loeff M, Aaby P, Whittle H, Bakker R, Buckner M, Dias F, White RG. Behaviour change and competitive exclusion can explain the diverging HIV-1 and HIV-2 prevalence trends in Guinea-Bissau. Epidemiol Infect 2007;epub

184. Diness BR, Fisker AB, Roth A, Yazdanbakhsh M, Sartono E, Whittle H, Nante JE, Lisse IM, Ravn H, Rodrigues A, Aaby P, Benn CS. Effect of high-dose vitamin A supplementation on the immune response to Bacille Calmette-Guerin vaccine. Am J Clin Nut 2007;86:1152-9

185. Wejse C, Olesen R, Rabna P, Kaestel P, Gustafson P, Aaby P, Andersen PL, Glerup H, Sodemann M. Serum 25-hydro-

xyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. AJCN 2007;86:1376-83

186. Aaby P, Jensen H. Response to Deborah Lehmann. TMIH 2007;12:1270-1

187. Biai S, Rodrigues A, Gomes M, Ribeiro I, Sodemann M, Alves F, Aaby P. Reduced in-hospital mortality after improved management of children under 5 years admitted to hospital with malaria: randomised trial. BMJ 2007 doi:10.1136/bmj.39345.467813.80

188. Kamper-Jorgensen M, Woodward A, Wohlfahrt J, Benn CS, Simonsen J, Hjalgrim H, Schmiegelow K. Childcare in the first 2 years of life reduces the risk of childhood acute lymphoblastic leukemia. Leukemia 2007;epub;2008;22:189-93

189. Jakobsen MS, Sodemann M, Biai S, Nielsen J, Aaby P. Promotion of exclusive breastfeeding is not likely to be cost effective in West Africa. A randomized intervention study from Guinea-Bissau. Acta Paediatr 2007;epub;2008;97:68-75

190. Veirum JE, Sodeman M, Biai S, Hedegård KL, Aaby P. Increased mortality in the year following discharge from a paediatric ward in Bissau, Guinea-Bissau. Acta Paediatr 2007;96:1832-38

191. Holmgren B, da Silva Z, Vastrup P, Larsen O, Andersson S, Ravn H, Aaby P. Mortality associated with HIV-1, HIV-2 and HTLV-1 single and dual infections in a middle-aged and older population in Guinea-Bissau. Retrovirology 2007;4:85 doi:10.1186/1742-4690-4-85

192. Whittle H, Aaby P. Measles. Weatherall DJ, Ledingham JGG, Warrell DA. Oxford Textbook of Medicine, 5th edition. Oxford: Oxford University Press (in press)

193. Ursing J, Kofoed PE, Rodrigues A, Rombo L, Gil JP. Plasmodium falciparum genotypes associated with chloroquine and amodiaquine resistance in Guinea-Bissau. Am J Trop Med Hyg 2007, 76:844-848.

194. Ursing J, Schmidt BA, Lebbad M, Kofoed, Dias F, PedroGil J, Rombo L. Chloroquine resistant P. falciparum prevalence is low and unchanged between 1990 and 2005 in Guinea-Bissau: An effect of high chloroquine dosage? Infect Genet Evol 2007; 7:555-61

195. Kofoed PE, Ursing J, Rodrigues A, Rombo L. Failures following initial treatment for uncomplicated malaria: Quinine as second line therapy. J Paediatr Inf Dis 2007; 2:121-126.

196. Kofoed PE. Severe malaria in African children; still a challenge. J Paediatr Inf Dis 2007; 2: 181-182.

197. Ursing J, Rombo L, Kofoed PE, Gil JP. Cariers, channels and chloroquine efficacy in Guinea-Bissau. Trends Parasitology 2007; epub

198. Kitchen M, Quigley MA, Mwinga AM, Fuchs D, Lisse IM, Porter JDH, McAdam KPWJ, Godfrey-Faussett P. HIV progression and predictors of mortality in a community-based cohort of Zambian Adults. J Int Assoc Phys AIDS Care 2007; epub

2008

199. Vannberg FO, Chapman SJ, Khor CC, Tosh K, Floyd S, Jackson-Sillah D, Crampin A, Sichali L, Bah B, Gustafson P, Aaby P, McAdam KP, Bah-Sow O, Lienhardt C, Sirugo G, Fine P, Hill AV. CD209 Genetic Polymorphism and Tuberculosis Disease. PLoS ONE 2008;3:e1388.

200. Rodrigues A, Schellenberg JA, Kofoed PE, Aaby P, Greenwood B. Changing pattern of malaria in Bissau, Guinea-Bissau. TMIH 2008; 13:410-17

201. Aaby P, Benn CS, Nielsen J, Ravn H. Sex-differential non-specific effects of BCG and DTP in Cebu, The Philippines. IJE 2008; epub

202. Stensballe LG, Hjuler T, Andersen A, Kaltoft M, Ravn H, Aaby P, Simoes EA. Hospitalization for respiratory syncytial virus infection and invasive pneumococcal disease in

Danish children aged <2 years: a population-based cohort study. Clin Infect Dis 2008;46:1165-71.

203. Hersoug L-G, Benn CS, Simonsen JB, Kamper-Jørgensen M, Linneberg A. Maternal employment in childcare institutions and the risk of infant wheeze and atopic dermatitis in the offspring. Paediatric Allergy Immunol 2008;epub

204. Rodrigues A, Aaby P, Mølbak K, Fischer TK. Rotavirus disease in Guinea-Bissau, West Africa – A review of longitudinal community and hospital studies. JID (in press)

205. Diness BR, Christoffersen D, Pedersen, Rodrigues A, Fischer TK, Andersen A, Whittle H, Yazdanbakhsh M, Aaby P, Benn CS. Effect of high-dose vitamin A supplementation at birth with BCG vaccine on rotavirus colonization and disease. A randomized prospective study in Guinea-Bissau. JID (in press)

206. Benn CS, Friis H, Wejse C. Should micronutrient supplementation be integrated in the case management of tuberculosis? JID 2008;197:1487-1489

207. Erikstrup E, Mortensen OH, Rinnow A, Fischer CP, Pedersen AM, Krogh-Madsen R, Lindegaard B, Erhardt J, Ullum H, Benn CS, Pedersen BK. RBP4-to-retinol ratio, but not total RBP4, is elevated in patients with type 2 diabetes. Diabetes, Obesity and Metabolism (in press)

208. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B, Andersson S, Aaby P. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau. Is HIV-2 disappearing? AIDS 2008;22:1195-1202

209. Benn CS, Diness BR, Roth A, Nante E, Fisker AB, Lisse IM, Yazdanbakhsh M, Whittle H, Rodrigues A, Aaby P. Effect of 50,000 IU vitamin A given with BCG vaccine on mortality in infants in Guinea-Bissau: randomised placebo controlled trial. BMJ 2008;336:1416-20

210. Benn CS, Fisker AB, Jørgensen MJ, Aaby P. Conflicting evidence for neonatal vitamin A supplementation. Vaccine 2008;26:4111-12

211. Garly ML, Trautner SL, Marx C, Danebod K, Nielsen J, Ravn H, Martins CL, Balé C, Aaby P, Lisse IM. Thymus size at 6 months of age and subsequent child mortality. J Pediatr 2008;epub

212. Norrgren H, Bamba S, Larsen O, Da Silva Z, Aaby P, Koivula T, Andersson S, MD, Increased prevalence of HTLV-1 in patients with pulmonary tuberculosis coinfected with HIV, but not in HIV-negative tuberculosis patients. JAIDS 2008;epub

213. Checkley W, Buckley G, Gilman RH, Assis AMO, Guerrant RL, Morris SS, Mølbak K, Valentiner-Branth P, Lanata CF, Black RE and The Childhood Malnutrition and Diarrhea Network. Multi-country analysis of the effects of diarrhea on childhood stunting. IJE 2008;epub

214. Martins CL, Garly ML, Balé C, Rodrigues A, Ravn H, Whittle HC, Lisse IM, Aaby P. Protective efficacy of standard Edmonston-Zagreb measles vaccination in infants aged 4.5 months: interim analysis of a randomised clinical trial. BMJ 2008;336:epub

215. Benn CS, Whittle H, Fisker A, Aaby P. Neonatal vitamin A supplementation in South Asia: Rapid implementation or understanding the variation? BMJ Rapid responses 2008;July 11

216. Sodemann M, Nielsen J, Veirum J, Jakobsen MS, Biai S, Aaby P. Hypothermia of newborns is associated with excess mortality in the first two months of life in Guinea-Bissau, West Africa. TMIH 2008;13:1-7

217. Benn CB, Martins C, Rodrigues A, Fisker AB, Christoffersen D, Aaby P. The effect of vitamin A supplementation administered with missing vaccines during national immunisation days in Guinea-Bissau. IJE 2008;epub

218. Wejse C, Gomes VF, Rabna P, Aaby P, Lisse I, Andersen PL, Glerup H, Sodemann M. Vitamin D as supplementary

treatment for tuberculosis – a randomised double-blind placebo-controlled trial. AJRCCM (in press)

219. Stensballe LG, Simonsen J, Thomsen SF, Larsen AMH, Hovmand SG, Aaby P, Kyvik KO, Skytthe A, Backer V, Bisgaard H. The causal direction in the association between respiratory syncytial virus hospitalization and asthma studied in a population-based prospective twin cohort. J Allergy Clin Immunol (in press)

220. Stensballe LG, Ravn H, Kristensen K, Agerskov K, Meakins T, Aaby P, Simoes E. Seasonal variation of maternally derived respiratory syncytial virus neutralizing antibody levels in cord-blood of Danish newborns and the association to RSV hospitalization in the infant population. J Ped (in print)

221. Stensballe LG, Ravn H, Kristensen K, Agerskov K, Meakins T, Aaby P, Simoes E. Respiratory syncytial virus (RSV) neutralizing antibodies in cord blood, RSV hospitalization, and recurrent wheeze. J Allergy Clin Immunol (in print)

222. Thomsen SF, Stensballe LG, Skytthe A, Kyvik KO, Backer V, Bisgaard H. Increased concordance of severe respiratory syncytial virus infection within identical twins. Pediatrics 2008;121(3):493-6.

223. Benn CS, Fisker AB, Rodrigues A, Ravn H, Sartono E, Whittle H, Yazdanbakhsh M, Aaby P. Sex-differential effect on infant mortality of oral polio vaccine administered with BCG at birth in Guinea-Bissau. A natural experiment. PLoS One (revision)

224. Ursing J, Kofoed PE, Rodrigues A, Bergqvist Y, Rombo L. Grossly overdosed chloroquine is well tolerated and routine in Guinea-Bissau. Antimicrobial Agents Chemotherapy (in press)

225. Roth A, Benn CB, Ravn H, Rodrigues A, Lisse IM, Yazdanbakhsh M, Whittle H, Aaby P. A randomised trial of the effect of revaccination with BCG in early childhood and mortality (submitted)

226. Aaby P, Roth A, Ravn H, Rodrigues A, Lisse IM, Yazdanbakhsh M, Whittle H, Benn CB. Divergent effects of revaccination with BCG in a randomized trial: Interactions with other vaccinations and micronutrients (submitted)

227. Kristensen K, Stensballe LG, Bjerre J, Roth D, Fisker N, Kongstad T, Svendsen AL, Nielsen BW. Risk factors for RSV hospitalisation in children with heart disease. Arch Dis Child (in press)

228. Thomsen SF, van der Sluis S, Stensballe LG, Posthuma D, Skytthe A, Kyvik KO, Duffy DL, Backer V, Bisgaard H. Exploring the association between severe respiratory syncytial virus infection and asthma: A registry-based twin study. Am J Resp Crit Care Med (in revision).

229. Rabna P, Andersen A, Wejse C, Oliveira I, Gomes VF, Haaland MB, Aaby P, Eugen-Olsen J. High mortality risk among individuals assumed to be TB-negative in Guinea-Bissau can be predicted using a simple test. (Submitted)