Co-administration of live measles and yellow fever vaccines and inactivated pentavalent vaccines is associated with increased mortality compared with measles and yellow fever vaccines only. An observational study from Guinea-Bissau

Ane Bærent Fisker[a,b*],Henrik Ravn[a,b], Amabelia Rodrigues[a], Marie Drivsholm Østergaard[a], Carlito Bale[a], Christine Stabell Benn[a,b], Peter Aaby[a,b]

a) Bandim Health Project, INDEPTH Network, Apartado 861, Bissau, Guinea-Bissau
b) Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark

Note: This is the author's version of a work that was accepted for publication in Vaccine. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was published in **Vaccine [Volume 32,Issue 5 (23-01-2014)] DOI: 10.1016/j.vaccine.2013.11.074**

E-mail adresses:
Ane Bærent Fisker: a.fisker@bandim.org
Henrik Ravn: hjn@ssi.dk
Amabelia Rodrigues: a.rodrigues@bandim.org
Marie Drivsholm Østergaard: mariedrivsholm@gmail.com
Carlito Bale: c.bale@bandim.org
Christine Stabell Benn: cb@ssi.dk
Peter Aaby: p.aaby@bandim.org

***Corresponding author:** Ane Bærent Fisker, a.fisker@bandim.org

Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark. Tel: +45 32 68 31 62; Fax +45 32 68 31 65

Words: 4100

ABSTRACT

Background: Studies from low-income countries indicate that co-administration of inactivated diphtheria-tetanus-pertussis (DTP) vaccine and live attenuated measles vaccine (MV) is associated with increased mortality compared with receiving MV only. Pentavalent (DTP-*H. Influenza type B-Hepatitis B)* vaccine is replacing DTP in many low-income countries and yellow fever vaccine (YF) has been introduced to be given together with MV. Pentavalent and YF vaccines were introduced in Guinea-Bissau in 2008. We investigated whether co-administration of pentavalent vaccine with MV and yellow fever vaccine has similar negative effects.

Methods: In 2007-11 we conducted a randomised placebo-controlled trial of vitamin A at routine vaccination contacts among children aged 6-23 months in urban and rural Guinea-Bissau. In the present study we included 2331 children randomised to placebo who received live vaccines only (MV or MV+YF) or a combination of live and inactivated vaccines (MV+DTP or MV+YF+Pentavalent). Mortality was compared in Cox proportional hazards models stratified for urban/rural enrolment adjusted for age and unevenly distributed baseline factors.

Results: While DTP was still used 685 children received MV only and 358 MV+DTP; following the change in programme, 940 received MV+YF only and 348 MV+YF+pentavalent. During 6 months of follow-up, the adjusted mortality rate ratio (MRR) for co-administered live and inactivated vaccines compared with live vaccines only was 3.24 (1.20-8.73). For MV+YF+pentavalent compared with MV+YF only, the adjusted MRR was 7.73 (1.79-33.4).

Conclusion: In line with previous studies of DTP, the present results indicate that pentavalent vaccine co-administered with MV and YF is associated with increased mortality.

Words: 248

Keywords: diphtheria-tetanus-pertussis vaccine, pentavalent vaccine, measles vaccine, child mortality

Highlights

- Inactivated diphtheria-tetanus-pertussis (DTP) vaccine co-administered with live measles vaccine (MV) is associated with increased mortality compared with MV only.
- One third of Guinean children receive delayed DTP vaccines with MV after 9 months of age.
- Pentavalent vaccine (DTP+HiB+HepB) has replaced DTP in many low-income countries.
- Pentavalent vaccine co-administered with live MV and yellow fever vaccine is also associated with increased mortality.

INTRODUCTION

The vaccination schedule in many low-income countries comprises Bacillus Calmette-Guérin (BCG[1]) and oral polio vaccine (OPV) at birth, 3 doses of whole-cell diphtheria-tetanus-pertussis (DTP) and OPV at 6, 10 and 14 weeks of age and measles vaccine (MV) at 9 months of age[1]. Vaccination coverage is normally assessed at 12 months of age[2]. The coverage for the third dose of DTP (DTP-3) is the primary indicator of the performance of the vaccination programme[3, 4]. Both observational studies[5, 6] and randomised trials[7, 8] indicate that the live BCG and MV are associated with much lower mortality than can be explained by prevention of tuberculosis and measles infection; these vaccines reduce susceptibility to diseases unrelated to the targeted infection. In contrast, the inactivated DTP vaccine has been associated with increased mortality in areas with herd immunity to pertussis[9]. These effects beyond target disease protection have been called "non-specific effects".

According to the recommended vaccine schedule, DTP-3 is provided more than 5 months before MV. However, vaccines are often given with delay and DTP is often co-administered with MV[10, 11]. In rural Guinea-Bissau one third of children receive DTP with or after MV[10]. Studies from low-income countries indicate that co-administration of DTP and MV is associated with increased mortality compared with MV only[12-15]. In a recent small randomised trial girls had impaired growth if they had been randomised to MV+DTP compared with MV only[16]. We have proposed the hypothesis that DTP vaccine provided with measles vaccine is associated with at least 50% higher mortality than measles vaccine alone[17]. We have argued that the focus on DTP-3 coverage rather than MV coverage leads to co-administration of DTP with MV or administration of DTP after MV and this may lead to increased child mortality[18, 19]

In recent years, DTP vaccine has gradually been replaced by the pentavalent DTP-*H. influenza type B*-*Hepatitis B* vaccine in low-income countries [4]. In 2008, pentavalent vaccine was introduced in Guinea-Bissau[19, 20]; in addition yellow fever vaccine (YF) was introduced to be given with measles vaccines at 9 months of age[19, 20]. The potential non-specific effects of pentavalent and YF vaccines have not been studied previously. Specifically it is not known whether pentavalent vaccine provided together with MV/YF has the same negative effects as DTP provided with MV.

In 2007-2011 the Bandim Health Project (BHP) in Guinea-Bissau conducted a randomised controlled trial of the effect of vitamin A versus placebo at vaccination contacts. We used the opportunity to

[1] **Abbreviations used:** BCG: Bacillus Calmette-Guérin vaccine; BHP: Bandim Health Project; DTP: Diphtheria-tetanus-pertussis vaccine; EPI: Expanded Programme on Immunisations; HDSS: Health and demographic surveillance system; MRR: Mortality rate ratio; MV: Measles vaccine; OPV: Oral polio vaccine; YF: Yellow fever vaccine

study the effect of co-administration of live and inactivated vaccines. Since vitamin A may interact with vaccines[21, 22] and the hypothesis of increased mortality of DTP+MV versus MV only stated specifically that it should be tested in settings in which no other interventions were given with DTP[17], we limited the analyses to the placebo group. We compared mortality of children who received a combination of live and inactivated vaccines (MV+DTP/MV+YF+pentavalent) with children who received only live vaccines (MV/MV+YF) at enrolment, pursuing the *a priori* hypothesis that receiving a combination of live and inactivated vaccines is associated with higher mortality[17].

METHODS

Setting and population

The study was observational. Children who had been randomised to placebo in a trial comparing vitamin A versus placebo at routine vaccination contacts after 6 months of age (clinicaltrials.gov, number NCT00514891)[22] entered the present study if they received MV, MV+YF, MV+DTP or MV+YF+pentavalent vaccine at enrolment. The study took place in the urban and rural areas surveyed through the health and demographic surveillance system (HDSS) of the BHP in Guinea-Bissau. When we initiated the vitamin A trial in August 2007, the Guinean Expanded Programme on Immunizations (EPI) schedule was BCG and OPV at birth, DTP with OPV at 6, 10 and 14 weeks and MV at 9 months of age. In August 2008, the schedule was altered: Pentavalent vaccine replaced DTP, and YF was added to MV at 9 months of age.

In the urban study area, BHP assistants routinely record all children vaccinated at the health centres and during outreach campaigns at the date of vaccination. In the rural area children are vaccinated by nurses at government health centres, during outreach and by the BHP team. BHP field assistants register all vaccines at the six-monthly home visits.

Enrolment into the trial and assessment of exposure

The trial is described in details elsewhere[22]. Briefly, children aged 6-23 months who were eligible to receive one or more vaccines were invited to participate. Most children were due to receive MV, but some were also missing DTP or OPV or other vaccines or combinations of vaccines. Exclusion criteria were vitamin A within the preceding month and being part of another randomised trial. Enrolments took place between 13 August 2007 – 28 December 2009 in the urban area and 11 September 2007 – 28 November 2010 in the rural area. Children due to be vaccinated were invited to participate at the three health centres in the urban study area and at outreach vaccination posts organised in the villages during the six-monthly visits in the rural area. At enrolment children were randomised to vitamin A oil (200,000 IU vitamin A as retinyl palmitate and 40 IU vitamin E per ml oil)

or placebo (40 IU vitamin E per ml oil). Depending on randomisation group children were give vitamin A or placebo oil orally; children aged 6-11 months received ½ ml oil, children 12-23 months received 1 ml oil. Children were subsequently vaccinated by a study nurse and the group assignment and vaccines received were noted on the enrolment form.

Vaccines were UNICEF certified delivered through the national programme. The vaccines were: DTP: Serum Institute of India, India and Bio Farma, Indonesia; Pentavalent: Quinvaxem from Berna Biotec, Korea; Easyfive from Panacea, India and Shan5 from Shantha, India; OPV: Polio Sabin, GlaxoSmithKline, Belgium and OPVERO, Sanofi Pasteur, France; MV: Measles Vaccine (Edmonston-Zagreb) from Serum Institute of India, India and Rouvax (Schwarz) from Sanofi-Pasteur, France and YF: Stamaril, Sanofi-Pasteur, France and Institut Pasteur de Dakar, Senegal.

During the conduct of the trial bi-annual vitamin A campaigns were conducted. In several of these campaigns vaccines were co-administered with vitamin A; in July 2009 MV was given with vitamin A; in May 2010, May 2011 and November 2011 OPV was given with vitamin A. In addition campaigns distributing OPV were conducted in March 2010, April 2010 and March 2011. Also an H1N1-influenza vaccination campaign was conducted in October 2010. In the urban area participation in campaigns was registered during the campaigns and through follow up visits to all children who had not been seen during the campaigns. In the rural area information was collected at the first visit after the campaign.

Outcomes examined

Enrolled children were followed though the HDSS. A study-specific interview was conducted 6 and 12 months after enrolment. In the urban area this was done by a special team, in the rural area it was integrated with the 6-monthly routine visits. In the urban area a subgroup of children were also followed intensively through an adverse events study[23] (seven visits during the first month) and a study of diarrhoeal morbidity (weekly visits during the first 6 months). When a death was registered an interview was conducted to determine the cause of death. Follow-up of children who died due to accidents was censored on the date of death.

Statistical analyses

Key information was double entered and inconsistencies resolved. Z-scores for weight-for-age, length-for-age, weight-for-length and arm-circumference-for-age were based on the 2006-WHO child growth standard[24] and calculated using the WHO Anthro version 3.1 macro for Stata[25]. Baseline characteristics were compared by chi-squared tests, t-tests or ranksum tests. Survival was assessed in

Cox proportional hazards models with time since vaccination as underlying time scale and adjusted for age as a continuous variable. The proportional hazards assumption was evaluated by log-log plots and Schoenfeld residuals. Analyses were performed using Stata11.2 (StataCorp, College Station, TX).

In the analysis comparing survival after the vaccination(s) given at enrolment, we censored follow up at registration of subsequent receipt of a vaccine. Since information on subsequent vaccines is better for children who survive than for those who die[26], follow-up was censored when the information was obtained rather than on the day the vaccine was received thus preventing a censoring bias since only children who survived to the date of inspection of the vaccination card could be censored. The analysis compared overall mortality for children who received a combination of live and inactivated vaccines (either MV+DTP or MV+YF+pentavalent vaccine) with the mortality of children who received live vaccines only (MV only or MV+YF only); analyses were also conducted separately for the new and the old EPI programme. All analyses were also conducted stratified by sex using an interaction term.

In the primary analysis, follow up time was censored 6 months after enrolment due to the high incidence of subsequent vaccinations. By censoring 6 months after enrolment we limited the effect of subsequent vaccinations. However, we also conducted an analysis with follow-up for 12 months[17] and a sensitivity analysis without censoring for subsequent vaccinations.

Estimates stratified for site of enrolment (urban / rural) are presented adjusted only for age ("crude") and adjusted also for sex, season of vaccination (rainy / dry), ethnic group (Balanta, Fula, Mandinga, Manjaco, Pepel or other), morbidity on the day of enrolment (any of the following symptoms: cough, fever, diarrhoea and vomiting), maternal education (none / any), whether the mother signed the form, and stunting (length-for-age z-score<-2).

In addition to the adjusted analyses described above, we conducted sensitivity analyses using an alternative approach to control for background covariates. We calculated a propensity score for each child using logistic regression based on the baseline information available for all children (age, sex, old or new vaccination program, place of enrolment, season, whether the mother signed the enrolment form, maternal ethnicity, maternal schooling, morbidity on the day of enrolment, anthropometric measurements (mid-upper-arm-circumference, weight-for-age and length-for-age)). All variables were measured prior to or on the day of enrolment. Variables were selected based on prior knowledge and assumptions about determinants for vaccination or availability of vaccinations: Poor growth has been associated with lower vaccination incidence[27], the new vaccination programme has made vaccines more accessible through outreach[19], rural Guinea-Bissau has lower

coverage than urban Guinea-Bissau[10, 28]. We assumed that mothers with formal education and ability to sign the forms would have higher coverage and that the practice of not vaccinating ill children would lead to lower coverage among these children. We furthermore included sex because the non-specific effects differ by sex[8] and may influence subsequent vaccination seeking behaviour. We then used the estimated propensity scores in two ways. First, we performed a one-to-one match on the propensity score by matching each child in the combined live and inactivated group to one child in the live vaccine group using the Stata function psmatch2 and the nearest neighbour method. We subsequently applied a Cox proportional hazards model to the matched children with time since vaccination as the underlying timescale. We analysed the matched data both un-stratified and stratified by the propensity score matched-pairs in the Cox proportional hazards model. Balance of baseline variables between the two vaccine groups in the matched data set was assessed by standardised differences of means and proportions and graphically by inspection of quantile-quantile plots for the continuous variables[29]. We tested whether balance could be improved by including interactions. This was not the case. The median difference in propensity score was 0.009, interquartile range 0.001 to 0.133. Second, we created quintiles based on the estimated propensity scores and applied a Cox proportional hazards model with time since vaccination as the underlying timescale stratified by propensity score quintiles. Provided that we have identified the background factors associated with receiving a combination of live and inactivated vaccines together, the resulting HR estimate from the matched analysis is to be interpreted as the average causal vaccine effect in the sub-population of children with the same distribution of background factors as among the children receiving both vaccines, while the HR from the stratified model is to be interpreted as the average causal vaccine effect among children with the same distribution of background factors as among the children in our sample which represents our population[30]. All analyses were conducted using Stata version 12.1.

Ethical considerations

The protocol for the parent trial was approved by the Ministry of Health in Guinea-Bissau and the Danish Central Ethical Committee gave its consultative approval (2006-7041-99).

Role of the funding source

The sponsors had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

RESULTS

Among 3800 children in the placebo arm of the vitamin A trial, 2447 received MV at enrolment. One hundred and sixteen children had received a combination of the new and old EPI vaccines and have been excluded (Figure 1). Among the remaining 2331 children, 1043 (45%) received vaccines according to the old EPI programme; 685 (66%) MV only and 358 MV co-administered with DTP (34%). The remaining 1288 children (55%) received vaccines according to the new EPI programme; 940 children (73%) MV+YF and 348 children (27%) MV+YF+pentavalent vaccines (Figure 1). Sixteen children (0.7%) had no follow up time and have been excluded from the comparison.

Most children (78% (550/702)) who received a combination of live and inactivated vaccines were enrolled in the rural area, whereas only 40% (646/1613) of children who received live vaccines only were enrolled in the rural area. Baseline characteristics stratified for site of enrolment are presented in Table 1. Children who received live vaccines only were younger in the urban area (Table 1). A similar tendency was seen for children in the rural area who received vaccines according to the old EPI programme but not for children who received vaccines according to the new EPI. Nutritional status of children who received a combination of live and inactivated vaccines in the old EPI programme was lower than for children who received live vaccines. For the new EPI programme poorer nutritional status for those who received a combination of live and inactivated vaccines was only observed in the urban area. The maternal educational level was lower for children who received a combination of live and inactivated vaccines than for children who received live vaccines in both the old and new EPI programme and in both the urban and rural areas, also reflected in the fact that fewer mothers could write their name on the inclusion form. Almost all children were breastfed at enrolment (Table 1).

During the 12 months of follow-up 44 deaths occurred: 19 among the children receiving live vaccines only and 25 among children who received a combination of live and inactivated vaccines. Four deaths were due to accidents (attacked by a bee swarm, traffic accident, drowning and iron poisoning), all in the live+inactivated vaccine group (2 MV+DTP; 2 MV+YF+Pentavalent). Of the children vaccinated according to the old EPI schedule, 18% (182/1035) had vaccines registered within 12 months of follow-up; this figure was as high as 64% (694/1080) in the new EPI programme due to many campaigns (Figure 1, Supplementary Table 1). Four deaths occurred after registration of a subsequent vaccine (2 MV+YF; 2 MV+YF+Pentavalent) (Figure 1).

Censoring for accident deaths and subsequent vaccines, the mortality rate was 44.0 per 1000 person years (PYRS) within the 6 months after enrolment for the combined live and inactivated vaccines group and 10.5/1000 PYRS in the live vaccine only group, yielding a crude MRR of 4.16 (1.58-10.9)

when adjusting for age and stratifying by place of enrolment (Table 2, Figure 2). Adjusted also for sex, season, ethnic group, morbidity on the day of enrolment, maternal education, whether the mother signed the form and stunting, the estimate changed to 3.24 (1.20-8.73) (Table 2). The adjusted MRR was 3.71 (1.14-12.0) in girls and 2.46 (0.51-11.9) in boys. Extending the follow up period to 12 months, the adjusted MRR was 1.89 (0.89-3.89). If we did not censor for accidents, the adjusted MRRs were 4.05 (1.54-10.6) and 2.52 (1.23-5.15) during 6 and 12 months of follow-up, respectively.

Adjusted for age, mortality was approximately two times higher in the rural than in the urban area, MRR=2.14 (0.83-5.48). However, the effect of receiving live and inactivated vaccines compared with live vaccines only was similar in the urban area (MRR=2.31 (0.42-12.7)) and in the rural area (MRR=3.95 (1.07-14.5), p for same effect=0.62) (Supplementary Table 2).

Stratified by EPI programme period, the adjusted MRR for MV+DTP compared with MV only was 1.56 (0.39-6.29) with 6 months of follow-up, and 1.14 (0.44-2.95) with 12 months of follow-up (Table 2). For MV+YF+pentavalent compared with MV+YF the adjusted MRR with 6 months of follow-up was 7.73 (1.79-33.4) and 4.85 (1.46-16.2) with 12 months of follow-up (Table 2, Figure 3).

Sensitivity analyses

When conducting the analyses without censoring at registration of subsequently received vaccines an additional four deaths were included in the 12 months follow-up while there were no additional deaths for the analyses of the first 6 month. The adjusted MRRs were: 3.08 (1.16-8.19) with 6 months of follow-up and 2.02 (1.02-4.02) with 12 months of follow-up.

The use of propensity score to match children in the live and inactivated group to their peers in the live vaccine only group matched 684/702 children. Fifteen of the 18 unmatched children were not matched due to missing information in variables used to derive the propensity score. The remaining three had high propensity scores (>0.7558) and were outside the supported range. In the crude analysis on the matched pairs, receiving live and inactivated vaccines together was associated with a 3.41 (1.11-10.5) times higher mortality than receiving live vaccines only with 6 months of follow up, while it was 2.05 (0.92-4.57) with 12 months of follow up. In the matched analysis performed stratified by matched pairs, the MRR was 4.00 (1.13-14.2) with 6 months of follow up and 3.40 (1.25-9.22) with 12 months (Supplementary Table 3). When the Cox regression model was performed stratified by quintiles of propensity score on the whole sample, the MRR was 3.13 (1.14-8.60) with 6 months of follow up and 1.85 (0.88-3.88) with 12 months of follow up (Supplementary Table 3).

DISCUSSION

Main findings

Controlled for background characteristics which differed between the two groups, children who received a combination of live and inactivated vaccines had a threefold higher mortality than children who received live vaccines only. The negative effect of combined live and inactivated vaccines was also observed for pentavalent+MV+YF compared with MV+YF. Hence, the negative effect of combining live and inactivated vaccines does not seem to be limited to DTP and MV.

Strengths and weaknesses

The present study used data collected within a randomised placebo-controlled trial of vitamin A supplementation at vaccination contacts, and the sample size was limited as only the subgroup receiving placebo and measles vaccine at enrolment was used. We did not include the vitamin A group as the hypothesis should be tested in settings where no other interventions were given with DTP[17] and we have shown in many previous studies that vitamin A and vaccines interact[21, 22, 31]. The study was observational and should be interpreted with caution. However, the hypothesis that DTP administered with or after MV is associated with higher mortality than MV only was published many years ago and has been supported in several datasets[12-15, 32, 33]. In the present study vaccination status was known from the day of administration as we administered the vaccines at enrolment. The BHP staff was carefully trained and intensively supervised, thus misclassification of vaccination status is unlikely.

We have used the adjusted Cox proportional hazards model to control for background covariates. We also used the propensity score methods as an alternative approach for control for background covariates; this did not alter the findings (Supplementary Table 3). However, neither the standard Cox regression nor the propensity score analyses rule out bias from unmeasured confounding.

Few children were lost to follow-up as the study was conducted within the BHP HDSS. However, the follow-up period was limited by frequent vaccination campaigns in 2009, 2010 and 2011. Due to the 6-monthly follow up visits in the rural areas, the information on subsequent vaccinations may have been collected months after administration. This may have diluted the effect of the vaccines received at enrolment.

It has been argued that the higher mortality after DTP+MV compared with MV only is explained by differential socio-economic status[34]. However, though the effect estimate became less strong, the

negative effect was still highly significant after adjustment for all the background variables which differed between the two vaccination groups. With the new EPI programme, the negative effect of combined vaccination was particularly strong in the rural areas where there were limited socio-economic differences between the two vaccine groups. Among children vaccinated with the new vaccines, receiving MV+YF+Pentavalent vaccines may have may have had a stronger negative effect in girls than in boys. The proportion of inactivated vaccines received after enrolment may contribute to this sex-difference since DTP - and presumably also pentavalent vaccine - administered after MV has been associated with increased mortality for girls[9, 32].

Interpretation

Vaccines used by the EPI have not been evaluated for their effect on overall child mortality and the current schedule is not based on trials which have demonstrated superiority in reducing mortality of one schedule over another schedule. Co-administration of DTP and MV has been deemed safe based on antibody responses[35, 36] and adverse events[37]. Though DTP/pentavalent vaccines should be given at six, 10 and 14 weeks of age, the vaccines are often given later; later vaccination contacts, for instance in relation to measles vaccination, are seen as an opportunity to provide missing doses of antigens scheduled at an earlier age[11]. This increases coverage, but may not reduce mortality.

Data from rural Guinea-Bissau has indicated that up to one third of children receive DTP with or after measles vaccine[10]. Though this proportion may be decreasing[19] many children still receive pentavalent vaccine with MV. The present study supports that the previously observed negative effect of combining the inactivated DTP vaccine and the live MV is also present in the new vaccination programme in which pentavalent vaccines has replaced DTP and YF is given with MV at 9 months of age. Hence, the negative effect of combining inactivated and live vaccines may be a general phenomenon. The present observation indicates that a large reduction in mortality could be achieved by not providing live and inactivated vaccines together: The adjusted MRR of 3.24 (1.20-8.75) translates into a 64% (6-329%) higher mortality after the age of measles vaccination in a population like the present where 29% of follow up time is lived in the live+inactivated vaccine group.

Consistency with previous studies

The present study is in line with previous observational studies which have demonstrated higher mortality and morbidity among children who have received DTP with MV[12-15, 33, 38]. The negative effect may have been more pronounced for girls, as also found in the only randomised trial of DTP co-administered with MV[16]. In line with a study from Malawi[34] we found lower

socioeconomic status of children who received DTP/pentavalent vaccines together with MV. However, our data indicate clearly that the differential mortality is not explained merely by socioeconomic factors.

Implications

The data is consistent in showing that DTP/pentavalent vaccine given with MV compared with MV alone as the most recent vaccination is associated with increased mortality. Furthermore, the data available suggest that the third dose of DTP/pentavalent vaccine only marginally increases the protection against pertussis, Hib and hepatitis B infections[39-43]. Hence, rather than delaying pentavalent vaccines or MV when the two vaccines are both missing, the best strategy would seem to be to give only MV and drop the missing pentavalent vaccine. Public Health authorities may want to test this in a randomised trial.

Conclusion

Co-administration of live and inactivated vaccines is associated with increased mortality compared with live vaccines only. Importantly, this pattern was also present with the new EPI programme using pentavalent rather than DTP and with the addition of YF vaccine. The current vaccination programme is based on assumptions about vaccine efficacy against specific pathogens and how this translates into an effect on survival if the disease burden is high. Combined administration of antigens is deemed safe based on sero-conversion studies, but randomised trials testing the effect on overall survival have not been conducted prior to implementing the current vaccination programme. Further studies are needed to create a vaccination policy which optimises the impact of vaccines on child survival.

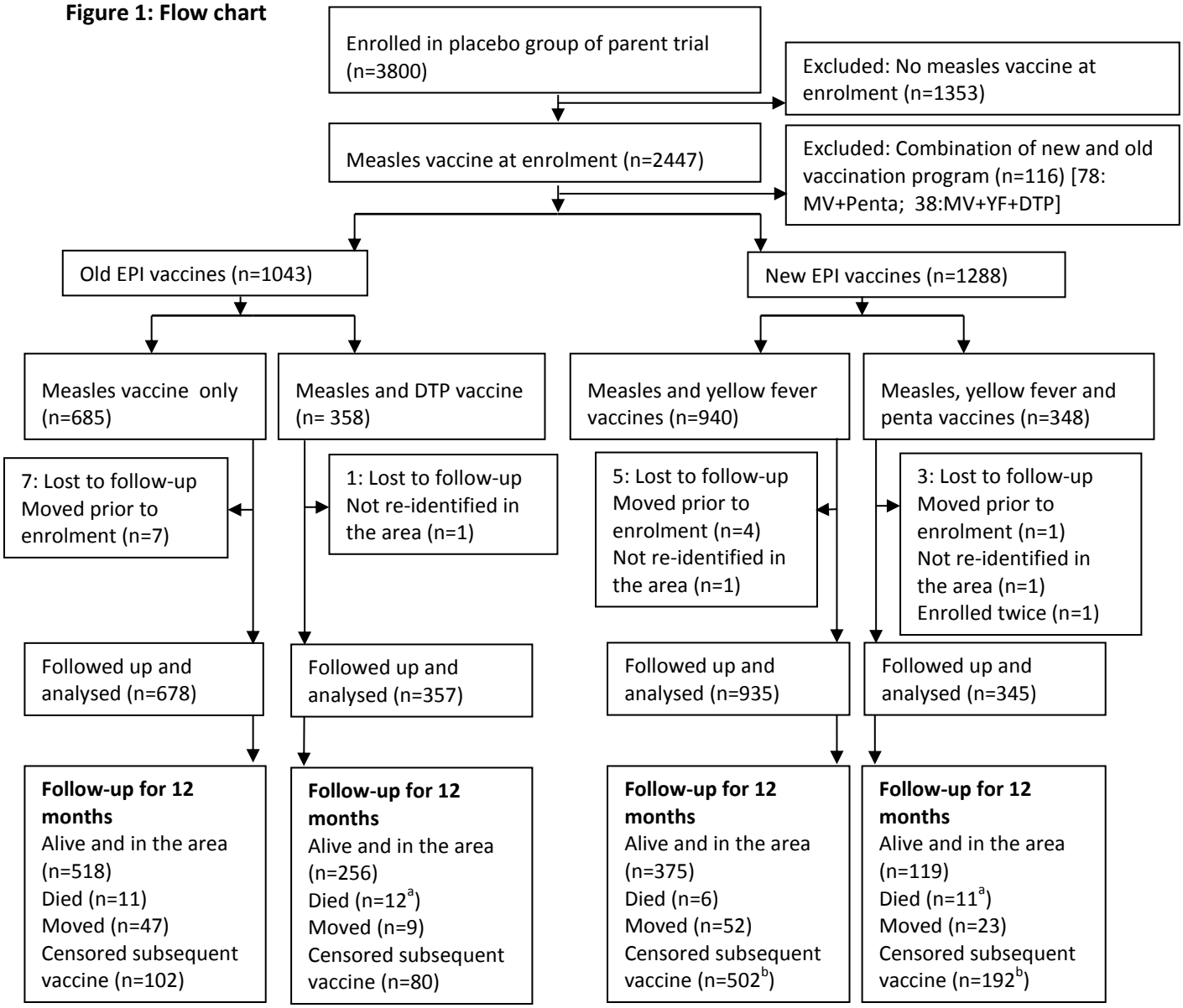
Conflicts of interest: None

Acknowledgement: We wish to thank all mothers and children participating in the trial, the dedicated staff at the BHP as well as all our collaborators at health centres, regional health departments and EPI managers. We thank Ibraima Balde, Manuel Fernandes, Mathias J Jørgensen, Linda Hornshøj, Julie Rasmussen, Emil D Christensen for their help in supervision of enrolments, data entry and follow-up.

Funding: This work was supported by European Research Council [ERC-2009-StG-243149], DANIDA [104.Dan.8-920]; European Union FP7 support for OPTIMUNISE [Health-F3-2011-261375] and the Danish Council of Independent Research [09-066317]. The Bandim Health Project received support from DANIDA and the Danish National Research Foundation via CVIVA [DNRF108]. PA holds a research professorship grant from Novo Nordisk Foundation.

Data sharing: No additional data available.

Figure 1: Flow chart



[a] Four deaths due to accidents censored (2 in the MV+DTP group, 2 in MV+YF+Penta group)

[b] Four deaths after registration of subsequent vaccines censored (2 in the MV+YF group, 2 in MV+YF+Penta group)

Figure 2: Cumulative mortality according to reception of live or live and inactivated vaccines.

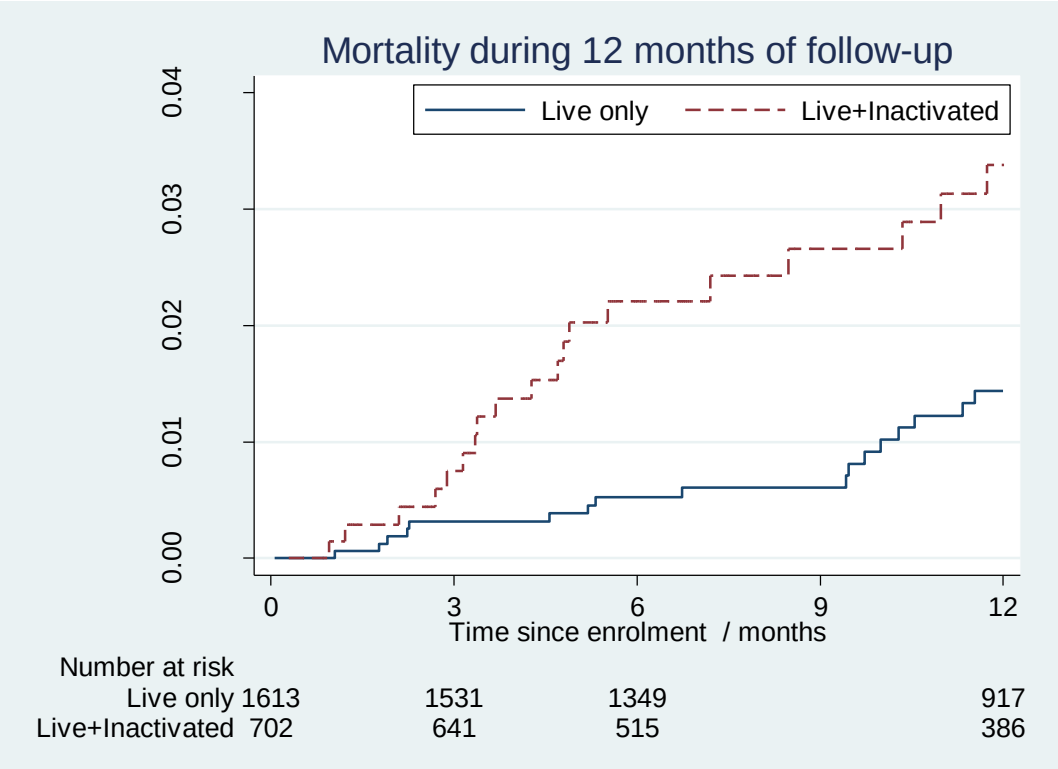


Figure 3: Cumulative mortality according to reception of MV+YF or MV+YF+Pentavalent vaccines.

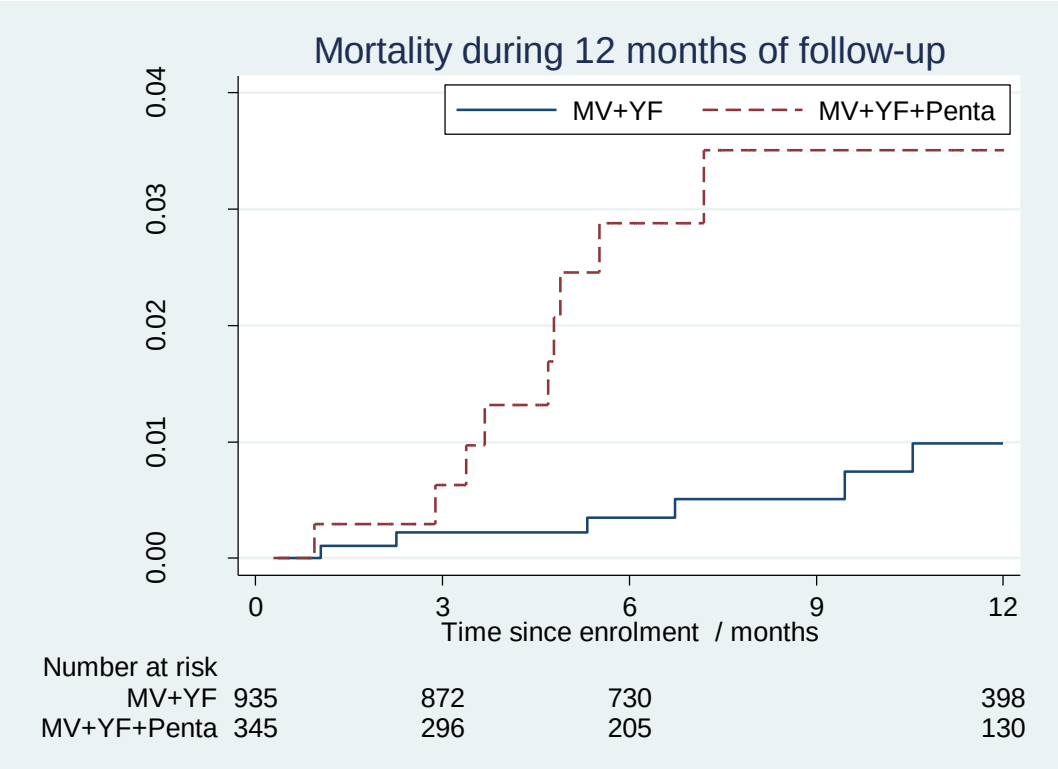


Table 1: The distribution of baseline characteristics between the live vaccine and live+inactivated vaccine groups[a]

	Old EPI programme						New EPI Programme					
	Rural			Urban			Rural			Urban		
	MV only	**MV + DTP**	**P-value[b]**	**MV only**	**MV + DTP**	**P-value[b]**	**MV + YF**	**MV + YF + Penta**	**P-value[b]**	**MV + YF**	**MV + YF + Penta**	**P-value[b]**
Number	341	328		337	29		305	222		630	123	
Age median (interquartile range) / months	13.1 (10.2-15.2)	13.6 (10.8-15.8)	0.08	10.2 (9.2-10.5)	11.1 (9.7-11.5)	0.004	12.3 (9.6-14.1)	11.4 (9.7-11.8)	0.002	10.3 (9.1-10.6)	11.5 (9.5-12.9)	<0.001
Male sex[c]	170 (50)	161 (49)	0.83	171 (51)	14 (48)	0.80	157 (51)	13 (46)	0.25	332 (53)	66 (54)	0.79
Enrolled in the dry season[c]	166 (49)	203 (62)	0.001	172 (51)	11 (40)	0.18	110 (36)	121 (55)	<0.001	209 (33)	54 (44)	0.02
Anthropometrics at enrolment												
Mean weight-for-age (SD)	-1.09 (1.26)	-1.27 (1.09)	0.05	-0.28 (1.18)	-0.76 (0.93)	0.04	-1.25 (1.23)	-1.18 (1.18)	0.51	-0.35 (1.10)	-0.57 (1.13)	<0.001
Mean length-for-age (SD)	-1.29 (1.33)	-1.58 (1.66)	0.01	-0.06 (1.25)	-0.18 (1.29)	0.64	-1.52 (1.29)	-1.50 (1.38)	0.88	-0.36 (1.28)	-0.40 (1.27)	0.71
Mean weight-for-length (SD)	-0.57 (1.28)	-0.62 (1.46)	0.63	-0.27 (1.18)	-0.87 (1.19)	0.01	-0.60 (1.26)	-0.51 (1.19)	0.41	-0.18 (1.12)	-0.50 (1.14)	0.004
Mean arm-circumference-for-age (SD)	-0.22 (1.22)	-0.35 (1.03)	0.15	0.18 (1.08)	-0.16 (1.05)	0.11	-0.49 (1.14)	-0.42 (1.07)	0.44	0.19 (1.06)	-0.04 (1.04)	0.03
Mean maternal arm-circumference mm (SD)	268 (38)	265 (30)	0.26	283 (37)	281 (37)	0.80	268 (29)	265 (27)	0.24	278 (36)	271 (36)	0.03
Breastfed at enrolment[c,d]	323 (96)	318 (98)	0.10	326 (98)	28 (100)	0.44	299 (99)	221 (100)	0.09	621 (99)	120 (99)	0.68
Morbidity on day of vaccination[c,d]												
Diarrhoea	37 (11)	65 (20)	0.001	16 (5)	4 (14)	0.04	14 (5)	22 (10)	0.02	41 (7)	10 (8)	0.46
Cough	67 (20)	78 (24)	0.18	86 (26)	9 (31)	0.52	31 (10)	33 (15)	0.10	150 (24)	34 (29)	0.27
Fever	80 (23)	106 (33)	0.009	21 (6)	3 (10)	0.39	34 (11)	23 (10)	0.78	41 (7)	15 (12)	0.03
Vomiting	22 (6)	26 (8)	0.44	6 (2)	1 (3)	0.53	8 (3)	11 (5)	0.16	16 (3)	6 (5)	0.14
Socioeconomic status												
Formal education of mother[d]	100 (30)	72 (22)	0.02	258 (79)	12 (50)	0.001	97 (33)	52 (24)	0.04	455 (79)	59 (54)	<0.001
Mother signed enrolment form	68 (20)	41 (13)	0.009	251 (75)	11 (38)	<0.001	47 (16)	19 (9)	0.02	426 (68)	57 (46)	<0.001
Ethnicity[d]												

	Balanta	76 (23)	131 (40)		28 (8)	3 (10)		75 (25)	71 (32)		63 (10)	11 (9)	
	Fula	66 (20)	54 (17)		36 (11)	9 (31)		57 (19)	28 (13)		99 (16)	34 (28)	
	Mandinga	40 (12)	25 (8)		26 (8)	4 (14)		55 (18)	40 (18)		48 (8)	13 (11)	
	Pepel	61 (18)	61 (19)		109 (32)	7 (24)		52 (17)	47 (21)		179 (28)	31 (25)	
	Manjaco/Mancanha	44 (13)	20 (6)		76 (23)	1 (3)		28 (9)	11 (5)		109 (17)	18 (15)	
	Other	46 (14)	35 (11)	<0.001	62 (18)	5 (17)	0.008	35 (12)	22 (10)	0.07	131 (21)	15 (12)	0.01
Age of mother median (years) interquartile range		25 (22-31)	27 (22-31)	0.12	26 (22-30)	27 (23-34)	0.29	26 (21-32)	27 (22-32)	0.26	26 (22-30)	26 (21-29)	0.42

[a] Values are numbers (percentages) unless stated otherwise

[b] P-value for test of no difference between groups

[c] Variables in 2 levels are presented by one level

[d] Values do not add up due to some having missing information

Table 2: Survival according to reception of live or a combination of live and inactivated vaccines

		Rate per 1000 PYRS (Deaths/PYRS)			
		Live	Live and Inactivated	Crude MRR (95% CI)[a]	Adjusted MRR (95% CI)[b]
Follow up for 6 months or registration of subsequent vaccine					
	All	10.5 (8 / 760)	44.0 (14 / 318)	4.16 (1.58-10.9)	3.24 (1.20-8.73)
	Boys	7.7 (3 / 390)	25.7 (4 / 156)	3.36 (0.71-15.9)	2.46 (0.51-11.9)
	Girls	13.5 (5 / 369)	61.7 (10 / 162)	4.53 (1.42-14.4)	3.71 (1.14-12.0)
Follow up for 6 months or registration of subsequent vaccine: Old EPI Programme: MV vs. MV+DTP					
	All	15.2 (5 / 329)	35.0 (6 / 172)	2.46 (0.62-9.68)	1.56 (0.39-6.29)
	Boys	6.1 (1 / 165)	23.6 (2 / 85)	4.16 (0.34-50.5.)	2.51 (0.20-30.9)
	Girls	24.3 (4 / 165)	46.1 (4 / 87)	2.03 (0.43-9.59)	1.31 (0.27-6.35)
Follow up for 6 months or registration of subsequent vaccine: New EPI Programme: MV+YF vs. MV+YF+Penta					
	All	7.0 (3 / 431)	54.6 (8 / 146)	7.37 (1.77-30.6)	7.73 (1.79 -33.4)
	Boys	8.9 (2 / 226)	28.2 (2 / 71)	3.00 (0.40-22.7)	2.74 (0.35-21.7)
	Girls	4.9 (1 / 205)	79.6 (6 / 75)	15.4 (1.74-137)	18.2 (1.97-168)
Follow up for 12 months or registration of subsequent vaccine					
	All	13.5 (17 / 1259)	36.1 (19 / 527)	2.17 (1.06-4.44)	1.86 (0.89-3.89)
	Boys	12.5 (8 / 639)	30.6 (8 / 261)	1.99 (0.71-5.53)	1.64 (0.57-4.67)
	Girls	14.5 (9 / 621)	41.4 (11 / 266)	2.31 (0.91-5.86)	2.05 (0.80-5.25)
Follow up for 12 months or registration of subsequent vaccine: Old EPI Programme: MV vs. MV+DTP					
	All	18.2 (11 / 606)	32.0 (10 / 313)	1.37 (0.54-3.46)	1.14 (0.44-2.95)
	Boys	13.2 (4 / 303)	31.8 (5 / 157)	1.88 (0.48-7.30)	1.57 (0.39-6.26)
	Girls	23.1 (7 / 303)	32.2 (5 / 155)	1.08 (0.52-3.60)	0.89 (0.26-3.04)
Follow up for 12 months or registration of subsequent vaccine: New EPI Program: MV+YF vs. MV+YF+Penta					
	All	9.2 (6 / 653)	42.0 (9 / 214)	4.13 (1.33-12.8)	4.85 (1.46-16.2)
	Boys	11.9 (4 / 336)	28.9 (3 / 104)	2.22 (0.46-10.6)	2.45 (0.47-12.8)
	Girls	6.3 (2 / 317)	54.4 (6 / 110)	7.66 (1.45-40.5)	9.26 (1.68-51.0)

[a]In Cox proportional hazards model with time since vaccination as underlying time scale, adjusted for age and stratified by urban / rural enrolment.

[b]In Cox proportional hazards model with time since vaccination as underlying time scale, adjusted for age and stratified by urban / rural enrolment, also adjusted for sex, season of vaccination, ethnic group, morbidity on the day of enrolment, maternal education, whether the mother signed the form and stunting.

A total of 35 children were excluded from the adjusted analysis due to missing information on stunting or ethnicity.

REFFERENCES

[1] UNICEF, World Health Organization. Immunisation Summary; A statistical reference containing data through 2009. Available at: http://www.childinfo.org/files/32775_UNICEF.pdf. 2011.
[2] Burton A, Monasch R, Lautenbach B, Gacic-Dobo M, Neill M, Karimov R, et al. WHO and UNICEF estimates of national infant immunization coverage: methods and processes. Bull World Health Organ. 2009;87:535-41.
[3] Global routine vaccination coverage, 2010. Wkly Epidemiol Rec. 2011;86:509-13.
[4] GAVI. GAVI Alliance Progress Report 2010. Available at: http://www.gavialliance.org/results/gavi-progress-reports/. 2011.
[5] Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. BMJ. 2000;321:1435-8.
[6] Roth A, Jensen H, Garly M-L, Djana Q, Martins CL, Sodemann M, et al. Low Birth Weight Infants and Calmette-Guérin Bacillus Vaccination at Birth. Pediatr Infect Dis J. 2004;23:544-50.
[7] Aaby P, Martins CL, Garly ML, Bale C, Andersen A, Rodrigues A, et al. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. BMJ. 2010;341:c6495.
[8] Aaby P, Benn CS. Non-specific and sex-differential effects of routine vaccines: what evidence is needed to take these effects into consideration in low-income countries? Human vaccines. 2011;7:120-4.
[9] Aaby P, Benn C, Nielsen J, Lisse IM, Rodrigues A, Ravn H. Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. BMJ open. 2012;2:e000707.
[10] Hornshoj L, Benn CS, Fernandes M, Rodrigues A, Aaby P, Fisker AB. Vaccination coverage and out-of-sequence vaccinations in rural Guinea-Bissau: an observational cohort study. BMJ open. 2012;2:e001509-e.
[11] Clark A, Sanderson C. Timing of children's vaccinations in 45 low-income and middle-income countries: an analysis of survey data. Lancet. 2009;373:1543-9.
[12] Aaby P, Ibrahim SA, Libman MD, 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.
[13] Aaby P, Jensen H, Walraven G. Age-specific changes in the female-male mortality ratio related to the pattern of vaccinations: an observational study from rural Gambia. Vaccine. 2006;24:4701-8.
[14] Aaby P, Vessari H, Nielsen J, Maleta K, Benn CS, Jensen H, et al. Sex differential effects of routine immunizations and childhood survival in rural Malawi. Pediatr Infect Dis J. 2006;25:721-7.
[15] Hirve S, Bavdekar A, Juvekar S, Benn CS, Nielsen J, Aaby P. Non-specific and sex-differential effects of vaccinations on child survival in rural western India. Vaccine. 2012.
[16] Agergaard J, Nante E, Poulstrup G, Nielsen J, Flanagan KL, Ostergaard L, et al. Diphtheria-tetanus-pertussis vaccine administered simultaneously with measles vaccine is associated with increased morbidity and poor growth in girls. A randomised trial from Guinea-Bissau. Vaccine. 2011;29:487-500.
[17] Fine PE, Smith PG. 'Non-specific effects of vaccines'--an important analytical insight, and call for a workshop. Trop Med Int Health. 2007;12:1-4.
[18] Aaby P, Benn CS. Assessment of childhood immunisation coverage. Lancet. 2009;373:1428.
[19] Fisker AB, Hornshoj L, Rodrigues A, Balde I, Fernandes M, Benn CS, et al. The new decade of vaccines: Improving DTP-3 coverage and reducing coverage for measles vaccine? An observational study of the introduction of new vaccines in Guinea-Bissau. 2013.
[20] GAVI. Proposal for NVS - YF, Penta support: Guinea-Bissau. Approved by GAVI on 01-08-2007. Available at: http://www.gavialliance.org/country/guinea-bissau/documents/#approvedproposal. GAVI alliance; 2007.
[21] 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.
[22] Fisker AB, Bale C, Rodrigues A, Balde I, Fernandes M, Jorgensen MJ, et al. A randomised trial of high-dose vitamin A at vaccination contacts after 6 months of age (Submitted). 2013.

[23] Fisker AB, Bale C, Jorgensen MJ, Balde I, Hornshoj L, Bibby BM, et al. High-dose vitamin A supplementation administered with vaccinations after 6 months of age: Sex-differential adverse reactions and morbidity. Vaccine. 2013;31:3191-8.
[24] World Health Organization. Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age:Methods and development. Geneva: World Health Organisation; 2006.
[25] World Health Organization. WHO Anthro (version 3.1, June 2010) and macros. 2010.
[26] Jensen H, Benn CS, Lisse IM, Rodrigues A, Andersen PK, Aaby P. Survival bias in observational studies of the impact of routine immunizations on childhood survival. Trop Med Int Health. 2007;12:5-14.
[27] Aaby P, Ravn H, Roth A, Rodrigues A, Lisse IM, Diness BR, et al. Early diphtheria-tetanus-pertussis vaccination associated with higher female mortality and no difference in male mortality in a cohort of low birthweight children: an observational study within a randomised trial. Arch Dis Child. 2012;97:685-91.
[28] Bale C, Garly ML, Martins C, Nielsen J, Whittle H, Aaby P. Risk factors for measles in young infants in an urban African area with high measles vaccination coverage. Pediatr Infect Dis J. 2011;30:689-93.
[29] Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med. 2009;28:3083-107.
[30] Williamson E, Morley R, Lucas A, Carpenter J. Propensity scores: from naive enthusiasm to intuitive understanding. Statistical methods in medical research. 2012;21:273-93.
[31] Benn CS, Aaby P, Nielsen J, Binka FN, Ross DA. Does vitamin A supplementation interact with routine vaccinations? An analysis of the Ghana Vitamin A Supplementation Trial. Am J Clin Nutr. 2009;90:629-39.
[32] Aaby P, Jensen H, Samb B, Cisse B, Sodemann M, Jakobsen M, et al. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. Lancet. 2003;361:2183-8.
[33] Aaby P, Biai S, Veirum JE, Sodemann M, Lisse I, Garly ML, et al. DTP with or after measles vaccination is associated with increased in-hospital mortality in Guinea-Bissau. Vaccine. 2007;25:1265-9.
[34] Jahn A, Floyd S, Mwinuka V, Mwafilaso J, Mwagomba D, Mkisi RE, et al. Ascertainment of childhood vaccination histories in northern Malawi. Trop Med Int Health. 2008;13:129-38.
[35] Marshall R, Habicht JP, Landrigan PJ, Foege WH, Delgado H. Effectiveness of measles vaccine given simultaneously with DTP. The Journal of tropical pediatrics and environmental child health. 1974;20:126-9.
[36] McBean AM, Gateff C, Manclark CR, Foster SO. Simultaneous administration of live attenuated measles vaccine with DTP vaccine. Pediatrics. 1978;62:288-93.
[37] Deforest A, Long SS, Lischner HW, Girone JA, Clark JL, Srinivasan R, et al. Simultaneous administration of measles-mumps-rubella vaccine with booster doses of diphtheria-tetanus-pertussis and poliovirus vaccines. Pediatrics. 1988;81:237-46.
[38] Biai S, Rodrigues A, Nielsen J, Sodemann M, Aaby P. Vaccination status and sequence of vaccinations as risk factors for hospitalisation among outpatients in a high mortality country. Vaccine. 2011;29:3662-9.
[39] van der Sande MA, Mendy M, Waight P, Doherty C, McConkey SJ, Hall AJ, et al. Similar long-term vaccine efficacy of two versus three doses of HBV vaccine in early life. Vaccine. 2007;25:1509-12.
[40] Griffiths UK, Clark A, Gessner B, Miners A, Sanderson C, Sedyaningsih ER, et al. Dose-specific efficacy of Haemophilus influenzae type b conjugate vaccines: a systematic review and meta-analysis of controlled clinical trials. Epidemiology and infection. 2012;140:1343-55.
[41] Bisgard KM, Rhodes P, Connelly BL, Bi D, Hahn C, Patrick S, et al. Pertussis vaccine effectiveness among children 6 to 59 months of age in the United States, 1998-2001. Pediatrics. 2005;116:e285-94.
[42] Muller AS, Leeuwenburg J, Voorhoeve AM. Pertussis in a rural area of Kenya: epidemiology and results of a vaccine trial. Bull World Health Organ. 1984;62:899-908.
[43] Nielsen AM, Larsen SO, Zoffmann H. [Whooping cough in Denmark among children under 1 year of age during 1980-1986]. Ugeskrift for laeger. 1991;153:1482-5.

Supplementary table 1: Censoring due to vaccines received during 12 months of follow-up

			n	Received one or more vaccines during follow-up n (%)	Routine vaccines			Campaign vaccines		
					MV	DTP / penta	YF	MV	OPV	H1N1
Old EPI programme	Rural	MV only	341	66 (19)	0	13	12	33	17	0
		MV+DTP	328	72 (22)	2	28	20	37	1	0
	Urban	MV only	337	36 (11)	0	13	18	3	2	0
		MV+DTP	29	8 (28)	0	7	0	1	0	0
New EPI programme	Rural	MV + YF	305	185 (61)	0	5	0	28	107	48
		MV+YF+Penta	222	122 (55)	0	20	0	40	48	28
	Urban	MV + YF	630	317 (50)	0	5	0	106	206	0
		MV+YF+Penta	123	70 (57)	0	32	0	22	16	0

Supplementary table 2: Survival according to reception of live or a combination of live and inactivated vaccines in the urban and rural areas

		Rate per 1000 PYRS (Deaths/PYRS)			
		Live	Live and Inactivated	Crude MRR (95% CI)[a]	Adjusted MRR (95% CI) [b]
Follow up for 6 months or registration of subsequent vaccine					
	All	10.5 (8 / 760)	44.0 (14 / 318)	4.16 (1.58-10.9)	3.24 (1.20-8.73)
	Urban	11.2 (5 / 448)	37.1 (2 / 54)	3.33 (0.64-17.31)	2.31(0.42-12.7)
	Rural	9.6 (3 / 312)	45.4 (12 / 264)	4.74 (1.34-16.8)	3.95 (1.07-14.5)
Follow up for 6 months or registration of subsequent vaccine: Old EPI Program: MV vs. MV+DTP					
	All	15.2 (5 / 329)	35.0 (6 / 172)	2.46 (0.62-9.68)	1.56 (0.39-6.29)
	Urban	12.4 (2 / 162)	90.4 (1 / 11)	7.43 (0.67-82.9)	4.86 (0.32-74.4)
	Rural	17.9 (3 / 168)	31.1 (5 / 161)	1.79 (0.43-7.50)	1.15 (0.26-5.07)
Follow up for 6 months or registration of subsequent vaccine: New EPI Program: MV+YF vs. MV+YF+Penta					
	All	7.0 (3 / 431)	54.6 (8 / 146)	7.37 (1.77-30.6)	7.73 (1.86-33.4)
	Urban	10.5 (3 / 286)	23.3 (1 / 43)	2.13 (0.23-21.8)	1.82 (0.17-19.3)
	Rural	0.0 (0 / 145)	67.6 (7 / 104)	p=0.002	NA
Follow up for 12 months or registration of subsequent vaccine					
	All	13.5 (17 / 1259)	36.1 (19 / 527)	2.17 (1.06-4.44)	1.86 (0.89-3.89)
	Urban	10.7 (8 / 750)	24.0 (2 / 83)	2.19 (0.47-10.4)	1.75 (0.36-8.56)
	Rural	17.7 (9 / 510)	38.3 (17 / 443)	2.16 (0.96-9.45)	1.89 (0.82-4.35)
Follow up for 12 months or registration of subsequent vaccine: Old EPI Programme: MV vs. MV+DTP					
	All	18.2 (11 / 606)	32.0 (10 / 313)	1.37 (0.54-3.46)	1.14 (0.44-2.95)
	Urban	9.9 (3 / 303)	51.4 (1 / 20)	4.91 (0.51-47.6)	3.32 (0.29-37.8)
	Rural	26.4 (8 / 303)	30.7 (9 / 293)	1.17 (0.45-3.04)	0.99 (0.37-2.67)
Follow up for 12 months or registration of subsequent vaccine: New EPI Program: MV+YF vs. MV+YF+Penta					
	All	9.2 (6 / 653)	42.0 (9 / 214)	4.13 (1.33-12.8)	4.85(1.46-16.2)
	Urban	11.2 (5 / 446)	15.7 (1 / 64)	1.44 (0.17-12.5)	1.47 (0.16-13.8)
	Rural	4.8 (1 / 207)	53.2 (8 / 150)	10.48 (1.30-84.2)	13.3 (1.59-112)

[a] In Cox proportional hazards model with time since vaccination as underlying time scale, adjusted for age and stratified by urban / rural enrolment.

[b] In Cox proportional hazards models with time since vaccination as underlying time, stratified by urban / rural enrolment and adjusted for age, sex, season of vaccination, ethnic group, morbidity on the day of enrolment, maternal education, whether the mother signed the enrolment form and stunting.

Supplementary Table 3: Results of sensitivity analyses using propensity score[a] methods to control for confounding

	Adjusted Cox model[b]	Matched by Propensity Score[c]	Matched by Propensity Score, stratified by pair[d]	Stratified by Propensity Score quintile[e]
Follow up for 6 months or registration of subsequent vaccine				
Mortality rate ratio (95%CI)	3.24 (1.20-8.71)	3.41 (1.11-10.5)	4.00 (1.13-14.2)	3.13 (1.14-8.60)
N	2280	1368	1368	2263
Deaths	22	17	17	22
PYRS	1062	634	634	1055
Follow up for 12 months or registration of subsequent vaccine				
Mortality rate ratio (95%CI)	1.86 (0.89-3.89)	2.05 (0.92-4.57)	3.40 (1.25-9.22)	1.85 (0.88-3.88)
N	2280	1368	1368	2263
Deaths	36	27	27	36
PYRS	1758	1041	1041	1747

a) A propensity score was calculated on the following baseline information: age, sex, old or new vaccination programme, place of enrolment, season, whether the mother signed the enrolment form, maternal ethnicity, maternal schooling, morbidity on the day of enrolment, anthropometric measurements (mid-upper-arm-circumference, weight-for-age and length-for-age).

b) Using a Cox proportional hazards model with time since vaccination as underlying time scale, adjusted for age and stratified by urban / rural enrolment, also adjusted for sex, season of vaccination, ethnic group, morbidity on the day of enrolment, maternal education, whether the mother signed the form and stunting to compare mortality.

c) Using propensity scores to match children in the live and inactivated group one-to-one to children in the live vaccine only group and using a Cox proportional hazards model with time since vaccination as underlying time scale to compare mortality in the matched sample.

d) Using propensity scores to match children in the live and inactivated group one-to-one to children in the live vaccine only group and using a Cox proportional hazards model time since vaccination as underlying time scale stratified by sample pair to compare mortality in the matched sample.

e) Using quintiles of propensity score as a stratifying variable in a Cox proportional hazards model with time since vaccination as underlying time scale to compare mortality.