



**The effect of vitamin A provided in campaign may depend on vaccination status: Observational study from Guinea-Bissau**

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2011-000448
Article Type:	Research
Date Submitted by the Author:	15-Oct-2011
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<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Epidemiology, Global health, Paediatrics, Public health, Nutrition & metabolism
Keywords:	EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES, Community child health < PAEDIATRICS, Paediatric infectious disease & immunisation < PAEDIATRICS, PUBLIC HEALTH

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3 **The effect of vitamin A provided in campaign may depend on vaccination status:**  
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5 **Observational study from Guinea-Bissau**  
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55 **Words:** 3782  
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**Abstract**

**Objective:** Vitamin A supplementation (VAS) is estimated to reduce all cause mortality by 24%. Previous studies indicate that the effect of VAS may vary with vaccination status.

We evaluated the effect of VAS provided in campaigns on child survival, overall and by sex and vaccination status at the time of supplementation.

**Design:** Observational cohort study

**Setting and Participants:** The study was conducted in the urban study area of the Bandim Health Project in Guinea-Bissau. We documented participation or non-participation in two national vitamin A campaigns in December 2007 and July 2008 for children between 6-35 months of age. Vaccination status was ascertained by inspection of vaccination cards. All children were followed prospectively.

**Outcome measures:** Mortality rates for supplemented and non-supplemented children were compared in Cox-models providing mortality rate ratios (MRR).

**Results:** We obtained information from 93% of 5567 children in 2007 and 90% of 5799 children in 2008. The VAS coverage was 58% in 2007 and 68% in 2008. Mortality in the supplemented group was 1.5% (44 deaths/2873 PYRS) and 1.6% (20 deaths/1260 PYRS) in the non-supplemented group (adjusted MRR=0.78 (0.46;1.34)). The effect was similar in boys and girls. Vaccination cards were seen for 86% in 2007 and 84% in 2008. The effect of VAS in children who had measles vaccine as their last vaccine (2814 children, adjusted MRR=0.34 (0.14;0.85)) differed from the effect in children who had diphtheria-tetanus-pertussis (DTP) vaccine as their last vaccine (3680 children, adjusted MRR=1.29 (0.52;3.22),  $p=0.04$  for interaction).

**Conclusion:** The effect of VAS differed by most recent vaccination, being beneficial after measles vaccine but not after DTP.

**Words:** 262

## Article summary

### Article focus

- Vitamin A supplementation (VAS) is estimated to reduce all cause mortality by 24%.
- The effect of VAS may vary with vaccination status, being beneficial with or after measles vaccine (MV) but not after diphtheria-tetanus-pertussis (DTP) vaccine.

### Key messages

- The effect of VAS is heterogeneous
- The effect of VAS varied with vaccination status: supplemented children had lower mortality than non-supplemented children when MV was the most recent vaccine, but not when DTP was the most recent vaccine.
- The effect of VAS tended to differ by season of supplementation.

### Strengths and limitations

Information was collected on the individual level, and the children were followed prospectively. Due to the observational nature of the study the comparison of supplemented and non-supplemented children should be interpreted with caution. However, a selection bias is unlikely to have worked in different directions for children who had DTP and MV as the most recent vaccine.

## Introduction

Vitamin A supplementation (VAS) is estimated to reduce all cause mortality by 24% when provided to children aged 6-59 months at 4-6 monthly intervals(1). However, a recent cluster randomised trial involving more than 1 million children in India failed to demonstrate a survival benefit in children randomised to vitamin A(2).

We have hypothesised that the effect of VAS varies with vaccination status because VAS amplifies the non-specific immunological effects of vaccines(3). Hence, VAS is beneficial in the time window when BCG (recommended at birth) and measles vaccine (MV, recommended at 9 months of age) are the most recent vaccines, but not while diphtheria-tetanus-pertussis vaccine (DTP, recommended at 6, 10, and 14 weeks of age) is the most recent vaccine(3). The hypothesis provides an explanation of the lack of a beneficial effect of VAS in children aged 1-5 months(4;5), the age group in which DTP is the predominant vaccine. The hypothesis also offers an explanation of why the large mortality reduction reported by the meta-analysis of the trials between 1986 and 1993(1) may no longer be reproducible; these trials were conducted before the Expanded Programme on Immunizations (EPI) reached high coverage(6). We recently reanalysed data from the 1989-91 VAS trial in Ghana which had reported an overall mortality reduction of 19% (2%;32%)(7). The reanalysis revealed that the survival advantage was limited to children with no health card (a proxy for being unvaccinated), and there were indications that this could be due to a harmful effect of VAS and DTP in girls(8).

WHO recommends vitamin A for all children aged 6-59 months distributed in campaigns or at vaccination contacts in areas where vitamin A deficiency is a public health problem(9;10). In Guinea-Bissau VAS is distributed in biannual campaigns, often linked to other interventions

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2  
3 such as distribution of mebendazole, oral polio vaccine (OPV) or other vaccines(11). Two  
4  
5 different strategies have been employed in the urban area: *fixed post campaigns* where the  
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7 mother brings her child to the campaign team and *door-to-door campaigns* where all houses  
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9 are visited by the campaign team. The fixed post campaigns require less staff, but have lower  
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11 coverage. In December 2007 and June/July 2008 fixed post campaigns distributing vitamin A  
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13 and mebendazole were conducted. We registered all children receiving VAS and  
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15 mebendazole during these campaigns and assessed survival prospectively to compare survival  
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17 in supplemented and non-supplemented children overall and by sex and vaccination status.  
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## 24 **Subjects and Methods**

### 25 *Setting*

26  
27 The Bandim Health Project (BHP) operates a health and demographic surveillance system  
28  
29 (HDSS) covering a population of 102,000 people in six suburban districts of Bissau, the  
30  
31 capital of Guinea-Bissau, West Africa. All households are visited monthly to register new  
32  
33 births, pregnancies and deaths. Children below the age of 3 are followed through 3-monthly  
34  
35 visits to ascertain vital status and vaccination status. At the first visit after birth information  
36  
37 on socio-economic status is collected. The indicators include maternal characteristics  
38  
39 (education and ethnicity) and household characteristics (type of roofing, availability of  
40  
41 bathroom and electricity). All children aged 6-35 months living in the urban study area on the  
42  
43 first day of the VAS campaigns in 2007 and 2008 were eligible for the present study. Since  
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45 children below 3 years of age are followed intensively through the HDSS, these children have  
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47 been the focus for the present study.  
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### 54 *Campaign information*

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3 National VAS campaigns were conducted by the Ministry of Health from 14-18 December  
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5 2007 and 30 June-4 July 2008. Children aged 6-11 months received 100,000 IU vitamin A,  
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7 children aged 12-35 months received 200,000 IU vitamin A and 250 mg mebendazole, and  
8  
9 children aged 36-59 months received 200,000 IU vitamin A and 500 mg mebendazole.  
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12 Trained BHP field assistants were present at all posts. The field assistants brought a list of all  
13  
14 children registered in the area and noted on this list whether a child had received VAS and  
15  
16 mebendazole at the post. The lists contained information on vaccines registered prior to the  
17  
18 campaign. If the vaccination card was seen, the assistant noted this on the list, verified the  
19  
20 already registered vaccines and updated the information if new vaccines had been received.  
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27 During the weeks after the campaigns all children who had not been seen during the  
28  
29 campaigns were visited. The caretaker was asked whether the child had received VAS  
30  
31 elsewhere. The vaccination card was seen and information on vaccines verified and updated.  
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33 If the child and caretaker were not present the household was visited up to three times: if no  
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35 one could provide information, the campaign status was classified as unknown.  
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41 During the 2007-campaign we discovered that the vitamin A capsules given in two of the six  
42  
43 districts had passed the expiry date one month before. These were immediately replaced by a  
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45 new batch. We sent two of the expired capsules to “as Vitas Oslo Innovation Center” (Oslo,  
46  
47 Norway) to have their vitamin A content measured. The vitamin A contents of the two  
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49 200,000 IU capsules were slightly lower than expected: 167,569 IU and 154,863 IU. Capsules  
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51 used in the 2008-campaign were all from a new stock.  
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57 *Vaccine information*  
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3 The vaccination schedule in Guinea-Bissau when this study was conducted was BCG and  
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5 OPV at birth, 3 doses of DTP vaccines at 6, 10 and 14 weeks of age and MV at 9 months of  
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7 age. A booster DTP vaccine at 18 months of age was given in the routine vaccination program  
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9 until March 2007. In August 2008 the DTP vaccine at 6, 10 and 14 weeks was replaced by the  
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11 pentavalent (DTP-HiB-HBV) vaccine and a yellow fever vaccine (YF) was added to be given  
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13 with MV at 9 months of age.  
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20 During the study period three trials of alternative vaccination strategies took place in the BHP  
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22 area. First, between October 2005 and October 2009, Trial A enrolled children aged 18  
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24 months in a randomised trial of booster DTP. Children who had completed their primary  
25  
26 immunisation schedule of 3 doses of DTP and a MV were randomised to receive booster  
27  
28 DTP+OPV or OPV only. Second, between August 2003 and April 2007, Trial B enrolled  
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30 children aged 4½ months in a randomised trial of early MV. Children were randomised to an  
31  
32 extra dose of MV at 4½ months or no vaccine; a subgroup of the controls received an extra  
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34 measles vaccine at 18 months of age(12). Third, between October 2005 and April 2008, Trial  
35  
36 C enrolled children aged at least 9 months who were delayed in relation to the vaccination  
37  
38 schedule and presented at the health centres to receive MV and the third DTP vaccine. They  
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40 were randomised to receive both vaccines and booster DTP at 18 months of age versus MV  
41  
42 only and no booster DTP(13). Furthermore, in May 2006 a measles vaccination campaign was  
43  
44 conducted in which children aged 6 months to 14 years were given a measles vaccine; thus  
45  
46 the oldest children in this study often have a MV received in this campaign as their most  
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48 recent vaccine.  
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57 For the present study, vaccine status was ascertained during the campaigns. Furthermore, as  
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59 part of the BHP routines, vaccines are registered daily at the 3 health centres in the study-area  
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3 and through the 3-monthly home visits, and as a part of the trials described above. We linked  
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5 the campaign information with information from the above sources, using only information  
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7 collected prior to the campaign. In case of discrepancy, information from the trials was  
8  
9 considered superior. If no trial information was available and if two of the other sources  
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11 agreed, this information was accepted. Vaccination information obtained during the campaign  
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13 was updated for 12% (1088/8812) of the children; 9% based on data collected at the health  
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15 centres, 1% based on routine surveillance data, and 2% based on trial data. Apart from in  
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17 Trial A, OPV is almost always given with BCG or DTP. Children who received OPV along  
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19 with another vaccine were classified according to the co-administered vaccine, i.e. DTP+OPV  
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21 and DTP were classified as DTP.  
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### 29 *Follow-up*

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31 All children below 3 years of age were followed by the HDSS. For children who died, a  
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33 locally adapted version of the INDEPTH verbal autopsy(14) was conducted. Cause of death  
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35 was determined by a local paediatrician.  
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### 41 *Statistical analyses*

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43 Children entered the analysis on the day we knew their VAS-campaign status (supplemented,  
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45 non-supplemented or no information). Thus, a child who was registered to have participated  
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47 in the campaign during the days of the campaign contributed time at risk from the day of  
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49 supplementation. A child who had campaign status registered after the campaign contributed  
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51 time from the day the information was obtained. Follow-up of children was censored when a  
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53 subsequent campaign was initiated (30 June 2008 for the 2007-campaign, 9 January 2009 for  
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55 the 2008-campaign), three years of age, migration or reception of VAS in a trial, whichever  
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57 came first. Some children were enrolled in a randomised trial of VAS at vaccination contacts  
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3 after 6 months of age (Trial registration: [clinicaltrials.gov](http://clinicaltrials.gov), NCT00514891), these children  
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5 were censored from follow-up on the date of receiving VAS in the trial (Figure 1).  
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10 We examined whether the VAS coverage varied by background factors by chi2 tests for  
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12 categorical variables and ranksum test for age at the time of the campaign. All three groups  
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14 (VAS, no VAS and no information) as well as the two main groups (VAS and no VAS) were  
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16 compared.  
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22 Survival was examined in Cox proportional hazards models with age as underlying time; thus  
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24 age was inherently controlled for in all models. The proportional hazards assumption was  
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26 tested using Schoenfeld residuals. Crude and adjusted estimates are presented. A priori we  
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28 had decided to adjust all estimates for sex and campaign, since the 2007-campaign took place  
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30 in the rainy season and the 2008-campaign took place in the dry season, and sex and season of  
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32 supplementation have been shown to be important determinants of the effect of VAS in our  
33  
34 previous studies(8;15;16). We would furthermore adjust the comparison of supplemented and  
35  
36 non-supplemented children for the background factors which were associated with  
37  
38 participation in the VAS campaigns. In all analyses we investigated whether the survival of  
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40 supplemented and non-supplemented children varied by sex and campaign (season). We  
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42 furthermore investigated interactions with vaccination status. Interaction between VAS and  
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44 potential effect modifies was evaluated by Wald statistics. Among children who had OPV as  
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46 the most recent vaccine, no deaths occurred among non-supplemented children. Therefore, the  
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48 potential interaction between VAS and OPV was tested by Mantel-Haenszel stratification.  
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## 56 57 **Results** 58 59 60

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3 In the 2007-campaign we followed 5567 (92%) of the 6026 children aged 6-35 months  
4 registered in the study area (Figure 1). For 7% (367/5567) we obtained no information on  
5 campaign status, since the family was travelling at all visits. Among the remaining 5200  
6 children, 3009 (58%) had received VAS and 2191 (42%) had not. In the 2008-campaign, we  
7 followed 5799 (93%) of 6210 children. For 10% (596/5799) we were unable to obtain  
8 information on campaign status. Among the remaining 5203 children, 3520 (68%) had  
9 received VAS and 1683 (32%) had not (Figure 1).  
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22 The distribution of background factors is shown in Table 1. Children for whom we had no  
23 information on campaign status had lower socioeconomic status, were more likely to be from  
24 the Muslim ethnic groups (Fula and Mandinga) and were younger. These children also tended  
25 to have higher mortality, crude MRR=2.17 (0.96; 4.94) (Table 2). In contrast, there were only  
26 few differences in background factors between supplemented and non-supplemented children.  
27 Non-supplemented children were more likely to have mothers with no formal education  
28 ( $p<0.001$ ) and to belong to the Muslim ethnic groups ( $p<0.001$ ) and more supplemented  
29 children had their vaccination card inspected ( $p<0.001$ ). Furthermore, more supplemented  
30 children were enrolled in trials and as a result more supplemented children had received OPV  
31 as the most recent vaccine in 2007 ( $p=0.05$ ). In 2008 the distribution of different vaccines did  
32 not differ between supplemented and non-supplemented children ( $p=0.23$ ) (Table 1).  
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### 50 *Effect of VAS*

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52 There was no significant difference in mortality between supplemented and non-  
53 supplemented children, the crude MRR being 0.93 (0.55;1.58). Adjusted for sex, campaign,  
54 maternal education, ethnicity and inspected vaccination card, the difference in mortality  
55 between supplemented and non-supplemented children was 0.78 (0.46;1.34) (Table 2). The  
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3 effect was similar in children aged 6-11 months who received 100,000 IU vitamin A (adjusted  
4 MRR=1.14 (0.41;3.19)) and children above 12 months who received 200,000 IU vitamin A  
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6 and 250 mg mebendazole (adjusted MRR=0.69 (0.36;1.29)) (p=0.41, test of interaction)  
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10 (Table 2). The effect did not differ for boys and girls (p=0.72).  
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15 Mortality was much higher in the rainy season after the 2008-campaign than in the dry season  
16 after the 2007-campaign; the adjusted MRR was 3.79 (2.13;6.74) comparing 2008 versus  
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18 2007. The difference between supplemented and non-supplemented tended to differ between  
19  
20 the two campaigns; the adjusted MRR was 0.38 (0.13;1.10) after the 2007-campaign and 1.02  
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22 (0.53;1.96) after the 2008-campaign (p=0.12, test of interaction). This difference may have  
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24 been strongest for girls; the adjusted MRR was 0.16 (0.02;1.59) after the 2007 campaign and  
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26 1.32 (0.44;3.94) after the 2008-campaign (p=0.11, test of interaction), whereas there was no  
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28 evidence of a difference in boys (p=0.50) (Table 2). Further adjusting for last vaccine at the  
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30 time of the campaign did not change the conclusions.  
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39 The difference between supplemented and non-supplemented children varied with maternal  
40 education. Supplementation was associated with lower mortality in children of educated  
41 mothers (adjusted MRR=0.41 (0.21;0.80)), but not in children of mothers with no schooling  
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43 (adjusted MRR=1.78 (0.60;5.31)) (p=0.02, test of interaction). The effect did not vary with  
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45 the other socioeconomic indicators presented in Table 1 (data not shown).  
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### 53 *Effect of VAS by vaccination status*

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55 Vaccination cards were seen for 86% of the children in 2007 and 84% in 2008, with more  
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57 cards seen for supplemented compared with non-supplemented children (2007: 90% vs. 81%,  
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3 p<0.001; 2008: 88% vs. 75%, p<0.001, Table 1); 9% (6/64) of the deaths in the study were  
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6 excluded due to no information on vaccination status.  
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10 The difference in mortality between supplemented and non-supplemented children varied  
11  
12 with vaccination status. In the 2814 children who had received MV as the most recent vaccine  
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14 prior to the campaign the adjusted MRR was 0.34 (0.14;0.85). In the 3680 children who had  
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16 received DTP as the most recent vaccine the adjusted MRR was 1.29 (0.52;3.22) (p=0.04 for  
17  
18 different effect in MV and DTP recipients). The pattern was similar in boys and girls (Table  
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20 3). The small group of non-supplemented children with OPV as the most recent vaccine had  
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22 lower mortality than all other groups (Table 3), resulting in a significant difference between  
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24 the effect of VAS in MV and OPV recipients (p=0.02).  
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### 31 *Cause of death*

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33 We aimed to examine the effect of VAS on all cause mortality. However, from May 2008 to  
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35 January 2009 a cholera epidemic occurred(17). An epidemic could potentially explain the  
36  
37 higher mortality after the 2008-campaign. Verbal autopsies were conducted for 66 of the 72  
38  
39 deaths, for the remaining six the family had moved prior to the interview. One death which  
40  
41 occurred after receiving VAS in 2008 was classified as due to cholera. Further 10 deaths  
42  
43 (15%) were due to diarrhoeal disease, 1 after the 2007-campaign (no VAS) and 9 after the  
44  
45 2008-campaign (8 VAS, 1 no VAS). The peak of the epidemic occurred in September  
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47 2008(17); five diarrhoeal deaths in 2008 occurred in August and September (all VAS) and  
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49 four deaths (3 VAS, 1 no VAS) occurred in the late epidemic in November and December  
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51 where few cholera cases were detected. The other causes of death were: prolonged disease  
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53 with failure to thrive and anaemia: 18 (27%, 14 VAS, 3 no VAS, 1 no information),  
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58 respiratory infection: 17 (26%, 9 VAS, 6 no VAS, 2 no information), malaria: 14 (21%, 5  
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3 VAS, 7 no VAS, 2 no information), fever of unknown origin: 3 (5%, 2 VAS, 1 no VAS),  
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5 cerebral palsy: 2 (3%, 1 VAS, 1 no VAS) and accident: 1 (2%, VAS). There were  
6  
7 significantly more malaria deaths among the non-supplemented and the children with no  
8  
9 information, the MRRs being 3.26 (1.03;10.3) and 5.43 (1.05;28.2), presumably reflecting  
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11 that they were less likely seek early treatment or to be absent in the rural areas where the  
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13 incidence of severe and untreated malaria would be higher.  
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## 20 Discussion

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22 Based on the marked mortality reductions seen in trial in the 1980s and 1990s, VAS has  
23  
24 become a high priority intervention, expected to reduce overall child mortality. This has been  
25  
26 challenged by a recent trial which found no effect of VAS in 1 million Indian children(2). In  
27  
28 our study, children receiving VAS in a campaign had 22% (-34%;54%) lower mortality  
29  
30 compared with non-supplemented children in the adjusted analysis. However, this is unlikely  
31  
32 to represent the true effect of VAS since there are many reasons that non-supplemented  
33  
34 children may have had higher mortality, e.g. selection bias, travelling to the rural areas, less  
35  
36 access to care and a higher risk of dying of malaria. More important, our observational study  
37  
38 identified differential effects of VAS by season and vaccination status; for example,  
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40 supplemented children did not have lower mortality than non-supplemented children in the  
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42 rainy season and among children who had DTP as the most recent vaccination.  
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### 50 *Strengths and weaknesses*

51  
52 The strengths of our study include that information was collected on the individual level, and  
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54 that the children were followed prospectively. By only allowing the children to contribute  
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56 observation time in the VAS or no VAS group from the day the information was obtained, we  
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3 avoided introducing survival bias in the analysis (i.e. obtaining positive information only from  
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5  
6 surviving children)(18).  
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10 Vitamin A content was assessed in the expired capsules which some campaign recipients  
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12 received in 2007. Though the content was lower than the original 200,000 IU, the content was  
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14 still high. Hence, we believe that all supplemented children received a relevant dose of  
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vitamin A.

Mortality was higher in children for whom we do not have information on the campaign  
participation, and also a substantial number of children died prior to us getting the  
information on their participation in the VAS campaign. The main reason for not getting any  
information was that the family had travelled to the rural areas, where mortality is higher(19).  
According to national data VAS coverage was considerably higher in the rural areas (Personal  
communication, Sidu Biai, WHO, Bissau). Hence, the children for whom we did not obtain  
information may have been supplemented in the rural areas, and if anything more of the  
deaths among travelling children may have occurred in supplemented than non-supplemented  
children.

#### *Consistency with other studies*

Apart from our previous studies from Bissau(11;16;19), there has been no evaluation of the  
impact of VAS campaigns on mortality based on individual level data. We have previously  
shown that the effect of VAS was more beneficial when provided with MV than with DTP  
vaccine(11). We have also gathered evidence that the effect of VAS versus placebo was more  
beneficial in girls with MV than in girls with DTP as their most recent vaccine(8). In the  
present study, we compared campaign participants with non-participants rather than VAS

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3 with placebo recipients, which may have introduced selection biases in the comparison of  
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5 VAS recipients and non-recipients. However, the varying effect by vaccination status is  
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7 unlikely to be explained by simple selection bias.  
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12 Mortality is usually 15% higher in the rainy (May-November) than dry season(20), and  
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14 though this may explain some of the differences in mortality after the 2007 and 2008  
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16 campaigns, it is unlikely to explain the 3-fold higher mortality. The difference may partly be  
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18 due to a more beneficial effect of VAS in the dry than in the rainy season (Table 2). We have  
19  
20 previously found strong differences in the effect of neonatal vitamin A supplementation by  
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22 season. In a randomised trial, neonatal VAS benefitted infant survival among normal birth-  
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24 weight neonates in the dry season, but was associated with increased mortality in the rainy  
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26 season(15). However, we found no seasonal differences in the effect of neonatal VAS to low  
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28 birth-weight infants(21), and no strong seasonal pattern was seen in Ghana where an analysis  
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30 by season has been reported(22). Though numbers were small, it may look as if VAS was  
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32 associated with an increased risk of dying of diarrhoea during the cholera epidemic in 2008.  
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41 The effect of VAS on mortality has most often been ascribed to prevention and treatment of  
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43 vitamin A deficiency(23). In the current study we found no evidence of a beneficial effect  
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45 limited to lower socioeconomic groups which presumably are the most vitamin A deficient.  
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#### 50 *Interpretation*

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52 The present study as well as previous observations support that the effect of VAS differs  
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54 depending on vaccination status being beneficial when provided after MV(3;8), but not after  
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56 DTP. This is unlikely to be explained by selection bias, since bias is unlikely to work in  
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3 different direction for children who had MV or DTP as their most recent vaccine. It is also  
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5 unlikely to be explained by differences in vitamin A deficiency.  
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10 We do not know what caused the tendency for a differential effect of VAS on mortality  
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12 between the two campaigns. Based on the verbal autopsy data, VAS may have been  
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14 associated with a higher risk of dying from cholera/diarrhoea during the cholera epidemic in  
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16 2008. The potential differential seasonal effects of VAS deserve further investigation.  
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### 22 *Implications and conclusion*

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24 Our present study highlights the importance of continuing to evaluate the effect of presumed  
25  
26 beneficial interventions. The circumstances under which the intervention is being  
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28 implemented may differ from the circumstances under which it was originally tested and  
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30 approved. The present study as well as several previous studies suggest that the effect of VAS  
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32 is beneficial when administered with or after MV(3;8;11;24) but not when administered with  
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34 or after DTP/Pentavalent(4;8;11;25-29). Hence, in a situation where children with DTP as the  
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36 most recent vaccination predominate, the overall effect of VAS campaigns could be negative.  
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38 From this perspective it may be no coincidence that the recent huge trial of one million  
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40 children in India, where children receive booster dose of DTP at 18 months of age(30),  
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42 showed no beneficial effect of biannual VAS(2).  
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50 Our research questions the current focus on up-scaling VAS and integrating interventions to  
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52 achieve higher coverage(31). We first need to understand under which conditions VAS does  
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54 not have a beneficial effect. In a recent meta-analysis(1;32) it is argued that no further  
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56 placebo-controlled trials are needed. The meta-analysis is essentially based on trials from the  
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58 late 1980s and early 1990s and to declare that no further trials are needed presupposes that it  
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3 can be shown that the effect has not changed over time. The only recent trials are the mega-  
4 trial from India showing no effect(2) and a placebo-controlled trial of VAS administered with  
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6 trial from India showing no effect(2) and a placebo-controlled trial of VAS administered with  
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8 vaccines in Guinea-Bissau also showing no overall but sex-differential effects (unpublished  
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10 data). Hence the effect of VAS may have changed, and placebo-controlled trials may be  
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12 needed to clarify whether season matters and with which vaccines VAS should be given.  
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## Acknowledgment

**Contributors:** ABF, PA and CSB planned the study. ABF, CB, IB, SB: Supervised the campaign registration and data entry and follow-up. CM was responsible for trial B and JA was responsible for trials A and C and both contributed the vaccination data. ABF was responsible for the statistical analysis with help from BMB. ABF wrote the first draft of the paper and has the primary responsibility for the final content. All authors read and approved the final manuscript.

**Conflicts of interest:** None

**Funding:** This work was supported by DANIDA (through grant 104.Dan.8-920), the Danish Medical Research Council, Graduate School of International Health, The Aase and Ejnar Danielsens Foundation, Danish Medical Associations Research foundation and Else and Mogens Wedell-Wedellsborgs Foundation. The Bandim Health Project received support from DANIDA and the Danish National Research Foundation. PA holds a research professorship grant from the Novo Nordisk Foundation. The sponsors had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

**Competing interests:** All authors have completed the Unified Competing Interest form at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) (available on request from the corresponding author) and declare (1) No financial support for the submitted work from anyone other than their employer; (2) No financial relationships with commercial entities that might have an interest in the submitted work; (3) No non-financial interests that may be relevant to the submitted work.

**Data sharing:** No additional data available.

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Table 1: Distribution of background factors between children followed in the two campaigns#

	2007					2008				
	VAS	No VAS	No information	P for different distribution		VAS	No VAS	No information	P for different distribution	
				All	VAS vs. no VAS				All	VAS vs. no VAS
Number	3009	2191	367			3520	1683	596		
Sex (Male) <sup>a</sup>	1514 (50)	1096 (50)	185 (50)	0.98	0.84	1780 (51)	861 (51)	297 (49)	0.84	0.69
Age at campaign / months (median (interquartile range))	20.0 (12.5;27.1)	19.6 (12.5;26.3)	17.1 (12.0;25.2)	0.001 / 0.001	0.08	19.5 (12.7;27.4)	19.5 (12.6;27.2)	19.1 (13.1;25.6)	0.09 / 0.24	0.47
<b>Vaccine information</b>										
Seen vaccination card <sup>a</sup>	2700 (90)	1765 (81)	34 (9)	<0.001	<0.001	3088 (88)	1259 (75)	15 (3)	<0.001	<0.001
<b>Last vaccine at the time of the campaign</b>										
BCG / unvaccinated	13 (0)	11 (1)	2 (6)	<0.001	0.05	18 (1)	15 (1)	0 (0)	0.53	0.23
OPV	606 (22)	352 (20)	4 (12)			760 (25)	306 (24)	5 (33)		
MV	927 (34)	604 (34)	10 (29)			923 (30)	361 (29)	5 (33)		
DTP	1095 (41)	736 (42)	14 (41)			1311 (42)	540 (43)	5 (33)		
DTP+MV	62 (2)	62 (4)	4 (12)			76 (2)	37 (3)	0 (0)		
<b>Socio-economic background</b>										
Electricity in the household				0.22	0.91				<0.001	0.09
Yes	854 (28)	634 (29)	85 (23)			1000 (28)	519 (31)	125 (21)		
No	2130 (71)	1539 (70)	280 (76)			2507 (71)	1154 (69)	466 (78)		
No information	25 (1)	18 (1)	2 (1)			13 (0)	10 (1)	5 (1)		
Bathroom				0.05	0.72				0.001	0.06
Inside the house	417 (14)	322 (15)	33 (9)			495 (14)	276 (16)	54 (9)		

Outside the house	2560 (85)	1850 (84)	330 (90)			3007 (85)	1395 (83)	537 (90)		
None	1 (0)	1 (0)	1 (0)			0 (0)	1 (0)	0 (0)		
No information	31 (1)	18 (1)	3 (1)			18 (1)	11 (1)	5 (1)		
Maternal education				<0.001	0.003				<0.001	<0.001
Any	1988 (66)	1354 (62)	169 (46)			2386 (68)	1000 (59)	301 (51)		
None	791 (26)	627 (29)	156 (43)			837 (24)	497 (30)	210 (35)		
No information	230 (8)	210 (10)	42 (11)			297 (8)	186 (11)	85 (14)		
Type of roofing				0.79	0.50				0.11	0.26
Straw	122 (4)	75 (3)	14 (4)			119 (3)	48 (3)	27 (5)		
Hard	2862 (95)	2098 (96)	351 (96)			3389 (96)	1625 (97)	564 (95)		
No information	25 (1)	18 (1)	2 (1)			12 (0)	10 (1)	5 (1)		
Ethnic group				<0.001	<0.001				<0.001	<0.001
Pepel	985 (33)	634 (29)	101 (28)			1067 (30)	456 (27)	190 (32)		
Fula/Mandinga	602 (20)	619 (28)	139 (38)			759 (22)	534 (32)	181 (30)		
Manjaco/Mancanha	627 (21)	380 (17)	41 (11)			703 (20)	269 (16)	86 (14)		
Other	795 (26)	558 (25)	86 (23)			991 (28)	424 (25)	139 (23)		
<b>Trial enrolments&amp;</b>										
Enrolled in trial A prior to campaign <sup>‡</sup>	1161 (39)	742 (34)	80 (22)	<0.001	0.001	1344 (38)	543 (32)	129 (22)	<0.001	<0.001
Enrolled in trial B prior to campaign <sup>‡</sup>	1329 (44)	852 (39)	90 (25)	<0.001	<0.001	1054 (30)	397 (24)	101 (17)	<0.001	<0.001
Enrolled in trial C prior to campaign <sup>‡</sup>	134 (4)	120 (5)	17 (5)	0.23	0.09	105 (3)	72 (4)	23 (4)	0.05	0.02

# Values are numbers (percentages) unless stated otherwise

‡ Variables in 2 levels are presented by one level

\* No information compared to VAS / No information compared to no VAS

& Trial A: RCT among 18 months old children: booster DTP+OPV vs. OPV only; Trial B: RCT among 4½ months old children: extra dose of MV at 4½ months vs. no vaccine +/- extra MV at 18 months of age. Trial C: RCT among 9 months old children due to receive MV+DTP3: DTP3+MV+booster DTP at 18 months vs. MV only



**Table 2: The effect of receiving VAS in a campaign, overall and by sex, campaign and age group**

	Rate per 1000 PYRS (Deaths / PYRS)	Crude Mortality Rate Ratio (95% CI)			Adjusted Mortality Rate Ratio (95% CI)*		
		All	Boys	Girls	All	Boys	Girls
<b>All</b>							
VAS	15.3 (44 / 2873)	0.93 (0.55;1.58)	0.85 (0.44;1.67)	1.07 (0.45;2.57)	0.78 (0.46;1.34)	0.73 (0.37;1.43)	0.89 (0.37;2.14)
No VAS	15.9 (20 / 1260)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
No information	32.5 (8 / 227)	2.17 (0.96;4.94)	2.04 (0.73;5.71)	2.40 (0.62;9.27)	2.10 (0.75;5.89)	1.96 (0.59;6.57)	2.35 (0.53;10.4)
<b>2007</b>							
VAS	4.4 (6 / 1352)	0.40 (0.14;1.15)	0.54 (0.15;1.85)	0.18 (0.02;1.69)	0.38 (0.13;1.10)	0.52 (0.15;1.79)	0.16 (0.02;1.59)
No VAS	10.9 (8 / 731)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
<b>2008</b>							
VAS	24.4 (38 / 1521)	1.04 (0.54;2.00)	0.88 (0.39;2.00)	1.37 (0.46;4.09)	1.02 (0.53;1.96)	0.86 (0.38;1.96)	1.32 (0.44;3.94)
No VAS	21.7 (12 / 529)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
P for same effect of VAS in 2007 and 2009		0.13	0.51	0.11	0.12	0.50	0.11
<b>Children aged 6-11 months</b>							
VAS	23.3 (16 / 687)	1.35 (0.48;3.76)	1.68 (0.47;6.07)	0.84 (0.15;4.66)	1.14 (0.41;3.19)	1.41 (0.40;5.10)	0.72 (0.13;3.98)
No VAS	17.0 (5 / 294)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
<b>Children aged 12-35 months</b>							
VAS	12.8 (28 / 2186)	0.81 (0.43;1.51)	0.62 (0.27;1.39)	1.19 (0.43;3.30)	0.69 (0.36;1.29)	0.53 (0.23;1.20)	0.98 (0.35;2.72)
No VAS	15.5 (15 / 966)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
P for same effect of VAS in younger and older children		0.40	0.20	0.74	0.41	0.21	0.76

\*Adjusted for sex, campaign, seen vaccination card, ethnicity and maternal education

Table 3: The effect of receiving VAS in a campaign, overall and by sex and vaccine status prior to the campaign#

			Crude				Adjusted*			
Campaign information	Rate / 1000 PYRS (Deaths/PYRS)	MRR (95%CI)	P for interaction vas and vaccine	Boys: MRR (95%CI)	Girls: MRR (95%CI)	MRR (95%CI)	P for interaction VAS and vaccine	Boys: MRR (95%CI)	Girls: MRR (95%CI)	
<b>Last received vaccine before the campaign#</b>										
OPV	VAS	14.1 (8 / 569)	P=0.09	0.02	P=0.05	P=0.19	NA	NA	NA	NA
	No VAS	0 (0 / 203)								
MV	VAS	11.3 (10 / 882)	(0.17;1.01)	Reference	(0.12;1.19)	(0.11;2.11)	(0.14;0.85)	Reference	(0.10;0.96)	(0.09;1.85)
	No VAS	26.9 (9 / 335)								
DTP	VAS	20.1 (21 / 1041)	(0.57;3.51)	0.06	(0.41;5.32)	(0.37;4.87)	(0.52;3.22)	0.04	(0.39;4.93)	(0.33;4.43)
	No VAS	14.5 (6 / 414)								
DTP+MV	VAS	16.5 (1 / 61)	(0.02;2.62)	0.68	0	NA	(0.02;2.30)	0.70	0	NA
	No VAS	63.3 (2 / 32)								

#Assessed among children with a seen vaccination card, thus excluding 6 deaths among children with no seen card (3 VAS, 3 No VAS).

\*Adjusted for sex, campaign, ethnicity and maternal education

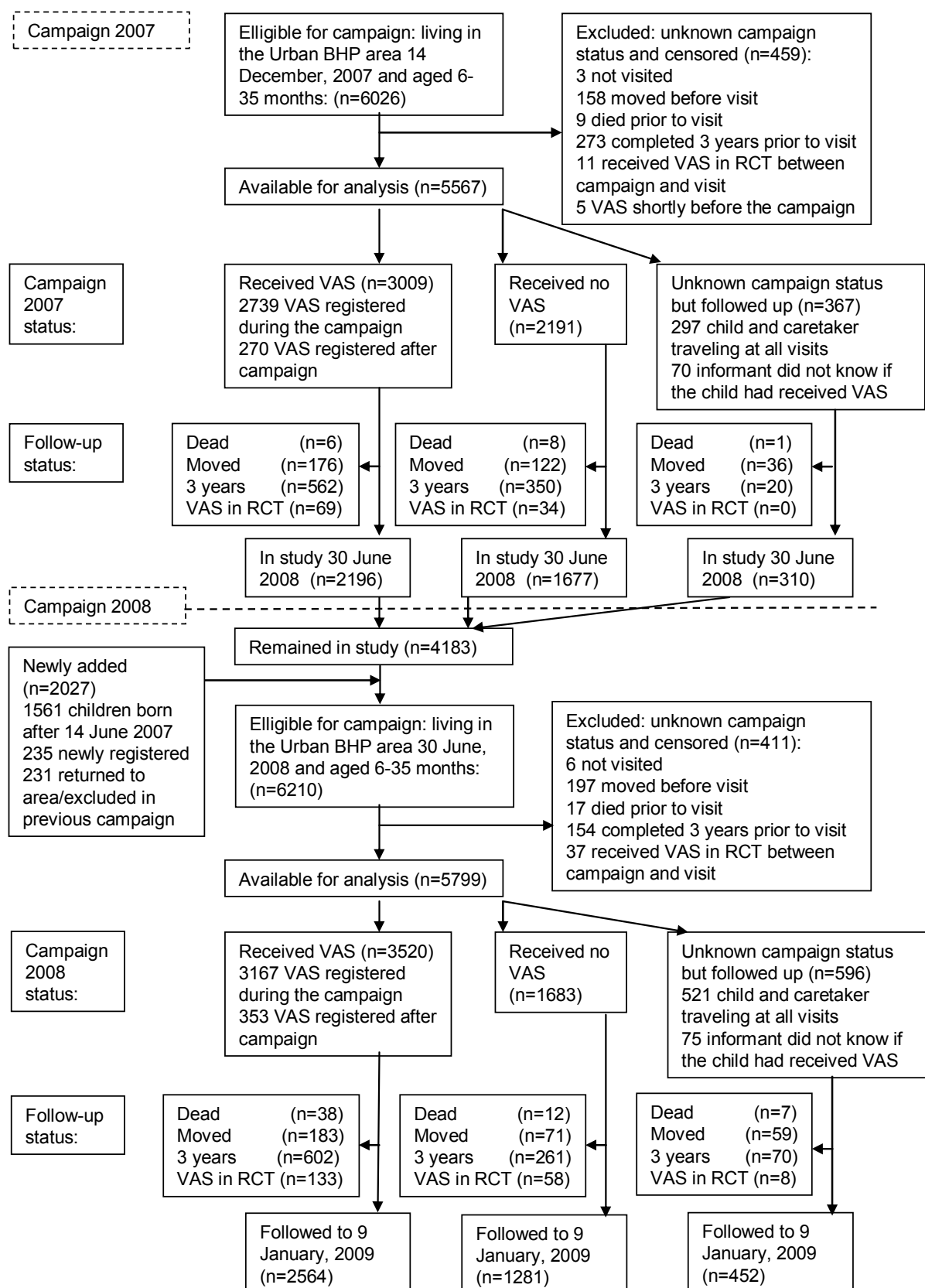
□57 children with BCG or no vaccination excluded due to small numbers (1 death: VAS)

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8 **Figure 1: Flowchart of children eligible for campaign participation**  
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Figure 1: Flowchart of children eligible for campaign participation





**Does the effect of vitamin A supplements depend on vaccination status? An observational study from Guinea-Bissau**

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2011-000448.R1
Article Type:	Research
Date Submitted by the Author:	24-Nov-2011
Complete List of Authors:	Fisker, Ane; Bandim Health Project, Bandim Health Project; University of Aarhus, Department of Biostatistics, Institute of Public Health Aaby, Peter; Bandim Health Project, Bandim Health Project Bale, Carlito; Bandim Health Project, Bandim Health Project Balde, Ibraima; Bandim Health Project, Bandim Health Project Biering-Sørensen, Sofie; Bandim Health Project, Bandim Health Project Agergaard, Jane; Aarhus University Hospital, Skejby Sygehus, Department of Infectious Diseases; Bandim Health Project, Bandim Health Project Martins, Cesario; Bandim Health Project, Bandim Health Project Bibby, Bo; University of Aarhus, Department of Biostatistics, Institute of Public Health Benn, Christine; Statens Serum Institut, Department of Epidemiology Research
<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Epidemiology, Global health, Paediatrics, Public health, Nutrition & metabolism
Keywords:	EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES, Community child health < PAEDIATRICS, Paediatric infectious disease & immunisation < PAEDIATRICS, PUBLIC HEALTH

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Does the effect of vitamin A supplements depend on vaccination status? An observational study from Guinea-Bissau

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**Words:** 4343

**Abstract**

**Objective:** Vitamin A supplementation (VAS) is estimated to reduce all cause mortality by 24%. Previous studies indicate that the effect of VAS may vary with vaccination status.

We evaluated the effect of VAS provided in campaigns on child survival overall and by sex and vaccination status at the time of supplementation.

**Design:** Observational cohort study

**Setting and Participants:** The study was conducted in the urban study area of the Bandim Health Project in Guinea-Bissau. We documented participation or non-participation in two national vitamin A campaigns in December 2007 and July 2008 for children between 6-35 months of age. Vaccination status was ascertained by inspection of vaccination cards. All children were followed prospectively.

**Outcome measures:** Mortality rates for supplemented and non-supplemented children were compared in Cox-models providing mortality rate ratios (MRR).

**Results:** We obtained information from 93% of 5567 children in 2007 and 90% of 5799 children in 2008. The VAS coverage was 58% in 2007 and 68% in 2008. Mortality in the supplemented group was 1.5% (44 deaths/2873 PYRS) and 1.6% (20 deaths/1260 PYRS) in the non-supplemented group (adjusted MRR=0.78 (0.46;1.34)). The effect was similar in boys and girls. Vaccination cards were seen for 86% in 2007 and 84% in 2008. The effect of VAS in children who had measles vaccine as their last vaccine (2814 children, adjusted MRR=0.34 (0.14;0.85)) differed from the effect in children who had diphtheria-tetanus-pertussis (DTP) vaccine as their last vaccine (3680 children, adjusted MRR=1.29 (0.52;3.22), p=0.04 for interaction).

**Conclusion:** The effect of VAS differed by most recent vaccination, being beneficial after measles vaccine but not after DTP.

**Words:** 262

## Article summary

### Article focus

- Vitamin A supplementation (VAS) is estimated to reduce all cause mortality by 24%.
- The effect of VAS may vary with vaccination status, being beneficial with or after measles vaccine (MV) but not after diphtheria-tetanus-pertussis (DTP) vaccine.

### Key messages

- The effect of VAS is heterogeneous
- The effect of VAS varied with vaccination status: supplemented children had lower mortality than non-supplemented children when MV was the most recent vaccine, but not when DTP was the most recent vaccine.
- The effect of VAS tended to differ by season of supplementation.

### Strengths and limitations

Information was collected on the individual level, and the children were followed prospectively. Due to the observational nature of the study the comparison of supplemented and non-supplemented children should be interpreted with caution. However, a selection bias is unlikely to have worked in different directions for children who had DTP and MV as the most recent vaccine.



## Introduction

Vitamin A supplementation (VAS) is estimated to reduce all cause mortality by 24% when provided to children aged 6-59 months at 4-6 monthly intervals(1). However, according to a preliminary report(2) of a recent cluster randomised trial involving more than 1 million Indian children VAS compared to no VAS was associated with no survival benefit. We have hypothesised that the effect of VAS varies with vaccination status because VAS amplifies the non-specific immunological effects of vaccines(3). Hence, VAS is beneficial in the time window when BCG (recommended at birth) and measles vaccine (MV, recommended at 9 months of age) are the most recent vaccines, but not while diphtheria-tetanus-pertussis vaccine (DTP, recommended at 6, 10, and 14 weeks of age) is the most recent vaccine(3). The hypothesis provides an explanation of the lack of a beneficial effect of VAS in children aged 1-5 months(4;5), the age group in which DTP is the predominant vaccine. The hypothesis also offers an explanation of why the large mortality reduction reported by the meta-analysis of the trials between 1986 and 1993(1) may no longer be reproducible; these trials were conducted before the Expanded Programme on Immunizations (EPI) reached high coverage(6). We recently reanalysed data from the 1989-91 VAS trial in Ghana which had reported an overall mortality reduction of 19% (2%;32%)(7). The reanalysis revealed that the survival advantage was limited to children with no health card (a proxy for being unvaccinated). Among vaccinated children there was a harmful effect of VAS among girls likely to receive DTP during follow-up(8).

WHO recommends vitamin A for all children aged 6-59 months distributed in campaigns or at vaccination contacts in areas where vitamin A deficiency is a public health problem(9;10). In Guinea-Bissau VAS is distributed in biannual campaigns, often linked to other interventions such as distribution of mebendazole, oral polio vaccine (OPV) or other vaccines(11). Two

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3 different strategies have been employed in the urban area: *fixed post campaigns* where the  
4 mother brings her child to the campaign team and *door-to-door campaigns* where all houses  
5 are visited by the campaign team. The fixed post campaigns require less staff, but have lower  
6 coverage. In December 2007 and June/July 2008 fixed post campaigns distributing vitamin A  
7 and mebendazole were conducted. We registered all children receiving VAS and  
8 mebendazole during these campaigns and assessed survival prospectively to compare survival  
9 in supplemented and non-supplemented children overall and by sex and vaccination status.  
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## 20 21 **Subjects and Methods**

### 22 *Setting*

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24 The Bandim Health Project (BHP) operates a health and demographic surveillance system  
25 (HDSS) covering a population of 102,000 people in six suburban districts of Bissau, the  
26 capital of Guinea-Bissau, West Africa. All households are visited monthly to register new  
27 births, pregnancies and deaths. Children below the age of 3 are followed through 3-monthly  
28 visits to ascertain vital status and vaccination status. At the first visit after birth information  
29 on socio-economic status is collected. The indicators include maternal characteristics  
30 (education and ethnicity) and household characteristics (type of roofing, availability of  
31 bathroom and electricity). All children aged 6-35 months living in the urban study area on the  
32 first day of the VAS campaigns in 2007 and 2008 were eligible for the present study. Since  
33 children below 3 years of age are followed intensively through the HDSS, these children have  
34 been the focus for the present study.  
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51 WHO classifies Guinea-Bissau as having a public health problem of VAD(12). We have  
52 previously found that 16% of 4-month-old children were vitamin A deficient (retinol binding  
53 protein (RBP) concentration equivalent to plasma retinol less than 0.70  $\mu\text{mol/L}$ )(13). During  
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3 2007-2008 we assessed vitamin A status in 181 children aged 9-17 months presenting for  
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5 vaccinations and found that 70% of the children had a RBP concentration equivalent to  
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7 plasma retinol less than 0.70 $\mu$ M (unpublished data).  
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### 10 11 *Campaign information*

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14 National VAS campaigns were conducted by the Ministry of Health from 14-18 December  
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16 2007 and 30 June-4 July 2008. Children aged 6-11 months received 100,000 IU vitamin A,  
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18 children aged 12-35 months received 200,000 IU vitamin A and 250 mg mebendazole, and  
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20 children aged 36-59 months received 200,000 IU vitamin A and 500 mg mebendazole.  
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23 Trained BHP field assistants were present at all posts. The field assistants brought a list of all  
24  
25 children registered in the area and noted on this list whether a child had received VAS and  
26  
27 mebendazole at the post. The lists contained information on vaccines registered prior to the  
28  
29 campaign. If the vaccination card was seen, the assistant noted this on the list, verified the  
30  
31 already registered vaccines and updated the information if new vaccines had been received.  
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36 During the weeks after the campaigns all children who had not been seen during the  
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38 campaigns were visited. The caretaker was asked whether the child had received VAS  
39  
40 elsewhere. The vaccination card was seen and information on vaccines verified and updated.  
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42 If the child and caretaker were not present the household was visited up to three times: if no  
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44 one could provide information, the campaign status was classified as unknown.  
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50 During the 2007-campaign we discovered that the vitamin A capsules given in two of the six  
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52 districts had been produced in November 2004 and had passed the expiry date one month  
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54 before. The capsules were immediately replaced by a new batch. We sent two of the expired  
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56 200,000 IU capsules to “as Vitas Oslo Innovation Center” (Oslo, Norway) to have their  
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3 vitamin A content measured. The vitamin A contents were slightly lower than expected:  
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5 167,569 IU and 154,863 IU. Capsules used in the 2008-campaign were all from a new stock.  
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### 8 9 10 *Vaccine information*

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12 The vaccination schedule in Guinea-Bissau when this study was conducted was BCG and  
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14 OPV at birth, 3 doses of DTP vaccines at 6, 10 and 14 weeks of age and MV at 9 months of  
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16 age. A booster DTP vaccine at 18 months of age was given in the routine vaccination program  
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18 until March 2007 after which it was only given in a randomised trial (Trial A below). In  
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20 August 2008 the DTP vaccine at 6, 10 and 14 weeks was replaced by the pentavalent (DTP-  
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22 HiB-HBV) vaccine and a yellow fever vaccine (YF) was added to be given with MV at 9  
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24 months of age.  
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30 During the study period three trials of alternative vaccination strategies took place in the BHP  
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32 area. First, between October 2005 and October 2009, Trial A enrolled children aged 18  
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34 months in a randomised trial of booster DTP. Children who had completed their primary  
35  
36 immunisation schedule of 3 doses of DTP and a MV were randomised to receive booster  
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38 DTP+OPV or OPV only. Second, between August 2003 and April 2007, Trial B enrolled  
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40 children aged 4½ months in a randomised trial of early MV. Children were randomised to an  
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42 extra dose of MV at 4½ months or no vaccine; a subgroup of the controls received an extra  
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44 measles vaccine at 18 months of age(14). Third, between October 2005 and April 2008, Trial  
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46 C enrolled children aged at least 9 months who were delayed in relation to the vaccination  
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48 schedule and presented at the health centres to receive MV and the third DTP vaccine. They  
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50 were randomised to receive both vaccines and booster DTP at 18 months of age versus MV  
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52 only and no booster DTP(15) (supplementary figure). Furthermore, in May 2006 a measles  
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54 vaccination campaign was conducted in which children aged 6 months to 14 years were given  
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3 an MV; thus the oldest children in the present study often had MV received in this campaign  
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5 as their most recent vaccine.  
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10 For the present study, vaccine status was ascertained during the campaigns. Furthermore, as  
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12 part of the BHP routines, vaccines are registered daily at the 3 health centres in the study-area  
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14 and through the 3-monthly home visits, and as a part of the trials described above. We linked  
15  
16 the campaign information with information from the above sources, using only information  
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18 collected prior to the campaign. In case of discrepancy, information from the trials was  
19  
20 considered superior. If no trial information was available and if two of the other sources  
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22 agreed, this information was accepted. Vaccination information obtained during the campaign  
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24 was updated for 12% (1088/8812) of the children; 9% based on data collected at the health  
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26 centres, 1% based on routine surveillance data, and 2% based on trial data. Except for  
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28 children included in Trial A, OPV is almost always given with BCG or DTP. In line with  
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30 previous studies(11;16), children who received OPV along with another vaccine were  
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32 classified according to the co-administered vaccine, i.e. children who received DTP alone  
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34 (161 children) and DTP+OPV (3519 children) were classified as DTP and children who  
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36 received MV+OPV (205 children) and MV alone (2609) were classified as MV.  
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### 43 *Follow-up*

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45 All children below 3 years of age were followed by the HDSS. For children who died, a  
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47 locally adapted version of the INDEPTH verbal autopsy(17) was conducted. Cause of death  
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49 was determined by a local paediatrician.  
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### 52 *Statistical analyses*

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3 Children entered the analysis on the day we knew their VAS-campaign status (supplemented,  
4 non-supplemented or no information). Thus, a child who was registered to have participated  
5 in the campaign during the days of the campaign contributed time at risk from the day of  
6 supplementation. A child who had campaign status registered after the campaign contributed  
7 time from the day the information was obtained. Follow-up of children was censored when a  
8 subsequent campaign was initiated (30 June 2008 for the 2007-campaign, 9 January 2009 for  
9 the 2008-campaign), three years of age, migration or reception of VAS in a trial, whichever  
10 came first. Some children were enrolled in a randomised trial of VAS at vaccination contacts  
11 after 6 months of age (Trial registration: [clinicaltrials.gov](http://clinicaltrials.gov), NCT00514891). These children  
12 were censored from follow-up on the date of receiving VAS in the trial (Figure 1).  
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27 We examined whether the background factors were evenly distributed between children in the  
28 VAS, no VAS and no information group, as well as between the two main groups (VAS and  
29 no VAS) using chi2 tests for categorical variables and ranksum test for age at the time of the  
30 campaign.  
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38 Survival was examined in Cox proportional hazards models with age as underlying time; thus  
39 age was inherently controlled for in all models. The proportional hazards assumption was  
40 tested using Schoenfeld residuals. Crude and adjusted estimates are presented. The 2007-  
41 campaign took place in the dry season and the 2008-campaign in the rainy season. A priori we  
42 had decided to adjust all estimates for sex and campaign, since sex and season of  
43 supplementation have been shown to be important determinants of the effect of VAS in our  
44 previous studies(8;18;19). We furthermore adjusted the comparison of supplemented and non-  
45 supplemented children for the background factors which were associated with participation in  
46 the VAS campaigns. In all analyses we investigated whether the survival of supplemented and  
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3 non-supplemented children varied by sex and campaign (season). We furthermore  
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5 investigated interactions with vaccination status. Interaction between VAS and potential  
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7 effect modifies was evaluated by Wald statistics. Among children who had OPV as the most  
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9 recent vaccine, no deaths occurred among non-supplemented children. Therefore, the  
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11 potential interaction between VAS and OPV was tested by Mantel-Haenszel stratification.  
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13 Finally, previous studies have indicated that the effect of VAS in girls depends on whether or  
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15 not they have received VAS on a previous occasion(20;21). We therefore investigated  
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17 whether survival after the 2008 campaign varied with reception of VAS in the 2007  
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19 campaign.  
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## 25 Results

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27 In the 2007-campaign we followed 5567 (92%) of the 6026 children aged 6-35 months  
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29 registered in the study area. Reasons for losses to follow-up are given in Figure 1. For 7%  
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31 (367/5567) we obtained no information on campaign status, since the family was travelling at  
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33 all visits. Among the remaining 5200 children, 3009 (58%) had received VAS and 2191  
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35 (42%) had not. In the 2008-campaign, we followed 5799 (93%) of 6210 children. For 10%  
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37 (596/5799) we were unable to obtain information on campaign status. Among the remaining  
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39 5203 children, 3520 (68%) had received VAS and 1683 (32%) had not (Figure 1). Median  
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41 length of follow-up and inter-quartile range was 6.2 (4.4-6.4) months for supplemented, 4.2  
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43 (3.2-5.1) months for non-supplemented and 2.9 (1.8-3.4) months for children for whom we  
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45 did not obtain information.  
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52 The distribution of background factors is shown in Table 1. Children for whom we had no  
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54 information on campaign status had lower socioeconomic status, were more likely to be from  
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56 the Muslim ethnic groups (Fula and Mandinga) and were younger. These children also tended  
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3 to have higher mortality, crude MRR=2.17 (0.96; 4.94) (Table 2). In contrast, there were only  
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5 few differences in background factors between supplemented and non-supplemented children.  
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7 Non-supplemented children were more likely to have mothers with no formal education  
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9 (p<0.001), to belong to the Muslim ethnic groups (p<0.001) and were less likely to have their  
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11 vaccination card inspected (p<0.001). Furthermore, more supplemented children were  
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13 enrolled in trials and as a result more supplemented children had received OPV as the most  
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15 recent vaccine in 2007 (p=0.05). In 2008 the distribution of different vaccines did not differ  
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17 between supplemented and non-supplemented children (p=0.23) (Table 1).  
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### 23 *Effect of VAS*

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25 There was no significant difference in mortality between supplemented and non-  
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27 supplemented children, the crude MRR being 0.93 (0.55;1.58). When the estimate was  
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29 adjusted for sex, campaign, maternal education, ethnicity and inspected vaccination card, the  
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31 difference in mortality between supplemented and non-supplemented children was 0.78  
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33 (0.46;1.34) (Table 2). The effect did not differ significantly for children aged 6-11 months  
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35 who received 100,000 IU vitamin A (adjusted MRR=1.14 (0.41;3.19)) and children above 12  
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37 months who received 200,000 IU vitamin A and 250 mg mebendazole (adjusted MRR=0.69  
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39 (0.36;1.29)) (p=0.41, test of interaction) (Table 2). The effect did not differ for boys and girls  
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41 (p=0.72).  
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Mortality was much higher in the rainy season after the 2008-campaign (57 deaths / 2208  
PYRS) than in the dry season after the 2007-campaign (15 deaths / 2154 PYRS); the adjusted  
MRR was 3.79 (2.13;6.74) comparing 2008 versus 2007. The difference between  
supplemented and non-supplemented tended to differ between the two campaigns; the  
adjusted MRR was 0.38 (0.13;1.10) after the 2007-campaign and 1.02 (0.53;1.96) after the



2008-campaign ( $p=0.12$ , test of interaction). This difference may have been strongest for girls; the adjusted MRR was 0.16 (0.02;1.59) after the 2007 campaign and 1.32 (0.44;3.94) after the 2008-campaign ( $p=0.11$ , test of interaction), whereas there was no evidence of a difference in boys ( $p=0.50$ ) (Table 2). Further adjusting for last vaccine at the time of the campaign did not change the conclusions.

The difference between supplemented and non-supplemented children varied with maternal education. Supplementation was associated with lower mortality in children of educated mothers (adjusted MRR=0.41 (0.21;0.80)), but not in children of mothers with no schooling (adjusted MRR=1.78 (0.60;5.31)) ( $p=0.02$ , test of interaction). The effect did not vary with the other socioeconomic indicators presented in Table 1 (data not shown).

#### *Effect of VAS by vaccination status*

Vaccination cards were seen for 86% of the children in 2007 and 84% in 2008, with more cards seen for supplemented compared with non-supplemented children (2007: 90% vs. 81%,  $p<0.001$ ; 2008: 88% vs. 75%,  $p<0.001$ , Table 1); 9% (6/64) of the deaths in the study were excluded due to no information on vaccination status.

The difference in mortality between supplemented and non-supplemented children varied with vaccination status. In the 2814 children who had received MV as the most recent vaccine prior to the campaign the adjusted MRR was 0.34 (0.14;0.85). In the 3680 children who had received DTP as the most recent vaccine the adjusted MRR was 1.29 (0.52;3.22) ( $p=0.04$  for different effect in MV and DTP recipients). The pattern was similar in boys and girls (Table 3). The small group of non-supplemented children with OPV as the most recent vaccine had lower mortality than all other groups (Table 3).

### *Effect of supplementation in the 2007-campaign on survival after the 2008-campaign*

Supplementation in the 2007-campaign was associated with improved survival after the 2008-campaign. Among 3520 children supplemented in 2008, 69% (2441) had been eligible for supplementation in 2007; 1435 (59%) had received VAS, 917 (38%) had not and 89 (4%) had no information on participation. After the 2008-campaign, children supplemented in both campaigns had lower mortality (8 deaths / 623 PYRS) than children who had not received VAS in 2007 but received VAS in 2008 (15 deaths / 394 PYRS), the adjusted MRR being 0.34 (0.14;0.80). This beneficial effect of prior VAS was more pronounced for girls (MRR=0.14 (0.03;0.68)) than for boys (MRR=0.59 (0.20;1.77)) (test of interaction between sex and VAS, p=0.14).

### *Cause of death*

We aimed to examine the effect of VAS on all cause mortality. However, from May 2008 to January 2009 a cholera epidemic occurred(22). This epidemic could potentially explain the higher mortality after the 2008-campaign. Verbal autopsies were conducted for 66 of the 72 deaths, for the remaining six the family had moved prior to the interview. One death which occurred after receiving VAS in 2008 was classified as due to cholera. Further 10 deaths (15%) were due to diarrhoeal disease, 1 after the 2007-campaign (no VAS) and 9 after the 2008-campaign (8 VAS, 1 no VAS). The peak of the epidemic occurred in September 2008(22); five diarrhoeal deaths in 2008 occurred in August and September (all VAS) and four deaths (3 VAS, 1 no VAS) occurred in the late epidemic in November and December where few cholera cases were detected. Campaign participation had no significant effect on the risk of death due to diarrhoea, the MRR being 1.87 (0.40;8.71). The other causes of death were: prolonged disease with failure to thrive and anaemia: 18 (27%, 14 VAS, 3 no VAS, 1

no information), respiratory infection: 17 (26%, 9 VAS, 6 no VAS, 2 no information), malaria: 14 (21%, 5 VAS, 7 no VAS, 2 no information), fever of unknown origin: 3 (5%, 2 VAS, 1 no VAS), cerebral palsy: 2 (3%, 1 VAS, 1 no VAS) and accident: 1 (2%, VAS).

There were significantly more malaria deaths among the non-supplemented and the children with no information, the MRRs being 3.26 (1.03;10.3) and 5.43 (1.05;28.2), presumably reflecting that they were less likely seek early treatment or to be absent in the rural areas where the incidence of severe and untreated malaria would be higher.

## Discussion

Based on the marked mortality reductions seen in trial in the 1980s and 1990s, VAS has become a high priority intervention, expected to reduce overall child mortality. However, according to the preliminary report, a recent trial VAS in one million Indian children has not shown the same beneficial effect(2).

In our study, children receiving VAS in a campaign had 7% (-58%;45%) lower mortality compared with non-supplemented children in the crude analysis and 22% (-34%;54%) lower mortality in the adjusted analysis. However, this is unlikely to represent the true effect of VAS since there are many reasons that non-supplemented children may have had higher mortality, e.g. selection bias, travelling to the rural areas, less access to care and a higher risk of dying of malaria. More important, our observational study identified differential effects of VAS by season and vaccination status; for example, supplemented children did not have lower mortality than non-supplemented children in the rainy season and among children who had DTP as the most recent vaccination.

### *Strengths and weaknesses*

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3 The strengths of our study include that information was collected on the individual level, and  
4 that the children were followed prospectively. By only allowing the children to contribute  
5 observation time in the VAS or no VAS group from the day the information was obtained, we  
6 avoided introducing survival bias in the analysis (i.e. obtaining positive information only from  
7 surviving children)(23).  
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16 Vitamin A content was assessed in the expired capsules which some campaign recipients  
17 received in 2007. Though the content was lower than the original 200,000 IU, the content was  
18 still high. Hence, we believe that all supplemented children received a relevant dose of  
19 vitamin A.  
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27 Mortality was higher in children for whom we do not have information on the campaign  
28 participation, and also a substantial number of children died prior to us getting the  
29 information on their participation in the VAS campaign. The main reason for not getting any  
30 information was that the family had travelled to the rural areas, where mortality is higher(24).  
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32 According to national data VAS coverage was considerably higher outside the capital and  
33 hence outside the urban study area (Personal communication, Sidu Biai, WHO, Bissau).  
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35 Hence, the children for whom we did not obtain information may have been supplemented in  
36 the rural areas, and if anything more of the deaths among travelling children may have  
37 occurred in supplemented than non-supplemented children.  
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#### 50 *Consistency with other studies*

51 Apart from our previous studies from Bissau(11;19;24), there has been no evaluation of the  
52 impact of VAS campaigns on mortality based on individual level data. We have previously  
53 shown that the effect of VAS was more beneficial when provided with MV than with DTP  
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3 vaccine(11). We have also gathered evidence that the effect of VAS versus placebo was more  
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5 beneficial in girls with MV than in girls with DTP as their most recent vaccine(8). In the  
6  
7 present study, we compared campaign participants with non-participants rather than VAS  
8  
9 with placebo recipients, which may have introduced selection biases in the comparison of  
10  
11 VAS recipients and non-recipients. However, the varying effect by vaccination status is  
12  
13 unlikely to be explained by simple selection bias. We had expected that a negative effect of  
14  
15 VAS with DTP would be most pronounced for girls(8;25;26). This was not the case.  
16  
17 However, recent studies have indicated that mortality after VAS is lowered in girls who have  
18  
19 received VAS on a previous occasion(20;21) and this was also the case in the present study.  
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21 Thus, repeated dosing may have alleviated the negative effects of VAS with DTP in girls.  
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27 Mortality is usually 15% higher in the rainy (May-November) than dry season(27), and  
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29 though this may explain some of the differences in mortality after the 2007 and 2008  
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31 campaigns, it is unlikely to explain the 3-fold higher mortality. The difference may partly be  
32  
33 due to a more beneficial effect of VAS in the dry than in the rainy season (Table 2). We have  
34  
35 previously found strong differences in the effect of neonatal vitamin A supplementation by  
36  
37 season. In a randomised trial, neonatal VAS benefitted infant survival among normal birth-  
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39 weight neonates in the dry season, but was associated with increased mortality in the rainy  
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41 season(18). However, we found no seasonal differences in the effect of neonatal VAS to low  
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43 birth-weight infants(26), and no strong seasonal pattern was seen in Ghana where an analysis  
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45 by season has been reported(28). Though numbers were small and the difference was not  
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47 statistically significant, VAS appeared to be associated with an increased risk of dying of  
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49 diarrhoea during the cholera epidemic in 2008. This contrasts with a recent meta-analysis  
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51 which estimates 28% (9%;43%) reduction in diarrhoea related mortality(1).  
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3 The effect of VAS on mortality has most often been ascribed to prevention and treatment of  
4 vitamin A deficiency(29). We have previously found poorer vitamin A status in children of  
5 non-educated mothers(13). In the current study we found no evidence of a beneficial effect  
6  
7 limited to lower socioeconomic groups which presumably are the most vitamin A deficient.  
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### 10 11 12 13 14 *Interpretation*

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16 The present study as well as previous observations support that the effect of VAS differs  
17 depending on vaccination status being beneficial when provided after MV(3;8), but not after  
18 DTP. This is unlikely to be explained by selection bias, since bias is unlikely to work in  
19 different direction for children who had MV or DTP as their most recent vaccine. It is also  
20 unlikely to be explained by differences in vitamin A deficiency.  
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30 We do not know what may have caused the observed tendency for a differential effect of VAS  
31 on mortality between the two campaigns. Previous studies have demonstrated seasonal  
32 variance in pathogen prevalences with distinct season-related epidemics of rota-virus (30),  
33 cryptosporidium(31), respiratory syncytial virus(32) as well as a malaria(33). The effect of  
34 VAS has been show to differ depending on the pathogen(34) and varying pathogen  
35 prevalences may therefore contribute to the seasonal differences. Based on the verbal autopsy  
36 data, VAS may have been associated with a higher risk of dying from cholera/diarrhoea  
37 during the cholera epidemic in 2008. An alternative explanation is the generally increased  
38 prevalence of infectious diseases in the rainy season, which may lead to impaired uptake and  
39 increased excretion of vitamin A(35). This could explain the lack of effect during the rainy  
40 season, but would not explain the vaccine differential effect. Regardless of the mechanism,  
41 the potential differential seasonal effects of VAS deserve further investigation.  
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### *Implications and conclusion*

Our present study highlights the importance of continuing to evaluate the effect of presumed beneficial interventions. The circumstances under which the intervention is being implemented may differ from the circumstances under which it was originally tested. The present study as well as several previous studies suggest that the effect of VAS is beneficial when administered with or after MV(3;8;11;36) but not when administered with or after DTP/Pentavalent(4;8;11;37-41). Hence, in a situation where children with DTP as the most recent vaccination predominate, the overall effect of VAS campaigns could be negative. From this perspective it may be no coincidence that the recent huge trial of one million children in India, a booster dose of DTP is recommended at 18 months of age(42), showed no beneficial effect of biannual VAS(2).

Our research questions the current focus on up-scaling VAS and integrating interventions to achieve higher coverage(43). We first need to understand under which conditions VAS does not have a beneficial effect. In a recent meta-analysis(1;44) it is argued that no further placebo-controlled trials are needed. The meta-analysis is essentially based on trials from the late 1980s and early 1990s and to declare that no further trials are needed presupposes that it can be shown that the effect has not changed over time. The only recent trials are the mega-trial from India showing no effect(2) and a placebo-controlled trial of VAS administered with vaccines in Guinea-Bissau also showing no overall but sex-differential effects (unpublished data). Hence the effect of VAS may have changed, and placebo-controlled trials may be needed to clarify whether season matters and with which vaccines VAS should be given.



## Acknowledgment

**Contributors:** ABF, PA and CSB planned the study. ABF, CB, IB, SB: Supervised the campaign registration, data entry and follow-up. CM was responsible for trial B and JA was responsible for trials A and C and both contributed the vaccination data. ABF was responsible for the statistical analysis with help from BMB. ABF wrote the first draft of the paper and has the primary responsibility for the final content. All authors contributed to and approved the final manuscript.

**Conflicts of interest:** None

**Funding:** This work was supported by DANIDA (through grant 104.Dan.8-920), the Danish Medical Research Council, Graduate School of International Health, The Aase and Ejnar Danielsens Foundation, Danish Medical Associations Research foundation and Else and Mogens Wedell-Wedellsborgs Foundation. The Bandim Health Project received support from DANIDA and the Danish National Research Foundation. PA holds a research professorship grant from the Novo Nordisk Foundation. The sponsors had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

**Competing interests:** All authors have completed the Unified Competing Interest form at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) (available on request from the corresponding author) and declare (1) No financial support for the submitted work from anyone other than their employer; (2) No financial relationships with commercial entities that might have an interest in the submitted work; (3) No non-financial interests that may be relevant to the submitted work.

**Data sharing:** No additional data available.



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**Table 1: Distribution of background factors between children followed in the two campaigns#**

	2007					2008				
	VAS	No VAS	No information	P for different distribution		VAS	No VAS	No information	P for different distribution	
				All	VAS vs. no VAS				All	VAS vs. no VAS
Number	3009	2191	367			3520	1683	596		
Sex (Male) <sup>‡</sup>	1514 (50)	1096 (50)	185 (50)	0.98	0.84	1780 (51)	861 (51)	297 (49)	0.84	0.69
Age at campaign / months (median (interquartile range))	20.0 (12.5;27.1)	19.6 (12.5;26.3)	17.1 (12.0;25.2)	0.001 / 0.001	0.08	19.5 (12.7;27.4)	19.5 (12.6;27.2)	19.1 (13.1;25.6)	0.09 / 0.24	0.47
<b>Vaccine information</b>										
Seen vaccination card <sup>‡</sup>	2700 (90)	1765 (81)	34 (9)	<0.001	<0.001	3088 (88)	1259 (75)	15 (3)	<0.001	<0.001
<b>Last vaccine at the time of the campaign</b>										
BCG / unvaccinated	13 (0)	11 (1)	2 (6)	<0.001	0.05	18 (1)	15 (1)	0 (0)	0.53	0.23
OPV	606 (22)	352 (20)	4 (12)			760 (25)	306 (24)	5 (33)		
MV	926 (34)	604 (34)	10 (29)			923 (30)	361 (29)	5 (33)		
DTP	1093 (41)	736 (42)	14 (41)			1311 (42)	540 (43)	5 (33)		
DTP+MV	62 (2)	62 (4)	4 (12)			76 (2)	37 (3)	0 (0)		
<b>Socio-economic background</b>										
Electricity in the household				0.22	0.91				<0.001	0.09
Yes	854 (28)	634 (29)	85 (23)			1000 (28)	519 (31)	125 (21)		
No	2130 (71)	1539 (70)	280 (76)			2507 (71)	1154 (69)	466 (78)		
No information	25 (1)	18 (1)	2 (1)			13 (0)	10 (1)	5 (1)		
Bathroom				0.05	0.72				0.001	0.06
Inside the house	417 (14)	322 (15)	33 (9)			495 (14)	276 (16)	54 (9)		

Outside the house	2560 (85)	1850 (84)	330 (90)			3007 (85)	1395 (83)	537 (90)		
None	1 (0)	1 (0)	1 (0)			0 (0)	1 (0)	0 (0)		
No information	31 (1)	18 (1)	3 (1)			18 (1)	11 (1)	5 (1)		
Maternal education				<0.001	0.003				<0.001	<0.001
Any	1988 (66)	1354 (62)	169 (46)			2386 (68)	1000 (59)	301 (51)		
None	791 (26)	627 (29)	156 (43)			837 (24)	497 (30)	210 (35)		
No information	230 (8)	210 (10)	42 (11)			297 (8)	186 (11)	85 (14)		
Type of roofing				0.79	0.50				0.11	0.26
Straw	122 (4)	75 (3)	14 (4)			119 (3)	48 (3)	27 (5)		
Hard	2862 (95)	2098 (96)	351 (96)			3389 (96)	1625 (97)	564 (95)		
No information	25 (1)	18 (1)	2 (1)			12 (0)	10 (1)	5 (1)		
Ethnic group				<0.001	<0.001				<0.001	<0.001
Pepel	985 (33)	634 (29)	101 (28)			1067 (30)	456 (27)	190 (32)		
Fula/Mandinga	602 (20)	619 (28)	139 (38)			759 (22)	534 (32)	181 (30)		
Manjaco/Mancanha	627 (21)	380 (17)	41 (11)			703 (20)	269 (16)	86 (14)		
Other	795 (26)	558 (25)	86 (23)			991 (28)	424 (25)	139 (23)		
<b>Trial enrolments&amp;</b>										
Enrolled in trial A prior to campaign <sup>a</sup>	1161 (39)	742 (34)	80 (22)	<0.001	0.001	1344 (38)	543 (32)	129 (22)	<0.001	<0.001
Enrolled in trial B prior to campaign <sup>a</sup>	1329 (44)	852 (39)	90 (25)	<0.001	<0.001	1054 (30)	397 (24)	101 (17)	<0.001	<0.001
Enrolled in trial C prior to campaign <sup>a</sup>	134 (4)	120 (5)	17 (5)	0.23	0.09	105 (3)	72 (4)	23 (4)	0.05	0.02

# Values are numbers (percentages) unless stated otherwise

<sup>a</sup> Variables in 2 levels are presented by one level

\* No information compared to VAS / No information compared to no VAS

& Trial A: RCT among 18 months old children: booster DTP+OPV vs. OPV only; Trial B: RCT among 4½ months old children: extra dose of MV at 4½ months vs. no vaccine +/- extra MV at 18 months of age. Trial C: RCT among 9 months old children due to receive MV+DTP3: DTP3+MV+booster DTP at 18 months vs. MV only

**Table 2: The effect on mortality of receiving VAS in a campaign, overall and by sex, campaign and age group**

	Rate per 1000 PYRS (Deaths / PYRS)	Crude Mortality Rate Ratio (95% CI)			Adjusted Mortality Rate Ratio (95% CI)*		
		All	Boys	Girls	All	Boys	Girls
<b>All</b>							
VAS	15.3 (44 / 2873)	0.93 (0.55;1.58)	0.85 (0.44;1.67)	1.07 (0.45;2.57)	0.78 (0.46;1.34)	0.73 (0.37;1.43)	0.89 (0.37;2.14)
No VAS	15.9 (20 / 1260)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
No information	32.5 (8 / 227)	2.17 (0.96;4.94)	2.04 (0.73;5.71)	2.40 (0.62;9.27)	2.10 (0.75;5.89)	1.96 (0.59;6.57)	2.35 (0.53;10.4)
<b>2007</b>							
VAS	4.4 (6 / 1352)	0.40 (0.14;1.15)	0.54 (0.15;1.85)	0.18 (0.02;1.69)	0.38 (0.13;1.10)	0.52 (0.15;1.79)	0.16 (0.02;1.59)
No VAS	10.9 (8 / 731)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
<b>2008</b>							
VAS	24.4 (38 / 1521)	1.04 (0.54;2.00)	0.88 (0.39;2.00)	1.37 (0.46;4.09)	1.02 (0.53;1.96)	0.86 (0.38;1.96)	1.32 (0.44;3.94)
No VAS	21.7 (12 / 529)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
P for same effect of VAS in 2007 and 2009		0.13	0.51	0.11	0.12	0.50	0.11
<b>Children aged 6-11 months</b>							
VAS	23.3 (16 / 687)	1.35 (0.48;3.76)	1.68 (0.47;6.07)	0.84 (0.15;4.66)	1.14 (0.41;3.19)	1.41 (0.40;5.10)	0.72 (0.13;3.98)
No VAS	17.0 (5 / 294)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
<b>Children aged 12-35 months</b>							
VAS	12.8 (28 / 2186)	0.81 (0.43;1.51)	0.62 (0.27;1.39)	1.19 (0.43;3.30)	0.69 (0.36;1.29)	0.53 (0.23;1.20)	0.98 (0.35;2.72)
No VAS	15.5 (15 / 966)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
P for same effect of VAS in younger and older children		0.40	0.20	0.74	0.41	0.21	0.76

\*Adjusted for sex, campaign, seen vaccination card, ethnicity and maternal education



**Table 3: The effect on mortality of receiving VAS in a campaign, overall and by sex and vaccine status prior to the campaign#**

			Crude				Adjusted*			
	Campaign information	Rate / 1000 PYRS (Deaths/PYRS)	MRR (95%CI)	P for interaction vas and vaccine	Boys: MRR (95%CI)	Girls: MRR (95%CI)	MRR (95%CI)	P for interaction VAS and vaccine	Boys: MRR (95%CI)	Girls: MRR (95%CI)
<b>Last received vaccine before the campaign#</b>										
OPV	VAS	14.1 (8 / 569)	P=0.09&	0.16	P=0.05&	P=0.19&	NA	NA	NA	NA
	No VAS	0 (0 / 203)								
MV	VAS	11.3 (10 / 882)	0.41 (0.17;1.01)	0.06	0.38 (0.12;1.19)	0.47 (0.11;2.11)	0.34 (0.14;0.85)	0.04	0.31 (0.10;0.96)	0.41 (0.09;1.85)
	No VAS	26.9 (9 / 335)								
DTP	VAS	20.1 (21 / 1041)	1.41 (0.57;3.51)	Reference	1.48 (0.41;5.32)	1.34 (0.37;4.87)	1.29 (0.52;3.22)	Reference	1.37 (0.39;4.93)	1.21 (0.33;4.43)
	No VAS	14.5 (6 / 414)								
DTP+MV	VAS	16.5 (1 / 61)	0.24 (0.02;2.62)	0.17	0	NA	0.21 (0.02;2.30)	0.16	0	NA
	No VAS	63.3 (2 / 32)								

#Assessed among children with a seen vaccination card, thus excluding 6 deaths among children with no seen card (3 VAS, 3 No VAS).

\*Adjusted for sex, campaign, ethnicity and maternal education

&Test for different mortality rate in supplemented and non-supplemented children

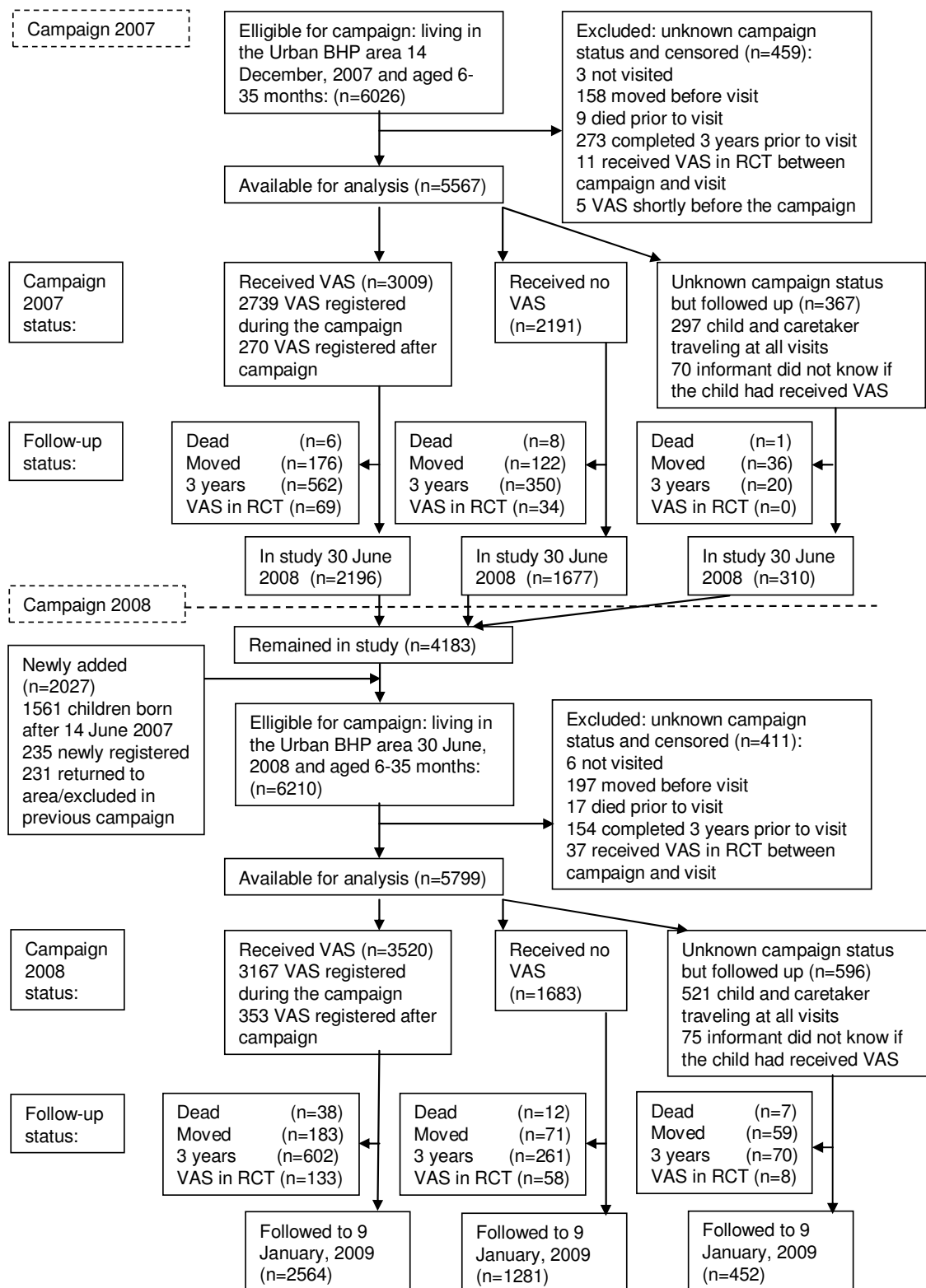
∝57 children with BCG or no vaccination excluded due to small numbers (1 death: VAS)



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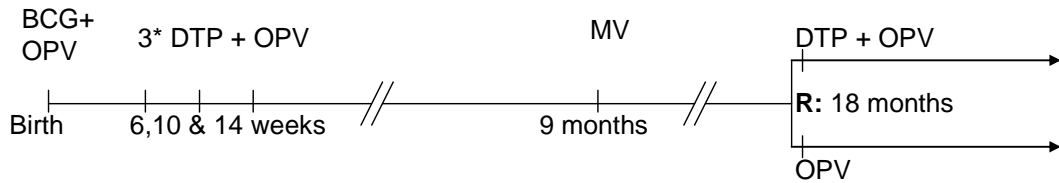
8 **Figure 1: Flowchart of children eligible for campaign participation**  
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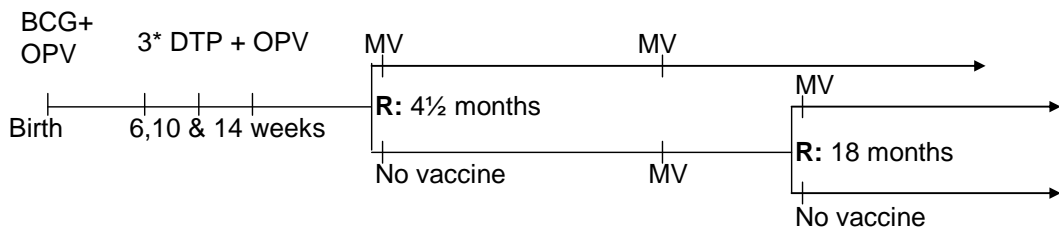
**Figure 1: Flowchart of children eligible for campaign participation**

**Supplementary Figure: Trial designs of vaccination trials conducted in the BHP study area during 2007 and 2008**

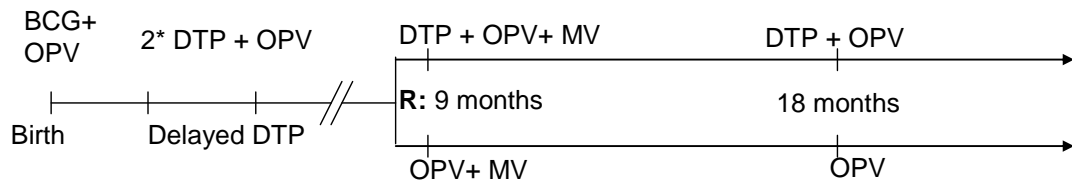
**Trial A**



**Trial B**



**Trial C**



R: Randomisation

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