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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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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. The authors 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. The authors documented participation or non-participation in two national vitamin A campaigns in December 2007 and July 2008 for children between 6 and 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 (MRRs).

Results: The authors 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 person-years) and 1.6% (20 deaths/1260 person-years) 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 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 diphtheria–tetanus–pertussis vaccine.

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 of this study

- 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 four to six monthly intervals.[1] However, according to a preliminary report[2] of a recent cluster randomised trial involving >1 million Indian children, VAS compared with 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 Immunisations reached high coverage.[6] We recently reanalysed data from the 1989 to 1991 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 different strategies have been employed in the urban area: *fixed post campaigns* where the mother brings her child to the campaign team and *door-to-door campaigns* where all houses are visited by the campaign team. The fixed post campaigns require less staff but have lower coverage. In December 2007 and June/July 2008, fixed post campaigns distributing vitamin A and mebendazole were conducted. We registered all children receiving VAS and mebendazole during these campaigns and assessed survival prospectively to compare survival in supplemented and non-supplemented children overall and by sex and vaccination status.

SUBJECTS AND METHODS

Setting

The Bandim Health Project (BHP) operates a health and demographic surveillance system (HDSS) covering a population of 102 000 people in six suburban districts of Bissau, the capital of Guinea-Bissau, West Africa. All households are visited monthly to register new births, pregnancies and deaths. Children below the age of 3 are followed through three monthly visits to ascertain vital status and vaccination status. At the first visit, after birth information on socioeconomic status is collected. The indicators include maternal characteristics (education and ethnicity) and household characteristics (type of roofing, availability of bathroom and electricity). All children aged 6–35 months living in the urban study area on the first day of the VAS campaigns in 2007 and 2008 were eligible for the present study. Since children below 3 years of age are followed intensively through the HDSS, these children have been the focus for the present study.

WHO classifies Guinea-Bissau as having a public health problem of VAS.[12] We have previously found that 16% of 4-month-old children were vitamin A deficient (retinol-binding protein concentration equivalent to plasma retinol <0.70 µmol/l).[13] During 2007–2008, we assessed vitamin A status in 181 children aged 6–17 months presenting for vaccinations and found that 70% of the children had a retinol-binding protein concentration equivalent to plasma retinol <0.70 µM (unpublished data).

Campaign information

National VAS campaigns were conducted by the Ministry of Health from 14 to 18 December 2007 and 30 June to 4 July 2008. Children aged 6–11 months received 100 000 IU vitamin A, children aged 12–35 months received 200 000 IU vitamin A and 250 mg mebendazole and children aged 36–59 months received 200 000 IU vitamin A and 500 mg mebendazole. Trained BHP field assistants were present at all posts. The field assistants brought a list of all children registered in the area and noted on this list whether a child had received VAS and mebendazole at the post. The lists contained information on vaccines registered prior to the campaign. If the vaccination card was seen, the assistant noted this on the list, verified the already registered vaccines and updated the information if new vaccines had been received.

During the weeks after the campaigns, all children who had not been seen during the campaigns were visited. The caretaker was asked whether the child had received VAS elsewhere. The vaccination card was seen and information on vaccines verified and updated. If the child and caretaker were not present, the household was visited up to three times, and if no one could provide information, the campaign status was classified as unknown.

During the 2007 campaign, we discovered that the vitamin A capsules given in two of the six districts had been produced in November 2004 and had passed the expiry date 1 month before. The capsules were immediately replaced by a new batch. We sent two of the expired 200 000 IU capsules to 'as Vitas Oslo Innovation Center' (Oslo, Norway) to have their vitamin A content measured. The vitamin A contents were slightly lower than expected: 167 569 IU and 154 863 IU. Capsules used in the 2008 campaign were all from a new stock.

Vaccine information

The vaccination schedule in Guinea-Bissau when this study was conducted was BCG and OPV at birth, three doses of DTP vaccines at 6, 10 and 14 weeks of age and MV at 9 months of age. A booster DTP vaccine at 18 months of age was given in the routine vaccination programme until March 2007 after which it was only

given in a randomised trial (trial A below). In August 2008, the DTP vaccine at 6, 10 and 14 weeks was replaced by the pentavalent (DTP−HiB−HBV) vaccine and a yellow fever vaccine was added to be given with MV at 9 months of age.

During the study period, three trials of alternative vaccination strategies took place in the BHP area. First, between October 2005 and October 2009, trial A enrolled children aged 18 months in a randomised trial of booster DTP. Children who had completed their primary immunisation schedule of three doses of DTP and an MV were randomised to receive booster DTP +OPV or OPV only. Second, between August 2003 and April 2007, trial B enrolled children aged 4.5 months in a randomised trial of early MV. Children were randomised to an extra dose of MV at 4.5 months or no vaccine; a subgroup of the controls received an extra MV at 18 months of age.[14] Third, between October 2005 and April 2008, trial C enrolled children aged at least 9 months who were delayed in relation to the vaccination schedule and presented at the health centres to receive MV and the third DTP vaccine. They were randomised to receive both vaccines and booster DTP at 18 months of age versus MV only and no booster DTP[15] (supplementary figure). Furthermore, in May 2006, a measles vaccination campaign was conducted in which children aged 6 months to 14 years were given an MV; thus, the oldest children in the present study often had MV received in this campaign as their most recent vaccine.

For the present study, vaccine status was ascertained during the campaigns. Furthermore, as part of the BHP routines, vaccines are registered daily at the three health centres in the study area and through the tri-monthly home visits and as a part of the trials described above. We linked the campaign information with information from the above sources, using only information collected prior to the campaign. In case of discrepancy, information from the trials was considered superior. If no trial information was available and if two of the other sources agreed, this information was accepted. Vaccination information obtained during the campaign was updated for 12% (1088/8812) of the children: 9% based on data collected at the health centres, 1% based on routine surveillance data and 2% based on trial data. Except for children included in trial A, OPV is almost always given with BCG or DTP. In line with previous studies,[11 16] children who received OPV along with another vaccine were classified according to the co-administered vaccine, that is, children who received DTP alone (161 children) and DTP+OPV (3519 children) were classified as DTP and children who received MV+OPV (205 children) and MV alone (2609) were classified as MV.

Follow-up

All children below 3 years of age were followed by the HDSS. For children who died, a locally adapted version of the INDEPTH verbal autopsy[17] was conducted. Cause of death was determined by a local paediatrician.

Statistical analyses

Children entered the analysis on the day we knew their VAS campaign status (supplemented, non-supplemented or no information). Thus, a child who was registered to have participated in the campaign during the days of the campaign contributed time at risk from the day of supplementation. A child who had campaign status registered after the campaign contributed time from the day the information was obtained. Follow-up of children was censored when a subsequent campaign was initiated (30 June 2008 for the 2007 campaign and 9 January 2009 for the 2008 campaign), 3 years of age, migration or reception of VAS in a trial, whichever came first. Some children were enrolled in a randomised trial of VAS at vaccination contacts after 6 months of age (Trial registration: clinicaltrials.gov, NCT00514891). These children were censored from follow-up on the date of receiving VAS in the trial (figure 1).

We examined whether the background factors were evenly distributed between children in the VAS, no VAS and no information group, as well as between the two main groups (VAS and no VAS) using χ^2 tests for categorical variables and rank-sum test for age at the time of the campaign.

Survival was examined in Cox proportional hazards models with age as underlying time; thus, age was inherently controlled for in all models. The proportional hazards assumption was tested using Schoenfeld residuals. Crude and adjusted estimates are presented. The 2007 campaign took place in the dry season and the 2008 campaign in the rainy season. A priori we had decided to adjust all estimates for sex and campaign since sex and season of supplementation have been shown to be important determinants of the effect of VAS in our previous studies.[8 18 19] We furthermore adjusted the comparison of supplemented and non-supplemented children for the background factors which were associated with participation in the VAS campaigns. In all analyses, we investigated whether the survival of supplemented and non-supplemented children varied by sex and campaign (season). We furthermore investigated interactions with vaccination status. Interaction between VAS and potential effect modifier was evaluated by Wald statistics. Among children who had OPV as the most recent vaccine prior to the campaign, no deaths occurred among children who did not receive VAS. Therefore, the potential interaction between VAS and OPV was tested by Mantel−Haenszel stratification. Finally, previous studies have indicated that the effect of VAS in girls depends on whether or not they have received VAS on a previous occasion.[20 21] We therefore investigated whether survival after the 2008 campaign varied with reception of VAS in the 2007 campaign.

RESULTS

In the 2007 campaign, we followed 5567 (92%) of the 6026 children aged 6−35 months registered in the study area. Reasons for losses to follow-up are given in figure 1.

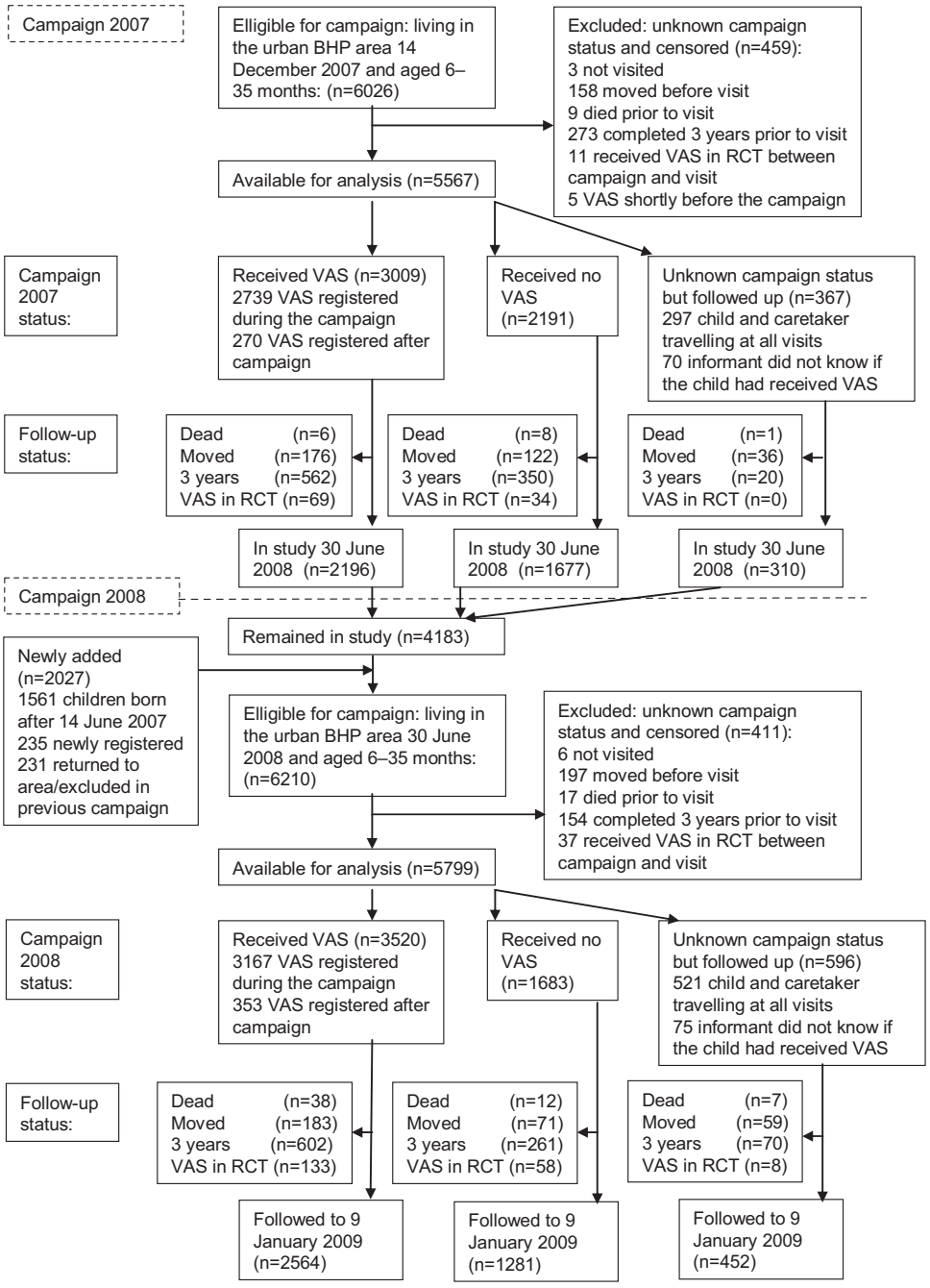

Figure 1 Flowchart of children eligible for campaign participation. BHP, Bandim Health Project; RCT, randomised controlled trial; VAS, vitamin A supplementation.

For 7% (367/5567), we obtained no information on campaign status since the family was travelling at all visits. Among the remaining 5200 children, 3009 (58%) had received VAS and 2191 (42%) had not. In the 2008 campaign, we followed 5799 (93%) of 6210 children. For 10% (596/5799), we were unable to obtain information on campaign status. Among the remaining 5203 children, 3520 (68%) had received VAS and 1683 (32%) had not (figure 1). Median length of follow-up and IQR was 6.2 (4.4–6.4) months for supplemented, 4.2 (3.2–5.1) months for non-supplemented and 2.9 (1.8–3.4) months for children for whom we did not obtain information.

The distribution of background factors is shown in table 1. Children for whom we had no information on campaign status had lower socioeconomic status, were more likely to be from the Muslim ethnic groups (Fula and Mandinga) and were younger. These children also tended to have higher mortality, crude mortality rate ratio (MRR)=2.17 (0.96; 4.94) (table 2). In contrast, there were only few differences in background factors between supplemented and non-supplemented children. Non-supplemented children were more likely to have mothers with no formal education ($p<0.001$), to belong to the Muslim ethnic groups ($p<0.001$) and were less likely to have their vaccination card inspected ($p<0.001$). Furthermore, more supplemented children were enrolled in trials and as a result more supplemented children had received OPV as the most recent vaccine in 2007 ($p=0.05$). In 2008, the distribution of

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

	2007					2008				
				p For different distribution					p for different distribution	
	VAS	No VAS	No information	All	VAS vs no VAS	VAS	No VAS	No information	All	VAS vs no VAS
Number	3009	2191	367			3520	1683	596		
Sex (male)†	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 (IQR)	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
Vaccine information										
Seen vaccination card†	2700 (90)	1765 (81)	34 (9)	<0.001	<0.001	3088 (88)	1259 (75)	15 (3)	<0.001	<0.001
Last vaccine at the time of the campaign										
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)		
Socioeconomic background										
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)		

Continued

Table 1 Continued

	2007					2008				
				p For different distribution					p for different distribution	
	VAS	No VAS	No information	All	VAS vs no VAS	VAS	No VAS	No information	All	VAS vs no VAS
Trial enrolments§										
Enrolled in trial A prior to campaign†	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†	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†	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 two levels are presented by one level.
‡No information compared with VAS/No information compared with no VAS.
§Trial A: RCT among 18-month-old children: booster DTP+OPV versus OPV only; trial B: RCT among 4.5-months-old children: extra dose of MV at 4.5 months versus 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 versus MV only.
DTP, diphtheria–tetanus–pertussis vaccine; MV, measles vaccine; OPV, oral polio vaccine; RCT, randomised controlled trial; VAS, vitamin A supplementation.

different vaccines did not differ between supplemented and non-supplemented children (p=0.23) (table 1).

Effect of VAS

There was no significant difference in mortality between supplemented and non-supplemented children, the crude MRR being 0.93 (0.55; 1.58). When the estimate was adjusted for sex, campaign, maternal education, ethnicity and inspected vaccination card, the difference in mortality between supplemented and non-supplemented children was 0.78 (0.46; 1.34) (table 2). The effect did not differ significantly for children aged 6−11 months who received 100 000 IU vitamin A (adjusted MRR=1.14 (0.41; 3.19)) and children above 12 months who received 200 000 IU vitamin A and 250 mg mebendazole (adjusted MRR=0.69 (0.36; 1.29)) (p=0.41, test of interaction) (table 2). The effect did not differ for boys and girls (p=0.72).

Mortality was much higher in the rainy season after the 2008 campaign (57 deaths/2208 person-years) than in the dry season after the 2007 campaign (15 deaths/2154 person-years); 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

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 MRR (95% CI)			Adjusted MRR (95% CI)*		
		All	Boys	Girls	All	Boys	Girls
All							
VAS	15.3 (44/2873)	0.93 (0.55 to 1.58)	0.85 (0.44 to 1.67)	1.07 (0.45 to 2.57)	0.78 (0.46 to 1.34)	0.73 (0.37 to 1.43)	0.89 (0.37 to 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 to 4.94)	2.04 (0.73 to 5.71)	2.40 (0.62 to 9.27)	2.10 (0.75 to 5.89)	1.96 (0.59 to 6.57)	2.35 (0.53 to 10.4)
2007							
VAS	4.4 (6/1352)	0.40 (0.14 to 1.15)	0.54 (0.15 to 1.85)	0.18 (0.02 to 1.69)	0.38 (0.13 to 1.10)	0.52 (0.15 to 1.79)	0.16 (0.02 to 1.59)
No VAS	10.9 (8/731)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
2008							
VAS	24.4 (38/1521)	1.04 (0.54 to 2.00)	0.88 (0.39 to 2.00)	1.37 (0.46 to 4.09)	1.02 (0.53 to 1.96)	0.86 (0.38 to 1.96)	1.32 (0.44 to 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
Children aged 6–11 months							
VAS	23.3 (16/687)	1.35 (0.48 to 3.76)	1.68 (0.47 to 6.07)	0.84 (0.15 to 4.66)	1.14 (0.41 to 3.19)	1.41 (0.40 to 5.10)	0.72 (0.13 to 3.98)
No VAS	17.0 (5/294)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Children aged 12–35 months							
VAS	12.8 (28/2186)	0.81 (0.43 to 1.51)	0.62 (0.27 to 1.39)	1.19 (0.43 to 3.30)	0.69 (0.36 to 1.29)	0.53 (0.23 to 1.20)	0.98 (0.35 to 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.
MRR, mortality rate ratio; PYRS, person-years; VAS, vitamin A supplementation.

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 person-years) than children who had not received VAS in 2007 but received VAS in 2008 (15 deaths/394 person-years), 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, one after the 2007 campaign (no VAS) and nine after the 2008 campaign (eight VAS and one 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 (three VAS and one 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, three no VAS and one no information); respiratory infection, 17 (26%, nine VAS, six no VAS and two no information); malaria, 14 (21%, five VAS, seven no VAS and two no information); fever of unknown origin, three (5%, two VAS and one no VAS); cerebral palsy, two (3%, one VAS and one no VAS) and accident, one (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), respectively, presumably reflecting that they were less likely to seek early treatment or to be absent in the rural areas where the incidence of severe and untreated malaria would be higher.

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)
Last received vaccine before the campaign§										
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	0.06	0.38	0.47	0.34	0.04	0.31	0.41
	No VAS	26.9 (9/335)	(0.17 to 1.01)		(0.12 to 1.19)	(0.11 to 2.11)	(0.14 to 0.85)		(0.10 to 0.96)	(0.09 to 1.85)
DTP	VAS	20.1 (21/1041)	1.41	Reference	1.48	1.34	1.29	Reference	1.37	1.21
	No VAS	14.5 (6/414)	(0.57 to 3.51)		(0.41 to 5.32)	(0.37 to 4.87)	(0.52 to 3.22)		(0.39 to 4.93)	(0.33 to 4.43)
DTP	VAS	16.5 (1/61)	0.24	0.17	0	NA	0.21	0.16	0	NA
+MV	No VAS	63.3 (2/32)	(0.02 to 2.62)				(0.02 to 2.30)			

*Assessed among children with a seen vaccination card, thus excluding six deaths among children with no seen card (three VAS and three no VAS).
†Adjusted for sex, campaign, ethnicity and maternal education.
‡Test for different mortality rate in supplemented and non-supplemented children.
§Fifty-seven children with BCG or no vaccination excluded due to small numbers (one death: VAS).DTP, diphtheria–tetanus–pertussis vaccine; MV, measles vaccine; MRR, mortality rate ratio; OPV, oral polio vaccine; PYRS, person-years; VAS, vitamin A supplementation.

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 1 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, for example, 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

The strengths of our study include that information was collected on the individual level and that the children were followed prospectively. By only allowing the children to contribute observation time in the VAS or no VAS group from the day the information was obtained, we avoided introducing survival bias in the analysis (ie, obtaining positive information only from surviving children).[23]

Vitamin A content was assessed in the expired capsules which some campaign recipients received in 2007. Though the content was lower than the original 200 000 IU, the content was still high. Hence, we believe that all supplemented children received a relevant dose of 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.[24] According to national data, VAS coverage was considerably higher outside the capital and hence outside the urban study area (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 19 24] 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 with placebo recipients, which may have introduced selection biases in the comparison of VAS recipients and non-recipients. However, the varying effect by vaccination status is unlikely to be explained by simple selection bias. We had expected that a negative effect of VAS with DTP would be most pronounced for girls.[8 25 26] This was not the case. However, recent studies have indicated that mortality after VAS is lowered in girls who have received VAS on a previous occasion,[20 21] and this was also the case in the present study. Thus, repeated dosing may have alleviated the negative effects of VAS with DTP in girls.

Mortality is usually 15% higher in the rainy (May to November) than in the dry season,[27] and though this may explain some of the differences in mortality after the 2007 and 2008 campaigns, it is unlikely to explain the threefold higher mortality. The difference may partly be due to a more beneficial effect of VAS in the dry than in the rainy season (table 2). We have previously found strong differences in the effect of neonatal VAS by season. In a randomised trial, neonatal VAS benefited infant survival among normal birth weight neonates in the dry season but was associated with increased mortality in the rainy season.[18] However, we found no seasonal differences in the effect of neonatal VAS to low birth weight infants[26] and no strong seasonal pattern was seen in Ghana where an analysis by season has been reported.[28] Though numbers were small and the difference was not statistically significant, VAS appeared to be associated with an increased risk of dying of diarrhoea during the cholera epidemic in 2008. This contrasts with a recent meta-analysis that estimates 28% (9%; 43%) reduction in diarrhoea-related mortality.[1]

The effect of VAS on mortality has most often been ascribed to prevention and treatment of vitamin A deficiency.[29] We have previously found poorer vitamin A status in children of non-educated mothers.[13] In the current study, we found no evidence of a beneficial effect limited to lower socioeconomic groups, which presumably are the most vitamin A deficient.

Interpretation

The present study as well as previous observations support that the effect of VAS differs depending on vaccination status being beneficial when provided after MV[3 8] but not after DTP. This is unlikely to be explained by selection bias since bias is unlikely to work in different direction for children who had MV or DTP as their most recent vaccine. It is also unlikely to be explained by differences in vitamin A deficiency.

We do not know what may have caused the observed tendency for a differential effect of VAS on mortality

between the two campaigns. Previous studies have demonstrated seasonal variance in pathogen prevalences with distinct season-related epidemics of rotavirus,[30] cryptosporidium,[31] respiratory syncytial virus[32] as well as a malaria.[33] The effect of VAS has been show to differ depending on the pathogen,[34] and varying pathogen prevalences may therefore contribute to the seasonal differences. Based on the verbal autopsy data, VAS may have been associated with a higher risk of dying from cholera/diarrhoea during the cholera epidemic in 2008. An alternative explanation is the generally increased prevalence of infectious diseases in the rainy season, which may lead to impaired uptake and increased excretion of vitamin A.[35] This could explain the lack of effect during the rainy season but would not explain the vaccine differential effect. Regardless of the mechanism, the potential differential seasonal effects of VAS deserve further investigation.

Implications and conclusions

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 1 million children in India, where 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.

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Competing interests None.

Contributors ABF, PA and CSB planned the study. ABF, CB, IB, SB-S 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.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data available.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

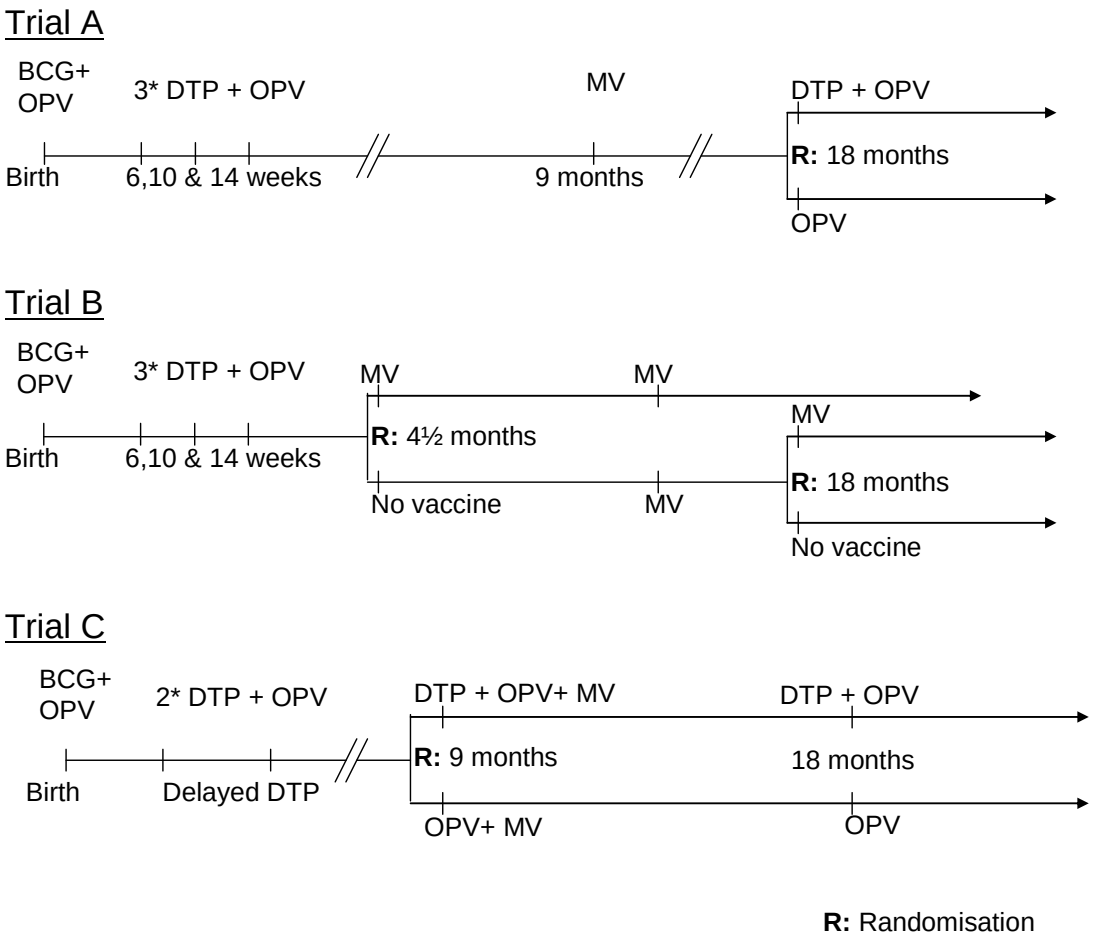
	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract *–page 1*
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found *–page 2*
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported – *page 3*
Objectives	3	State specific objectives, including any prespecified hypotheses *–page 3*
Methods		
Study design	4	Present key elements of study design early in the paper *–page 3-5*
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection *–page 4*
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *–page 4+6*
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed -*NA*
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable *–page 2,5-7*
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group *–page 4-6*
Bias	9	Describe any efforts to address potential sources of bias *–page 6*
Study size	10	Explain how the study size was arrived at *–page 4*
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why *–page 6*
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding *– page 6*
		(*b*) Describe any methods used to examine subgroups and interactions *–page 7*
		(*c*) Explain how missing data were addressed *–page 4*
		(*d*) If applicable, explain how loss to follow-up was addressed *NA*
		(*e*) Describe any sensitivity analyses *NA*
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed – *Figure 1*
		(b) Give reasons for non-participation at each stage – *Figure 1*
		(c) Consider use of a flow diagram– *Figure 1*
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders – *Table 1*
		(b) Indicate number of participants with missing data for each variable of interest – *Table 1*
		(c) Summarise follow-up time (eg, average and total amount) – *Table 2+3*
Outcome data	15*	Report numbers of outcome events or summary measures over time – *Table 2+3*

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included) – *Table 2+3*
		(*b*) Report category boundaries when continuous variables were categorized *NA*
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period *NA*
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses –*page 6*
Discussion		
Key results	18	Summarise key results with reference to study objectives –*page 9*
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias –*page 10*
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence –*page 11*
Generalisability	21	Discuss the generalisability (external validity) of the study results –*page 11*
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based –*page 13*

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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



R: Randomisation