Background
WHO recommends high-dose Vitamin A supplementation (VAS) at vaccination contacts after 6 months of age. It has not been studied whether the effect of VAS on mortality depends on the type of vaccine. We have hypothesized that VAS administered with measles vaccine (MV) is more beneficial than VAS with diphtheria–tetanus–pertussis (DTP) vaccine. We assessed the effect of VAS administered with different vaccines during national immunization days (NIDs).

Methods
In 2003, VAS was distributed during NIDs in Guinea-Bissau. Children 6 months or older were given VAS, and if they were missing vaccines, these were often given as well. We compared survival between children who had received VAS alone, VAS with DTP or DTP + MV, or VAS with MV. We also compared the survival between participants and non-participants. We followed 6- to 17-month old children until 18 months of age and analysed survival in Cox models.

Results
Twenty of 982 VAS-recipients died during follow-up. The mortality rate ratio (MRR) for VAS with DTP + MV or VAS with DTP was 3.43 (1.36–8.61) compared with VAS only. There were no deaths among those who received VAS with MV alone (P = 0.0005 for homogeneity of VAS effects). Children who received VAS with DTP had higher mortality than non-participants who did not receive VAS [MRR = 3.04 (1.31–7.07)].

Conclusion
The study design does not allow for definite conclusions. However, the results are compatible with our a priori hypothesis that VAS is more beneficial when given with MV and potentially harmful when given with DTP. Randomized trials testing the impact on mortality of the current WHO policy seem warranted.

Keywords
Vitamin A, diphtheria–tetanus–pertussis vaccine, measles vaccine, child mortality, low income populations

Introduction
The WHO recommends high-dose vitamin A supplementation (VAS) at vaccination contacts after 6 months of age. The recommendation is based on several large intervention trials showing VAS to be associated with a 23–30% reduction in overall
mortality in children aged 6 months to 5 years of age. However, none of those studies administered VAS simultaneously with vaccines, and hence, it is unknown whether the current WHO policy of providing VAS in combination with vaccines has a similar mortality-reducing effect as VAS administered alone. In particular, it has not been studied whether VAS is equally beneficial when administered with measles vaccines (MV) or with other vaccines such as diphtheria–tetanus–pertussis (DTP) vaccine.

We have previously hypothesized that VAS and DTP vaccine may interact negatively. The hypothesis was, among others, based on the observation that the effect of VAS given between 1 and 5 months of age, i.e. the time window in which DTP vaccine is administered, is significantly different from the beneficial effect of VAS given at birth or after 6 months of age. To date, no study has reported a beneficial effect of VAS between 1 and 5 months of age (Table 1).

In Guinea-Bissau, VAS has been distributed in connection with yearly national immunization days (NIDs). Mostly, VAS has been given with oral polio vaccine (OPV). However, the NIDs in 2003 were different. Children aged 6–60 months were given VAS, and if vaccines were missing, these were administered at the same time. Thus, children received either VAS alone, VAS with DTP, VAS with MV or VAS with DTP + MV.

In the study area of the Bandim Health Project (BHP), study assistants accompanied the health workers and registered all children and their treatment during the NIDs. All study area children were followed routinely through the BHP's demographic surveillance system. We took the opportunity to study the potential differential effects of VAS on mortality when administered with different vaccines. We focused our study on 6- to 17-month old children, since they were too young to have received VAS in previous campaigns. We followed up the children till the age of 18 months, since many children receive DTP booster vaccine at the age of 18 months or were enrolled in a BCG revaccination study at the age of 19 months.

### Methods

#### Setting

The BHP has a demographic surveillance system in six suburban districts of the capital of Guinea-Bissau and covers approximately 90,000 inhabitants. Until 3 years of age, all children are visited every third month to obtain information on vital status, hospitalizations, vaccinations, measles infections, etc. Mid-upper-arm circumference (MUAC) is measured. Information on vaccinations and survival is also registered by the BHP at the three health centres in the study area and at the only paediatric ward in the country.
As recommended by the WHO, children in Bissau are to receive BCG and OPV at birth, three DTP/OPV vaccines at 6, 10 and 14 weeks of age and MV at 9 months of age. The WHO recommendations for VAS after 6 months of age are not implemented in Guinea-Bissau; instead, VAS is being delivered during NIDs, usually in combination with OPV.

Procedures
The Ministry of Health in Guinea-Bissau has organized yearly immunization days since 2001. The NIDs in November 2003 differed from previous and later campaigns offering VAS and OPV. In 2003, all children aged 6–60 months were offered VAS. Additionally, infants <12 months of age should receive missing vaccines, since they are the target group for the vaccination programme. In contrast to the previous NIDs, the NIDs in 2003 were not organized as door-to-door visits; instead, all children were called to fixed vaccination posts. These posts were staffed by three to four health workers. The mothers were told to bring the vaccination card. In the study area of the BHP, trained fieldworkers were present at all posts with a list of all children in the area. The field worker compared the information on the list with the vaccination card of the child. Missing information was noted on the list as well as the information regarding the vaccines the child received that day. If a mother had not brought a vaccination card, the child was vaccinated if the child was missing one or more vaccines on the list and as reported by the mother.

Children aged 6–60 months were eligible for VAS if they had not received VAS within the last month and were not enrolled in an ongoing trial of VAS at birth.13 Children below 12 months of age received 100 000 IU VAS as recommended by the WHO. Provided maternal consent was given, children aged 12 months and older were randomized to 200 000 IU of VAS as recommended by WHO or half of this dose. VAS was distributed orally from gelatine capsules containing retinyl palmitate (International Dispensary Association, The Netherlands).

Children missing one or more DTP vaccines were offered DTP vaccine together with VAS. Children who were above 9 months of age and missing MV were offered a MV together with VAS. Some children above 9 months of age missed DTP as well as MV and were given both.

The NIDs were not well organized. First, the public information level was low and many mothers never heard of the campaign, and therefore did not attend. Second, apparently there was confusion among the health workers as to who should receive missing vaccines. Some teams apparently had misunderstood and provided missing vaccines also to children above 12 months of age. Some teams ran out of vaccines or syringes. Some calculated the age wrongly. As a result, some children who were missing one or more vaccines according to the schedule did not receive any vaccines.

Follow-up
We followed all study area children for mortality until 18 months of age, at which age most received booster doses of DTP and OPV and were enrolled in a study of revaccination with BCG.14 Information on survival and vaccination status was obtained from the BHP registration system. If a child died, a simple interview was performed to establish the cause of death. Children in the BHP registration system, but not seen during the NIDs, were visited by a field assistant during the months after the NIDs to ascertain the reason for non-participation.

Nutritional status
We utilized the information on MUAC from the routine visits every 3 months to assess the nutritional status in the subgroup of children who had had their MUAC measured within 3 months before the NIDs.

Statistical analyses
Mortality rates (MR) were presented per 1000 person-years of risk (pyrs). Survival was assessed in Cox proportional hazards models with age as the underlying time variable. Thus, age was inherently controlled for in the model. Effects are shown as MR ratios (MRR) with 95% CI. Children were censored at 18 months of age or if they moved. In a second analysis, we censored children when they got a subsequent vaccine. Three children who died due to an accident were censored in the analysis at the time of death. Eight children who developed a measles rash within the 2 weeks after supplementation were excluded, since they were considered infected prior to the NIDs. Among them, a recipient of VAS + MV died 12 days after supplementation.

All comparisons between recipients of VAS + MV vs other groups were done using a stratified log-rank test, since there were no deaths in that group.

All analyses were adjusted for suburb (Bandim, Belem/Mindara or Cuntum) and maternal schooling (none, any or unknown). The comparisons of participants and non-participants were furthermore adjusted for ethnicity (Pepel, Fula and others).

We tested the size of the estimates with and without control for nutritional status in the subgroup that had MUAC measured within 3 months prior to the NIDs. This was done by adding to the model MUAC and age at measurement of MUAC as continuous variables.

Children above 12 months of age were randomized to full or half the WHO recommended VAS dose. The trial is registered with clinicaltrials.gov, number NCT00168623. We have previously shown that half the recommended dose was more beneficial for girls.15 The results of the randomized trial will be presented separately. This observational subgroup
study of children between 6 and 17 months of age did not have power to investigate the association between dose of VAS and mortality. Among the 648 children who were above 12 months of age, eight deaths occurred, five in the group receiving the low dose (three boys and two girls) and three in the group receiving the high dose (two boys and one girl). We did not consider dose further in the analyses.

Results

Figure 1 shows the flow chart of infants enrolled in the study. Table 2 shows the baseline characteristics by treatment group. Treatment was associated with suburb, age of the child and maternal education, and with MUAC. We adjusted all subsequent analyses for age, suburb and maternal education. The analyses including all children were furthermore adjusted for sex. In the subgroup of children from whom a valid MUAC was available, we adjusted the estimates for MUAC.

The effect on mortality of VAS according to vaccination

Twenty (MR = 50/1000 pyrs) of the 982 children died during follow-up. Compared with children receiving VAS alone, the MRR for children receiving VAS with DTP + MV was 3.60 (95% CI = 1.22–11); for VAS with DTP alone, it was 3.19 (0.89–11). The MRR for VAS with MV alone was 0. Since the effect of VAS given with DTP was similar to that of VAS given with DTP + MV, we combined the two groups to gain power to study the effect of VAS given with different vaccines. As seen in Table 3, there was a negative effect of receiving VAS with any DTP in contrast to the effect of receiving VAS with MV alone.

The results were unchanged if we included only the 835 (85%) children who had their vaccination card seen at inclusion; VAS with any DTP was associated with a MRR of 4.10 (1.48–11) compared with VAS alone (P = 0.0003 for homogeneity of VAS with DTP vs VAS with MV).

Three deaths occurred after a subsequent vaccine had been received. When censoring on the date of the next vaccination, the estimate for VAS with any DTP vs VAS alone became 2.82 (1.04–7.66) (P = 0.0003 for homogeneity of VAS with DTP vs VAS with MV).

MUAC had been measured within 3 months in 657 (67%) of the children. The proportion of children who had their MUAC measured was comparable in the different treatment groups (P = 0.21). Among children who had had their MUAC measured, 12 children died. Compared with receiving VAS alone, the estimate of children receiving VAS with DTP + MV was 2.95 (0.57–15); for VAS with DTP alone it was 2.82 (0.56–14), and for VAS with any DTP it was 2.88 (0.83–10). MUAC tended to be lower among those who received VAS with DTP + MV compared with children who received VAS only (P = 0.07). However, control for MUAC did not change the estimates much, though the confidence intervals were naturally wider; VAS with DTP + MV: 2.82 (0.53–15); VAS with DTP alone: 2.45 (0.46–13); VAS with any DTP: 2.62 (0.72–9.50).

The effect of DTP without VAS

A total of 532 infants came to the health post but did not receive VAS, because they took part in a VAS-at-birth trial (Figure 1). They received vaccines if...

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*Three children received only OPV, none died.

Figure 1  Flow diagram. Children aged 6–17 months during national immunization days (Guinea-Bissau, 2003–2004)
missing, like the other participants. Eleven children died (MR = 29/1000 pyrs); among 51 DTP recipients, two children died (MR = 55/1000 pyrs).

Comparison of VAS recipients and non-participants
Seventeen children received VAS elsewhere. Among the remaining 878 children who did not participate in the NIDs, 20 (MR = 50/1000 pyrs) died. This resulted in a MRR for participants vs non-participants of 1.11 (0.59–2.08) controlled for suburb, maternal education, sex and ethnicity. There was no difference in MUAC between participants and non-participants (P = 0.51).

Excluding children who were away/travelling (n = 441) or too ill to participate (n = 48) among the non-participants, the MRR for participants vs non-participants was 2.05 (0.76–5.54). Comparing the VAS plus any DTP group with the non-participants, the MRR was 3.04 (1.31–7.07). There were no differences between the two sexes.

Discussion
As VAS is an important immunomodulator, several groups have studied whether simultaneous administration of VAS influences the immune response to specific vaccines. Apart from one study, no evidence was found for a negative effect of VAS on the immune response to OPV and MV, and VAS may even be associated with increased antibody responses to Hepatitis B vaccine and to MV, particularly in boys. No study, however, has addressed the effect

Table 2 Baseline characteristics of children receiving VAS alone or with missing vaccines and of non-participants (Guinea-Bissau, 2003)

| Participants | VAS alone | VAS + DTP | VAS + MV + DTP | VAS + MV | No VAS | N | Non-participants |  |
|--------------|-----------|-----------|----------------|----------|--------|---|------------------|---|
| N            | 730       | 48        | 88             | 116      | 531    | 895|                  |   |
| Median age, months (10–90 percentiles) | 13.5 (8.0–17.2) | 12.6 (7.3–17.0) | 12.9 (8.9–17.0) | 10.5 (8.7–16.3) | 8.9 (6.6–11.5) | 11.3 (7.5–16.7) | |
| Sex | | | | | | | |
| Boys | 368 (50%) | 23 (48%) | 52 (59%) | 59 (51%) | 277 (52%) | 448 (50%) | |
| Girls | 362 (50%) | 25 (52%) | 36 (41%) | 57 (49%) | 254 (48%) | 447 (50%) | |
| Suburb | | | | | | | |
| Bandim | 283 (39%) | 21 (44%) | 44 (50%) | 37 (32%) | 306 (58%) | 398 (44%) | |
| Belem/Mindara | 172 (24%) | 5 (10%) | 5 (6%) | 10 (9%) | 102 (19%) | 151 (17%) | |
| Cuntum | 275 (38%) | 22 (46%) | 39 (44%) | 69 (59%) | 123 (23%) | 346 (39%) | |
| Ethnicity | | | | | | | |
| Pepels | 220 (30%) | 15 (31%) | 35 (40%) | 32 (28%) | 164 (31%) | 258 (29%) | |
| Fulas | 81 (11%) | 8 (17%) | 10 (11%) | 15 (13%) | 59 (11%) | 163 (18%) | |
| Others | 429 (59%) | 25 (52%) | 43 (49%) | 69 (59%) | 308 (58%) | 474 (53%) | |
| Maternal schooling | | | | | | | |
| No | 193 (26%) | 16 (33%) | 45 (51%) | 41 (35%) | 101 (19%) | 275 (31%) | |
| Yes | 446 (61%) | 26 (54%) | 32 (36%) | 67 (58%) | 354 (67%) | 500 (56%) | |
| Unknown | 91 (12%) | 6 (13%) | 11 (13%) | 8 (7%) | 76 (14%) | 120 (13%) | |
| Mean mid-upper-arm-circumference (SD) | 145.6 (12.4) | 142.7 (10.8) | 142.3 (12.6) | 143.5 (12.5) | 144.1 (12.3) | 143.6 (11.9) | |

aSignificant differences in age between VAS alone and VAS + DTP (t-test = 0.01) and VAS + MV (<0.0001) and between VAS + MV and VAS + DTP + MV (P = 0.003). Non-participants younger than participants (P < 0.0001). No-VAS recipients younger than VAS recipients (P < 0.0001).

bP for equal distribution among participants < 0.0001. P for equal distribution between non-participants and participants = 0.06.

P for equal distribution between non-participants and participants < 0.0001. P for equal distribution between no-VAS recipients and VAS recipients < 0.0001.

cP for equal distribution among participants < 0.0001. P for equal distribution among participants < 0.0001. P for equal distribution between no-VAS recipients and VAS recipients < 0.0001.

dControlled for age, MUAC tended to be higher in VAS alone compared with VAS + DTP + MV (P = 0.07). No-VAS recipients higher MUAC than VAS recipients (P = 0.02).
Table 3 Effect on mortality of receiving vitamin A supplementation with different types of vaccines (Guinea-Bissau 2003–04)

|                | All                      | Boys                     | Girls                    |
|----------------|--------------------------|--------------------------|--------------------------|
|                | Deaths/pyrs (MR)         | MRR                      | Deaths/pyrs (MR)         | MRR                      | Deaths/pyrs (MR)         | MRR                      |
| VAS alone      | 12/282.4 (42)            | 1 (ref)                  | 6/141.0 (43)             | 1 (ref)                  | 6/141.5 (42)             | 1 (ref)                  |
| VAS + any DTP  | 8/57.2 (140)             | 3.43 (1.36–8.61)         | 5/28.5 (175)             | 4.71 (1.17–16)           | 3/28.7 (105)             | 2.21 (0.52–9.44)         |
| VAS + MV       | 0/59.3 (N/A)             | 0 deaths                 | 0/30.8 (N/A)             | 0 deaths                 | 0/28.5 (N/A)             | 0 deaths                 |
| P for homogeneity | 0.0005                  | 0.007                    |                          |                          | 0.03                     |                          |
| VAS + any DTP  |                          |                          |                          |                          |                          |                          |
| VAS + MV       |                          |                          |                          |                          |                          |                          |

*aMR = deaths/1000 pyrs.

*Tested using stratified log-rank test. All estimates and P-values are adjusted for age, suburb and maternal schooling. The overall estimate was furthermore adjusted for sex.

on mortality of VAS given with vaccines. In the present study, VAS with DTP was associated with increased mortality when compared with VAS alone. This contrasted with the effect of VAS with MV, which if anything was associated with decreased mortality.

The present study was observational. Participation was voluntary and depended on the mother bringing the child to a vaccination post, all children received VAS unless they were in a VAS-at-birth trial and eligible children were not randomized to a vaccine. There are considerable differences between the baseline characteristics of the different groups, especially the small group of children missing both DTP and MV seemed to be different. We have attempted to control for potential confounders, and it should be noted that age is inherently controlled for in the Cox models we used. Nonetheless, there could be considerable residual confounding, and all comparisons between participants and non-participants and between vaccine recipients and non-vaccine-recipients should be judged with caution. However, there are several observations in the present study, which suggest that our findings are not merely the result of confounding.

First, it could be speculated that those who received vaccines during the campaign had more non-compliant mothers (who had not followed the normal vaccination schedule) and therefore a higher risk of dying. In spite of control for maternal schooling and nutritional status, this could have confounded our comparison of VAS with any DTP vs VAS alone. However, this would not explain the contrasting effects of VAS given with any DTP vs VAS given with MV. Though the group missing DTP as well as MV may have been even less compliant than the group missing MV alone, we would not expect it to fully explain the contrasting effects of VAS given with DTP + MV vs VAS given with MV. Furthermore, if a different a priori risk of dying in the different treatment groups explained their different mortality, the mortality ratios should be reduced importantly by control for nutritional status. Since this did not happen, the increased mortality in the VAS with any DTP group does not merely seem to be due to confounding, but may be related to treatment.

Second, we know from previous studies that travelling or ill children are more likely to die, and in the present study, the non-participants who were travelling or too ill to participate in the campaign had high mortality. However, non-participants did not have the expected higher mortality compared with participants who received VAS with any DTP. On the contrary, participants who received VAS with any DTP had considerably higher mortality than non-participants. This was not the case for participants who had received VAS with MV. It could be argued that it was only mothers who perceived their children in need of treatment who participated in the NIDs. We did not register illness among participants, but mothers in Guinea-Bissau would normally not bring ill children for vaccinations, as also reflected by the fact that illness in the child was given as a reason for non-participation. Furthermore, a higher proportion of frail children among those coming for VAS and vaccination would not explain why it was only children receiving VAS with DTP who did not benefit from the campaign.

As DTP is associated with increased mortality in girls, and MV is associated with decreased mortality, particularly in girls, we would a priori expect to find decreased mortality after MV compared with DTP. However, in the present study, the difference in mortality between MV and DTP recipients was far larger than usually observed. Furthermore, among the children who did not receive VAS because they were in a VAS-at-birth trial, the MR (55/1000 pyrs) after DTP alone was lower than the MR among children who received VAS with DTP [140/1000 pyrs (Table 3)], suggesting that VAS + DTP may be associated with higher mortality than DTP alone.

Hence, in the present study, we found several indications that VAS given with DTP had negative effects in contrast to VAS given with MV alone.
However, we cannot exclude residual confounding in the comparisons of participants and non-participants and between recipients of different vaccines. Furthermore, the number of deaths was small, and it could be argued that it was a chance finding. Based on this study alone, no conclusions regarding the potential interactions between VAS and vaccines can be made. However, it should be noted that all currently available data indicate that the combination of VAS and DTP may be problematic, and we had a priori formulated the hypothesis of a negative effect. 4

The WHO recommends VAS at vaccination contacts after the age of 6 months. The first vaccine to be received after 6 months of age would normally be MV. However, many children come late for vaccinations. In Guinea-Bissau, approximately one-third of all children in the urban area and two-thirds in the rural receive at least one DTP vaccine after 6 months of age. Thus, in practice, there will be children coming for MV, DTP or DTP plus MV for their first vaccination contact after 6 months of age. Based on the present evidence, the WHO VAS policy may not be beneficial for children whose first vaccination after 6 months is a DTP vaccine. Large, randomized studies of the impact on survival of the current WHO recommendation of providing VAS at all vaccination contacts after 6 months of age seem warranted.

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**KEY MESSAGES**

- WHO recommends Vitamin A supplementation at vaccination contacts, but it has not been studied whether the effect of VAS on mortality depends on the type of vaccine administered at the same time.
- In this observational study, we found higher mortality among those who received VAS with DTP vaccine in contrast to those who received VAS only or VAS with measles vaccine.

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