Effects of the introduction of new vaccines in Guinea-Bissau on vaccine coverage, vaccine timeliness, and child survival: an observational study

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Summary

Background In 2008, the GAVI Alliance funded the introduction of new vaccines (including pentavalent diphtheria-tetanus-pertussis [DTP] plus hepatitis B and *Haemophilus influenzae* type b antigens) in Guinea-Bissau. The introduction was accompanied by increased vaccination outreach services and a more restrictive wastage policy, including only vaccinating children younger than 12 months. We assessed coverage of all vaccines in the Expanded Program on Immunizations before and after the new vaccines' introduction, and the implications on child survival.

Methods This observational cohort study used data from the Bandim Health Project, which has monitored vaccination status and mortality in randomly selected village clusters in Guinea-Bissau since 1990. We assessed the change in vaccination coverage using cohort data from children born in 2007 and 2009; analysed the proportion of children who received measles vaccine after 12 months of age using data from 1999–2006; and compared child mortality after age 12 months in children who had received measles vaccine and those who had not using data from 1999 to 2006.

Findings The proportion of children who were fully vaccinated by 12 months of age was 53% (468 of 878) in the 2007 cohort and 53% (467 of 879) in the 2009 cohort (relative risk [RR] 1·00, 95% CI 0·89–1·11). Coverage of DTP-3 and pentavalent-3 increased from 73% (644 of 878) in 2007 to 81% (712 of 879) in 2009 (RR 1·10, 95% CI 1·04 –1·17); by contrast, the coverage of measles vaccination declined from 71% (620 of 878) to 66% (577 of 879; RR 0·93, 0·85–1·01). The effect of the changes was significantly different for DTP-3 coverage compared with measles vaccine coverage (p=0·002). After 12 months of age, the adjusted mortality rate ratio was 0·71 (95% CI 0·56–0·90) for children who had received measles vaccine compared with those who had not (0·59 [0·43–0·80] for girls and 0·87 [0·62–1·23] for boys).

Interpretation The introduction of the new vaccination programme in 2008 was associated with increased coverage of DTP, but decreased coverage of measles vaccine. In 1999–2006, child mortality was higher in children who had not received measles vaccine than in those who had.

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Introduction

The vaccination schedule in many low-income countries includes BCG and oral polio vaccine (OPV) at birth, three doses of whole-cell diphtheria-tetanus-pertussis vaccine (DTP-3) and three doses of OPV at 6, 10, and 14 weeks of age, and measles vaccine at 9 months of age.[1] Just over a decade ago the GAVI Alliance began supporting the introduction of pentavalent (DTP plus hepatitis B and *Haemophilus influenzae* type b antigens) and yellow fever vaccines in many low-income countries, and within the past few years has also supported the introduction of pneumococcal and rotavirus vaccines. Vaccine coverage is normally assessed at 12 months of age,[2] and coverage for DTP-3 is usually used as the main performance indicator for a vaccine programme.[3–6] The focus on DTP-3 as the performance indicator has meant that DTP-3 coverage has increased—eg, 58% of African countries had higher coverage for measles vaccine than for DTP-3 in 1985, but in 2009 this had changed to 68% having a higher coverage for DTP-3 than for measles vaccine.[1]

In addition to providing protection against the targeted disease, vaccines used in child health programmes affect the susceptibility to other pathogens, with effects on mortality.[7] WHO's Strategic Advisory Group of Experts (SAGE) on immunisation has reviewed these so-called non-specific effects of vaccines and recommended further research.[8,9] In brief, many observational studies and randomised trials have shown that measles vaccine as the most recent vaccination is associated with a marked reduction in child mortality—a reduction that is considerably larger than can be explained by prevention of measles infection alone.[10–14] DTP as the most recent vaccination does not seem to have similar beneficial non-specific effects.[15–18] We postulate that if the focus on DTP-3 has lowered the proportion of children who have had measles vaccine as their most recent vaccination, it might have had a detrimental effect on child health.[14,19]

To reach the ambitious goal of 90% national vaccination coverage by 2010, WHO has joined forces with several other organisations—GAVI in particular—to improve vaccination coverage.[20] By 2010, GAVI had funded the introduction of the pentavalent vaccine in 59 countries worldwide.[4] Also, as part of GAVI's performance-based funding, GAVI funds were distributed as a financial reward to countries in which DTP-3 coverage exceeded the targeted level.[21]

In 2008, Guinea-Bissau received support from GAVI to replace DTP with pentavalent vaccine, and yellow fever vaccine was introduced to be given together with measles vaccine. The introduction was accompanied by support for outreach strategies to supplement the routine vaccination services at health centres.[22] Furthermore, emphasis was increased on the strengthening and standardisation of the performance statistics for coverage by 12 months of age, and for wastage,[23] which led to a greater focus on not wasting doses of vaccines. For example, when pentavalent and yellow fever vaccines were introduced, the Expanded Program on Immunizations (EPI) in Guinea-Bissau aimed to reduce wastage for the ten-dose measles vaccine (and yellow fever) vials from 40% to 25%.[23] Contrary to WHO recommendations,[24] the national EPI therefore implemented a policy of not opening a ten-dose vial of the live attenuated measles or yellow fever vaccine (which have to be discarded 6 h after reconstitution) unless six or seven children were present to receive the vaccine. Between October, 2008, and November, 2009, we interviewed 87 EPI managers and nurses responsible for vaccination in the areas of Guinea-Bissau surveyed by the Bandim Health Project about how many children were required for them to open a ten-dose measles vaccine vial. The median number was six children (IQR five to eight; unpublished data). In practice, this policy means that measles vaccine was not given during all outreach sessions; instead, mothers were asked to take their child to a health centre for vaccination or to come to a later outreach session, where enough children might be present to open a vial. Furthermore, practice changed in 2008 so that children in Guinea-Bissau were not given EPI vaccines after 12 months of age because they were outside the target group and vaccines given to them would count as waste. Therefore, children have only 3 months, between the ages of 9 and 11 months, to receive measles vaccine. Both DTP (ten-dose vials of inactivated vaccine, which can be kept open for up to 28 days), and pentavalent vaccines (single-dose vial) were opened if only one child was present.

In this study, we assessed the effect of these changes in the vaccination practices associated with the introduction of new vaccines on vaccination timeliness and coverage in rural Guinea-Bissau, and the potential implications for child survival.

Methods

Study design and participants

This study was an observational cohort study that used data from the Bandim Health Project (BHP), which monitors mortality and vaccination coverage in a health and demographic surveillance system in Guinea-Bissau.[10] Within this system, BHP follows 182 randomly selected geographic clusters initially consisting of 100 women of fertile age and their children in rural Guinea-Bissau. The clusters were selected using methods recommended by the EPI for surveys of immunisation coverage; 20 clusters of 100 women in each of the eight larger health regions have been sampled, and ten and 12 clusters in the two smallest regions. 100 clusters have been under surveillance since 1990, and the remaining 82 were added in 2006. Clusters are visited every 6 months and all pregnancies, births, deaths, and migrations are recorded at these visits. The cohort followed up in the clusters is open: new children and women enter when they take up residence in the area, are born, or become of fertile age. When a new woman is registered, information about age, ethnicity, and schooling is obtained. At all visits, a record is made of children's nutritional status (by measurement of mid-upper-arm circumference), whether the child has a vaccination card, whether the card was seen, and the dates of all vaccinations. Since September, 2007, the BHP teams have been accompanied by a nurse who offers vaccinations to children who are missing routine vaccines; a vaccination card is provided to children who do not yet have a card.

Separate ethical approval was not necessary for this study because the observational data was derived from the demographic surveillance system that has been in place since 1990 at the request of the Guinea-Bissau Ministry of Health.

Procedures

To assess the effect of policy changes for the coverage of different vaccines, we used three different approaches: first, we used data from all 182 clusters under surveillance by the BHP in 2007 (before the introduction of new vaccines) and in 2009 (after the introduction) to assess how increased outreach services affected coverage; second, we used data from 1999 to 2006 from the 100 clusters followed up since 1990 (before the change in programme) to assess the proportion of children who received measles vaccine after 12 months of age during this period; and third, using data from 1999 to 2006, we compared survival after 1 year of age in children who had received measles vaccine versus those who had not.

To assess vaccination coverage, we selected two cohorts of children. The 2007 cohort consisted of children born between January and April, 2007, thus reaching 12 months of age before the introduction of the new vaccines (in September, 2008) and before a shortage of DTP vaccines occurred (in May, 2008, the stock of DTP ran out and the introduction of the new vaccines was delayed until September, 2008, because of a shortage of petroleum for refrigerators, which interrupted the cold chain). The 2009 cohort consisted of children born in the same geographical clusters between January and April, 2009, who were eligible to receive the new vaccines

financed through GAVI. In the two southern regions of Guinea-Bissau (Tombali and Quinara), another research organisation aimed to ensure full vaccination coverage in selected villages by doing monthly mobile clinics.[25] This approach was not representative of the national programme; therefore, children living in these villages were excluded from the assessment of coverage.

To assess vaccination coverage by 12 months of age as done in EPI surveys, we used vaccine information obtained from children aged 12–23 months. Since the national policy was to provide vaccines only to children aged younger than 12 months, we considered only doses received before 12 months, even though BHP had vaccinated older children. Thus the denominator was the number of children with a vaccination card seen between the ages of 12 and 23 months and the numerator was the number of children having received a given vaccine by age 12 months. Similarly, of children whose vaccination card was not seen between the ages of 12 and 23 months, we assessed vaccination coverage by 12 months of age in those who had a vaccination card inspected between the ages of 24 and 35 months. We did not use recall data for children for whom a vaccination card was not seen within the relevant time periods; when the card could not be seen it was nearly always because the mother was not present.

Key vaccine coverage outcomes were the proportion of children who were fully vaccinated (ie, had received BCG, three doses of OPV and DTP or pentavalent vaccine, and measles vaccine before 12 months of age); vaccination coverage and median age of vaccination for all vaccines received before 12 months of age except for yellow fever, which was only given in the 2009 cohort; and the proportion of children who received the same vaccine on a specific day within a village (since the policy of opening a ten-dose vial only when six to seven children were present would lead to more children within a given village returning to receive the same vaccination on the same day).

To assess how the policy of not providing measles vaccine after 12 months of age affected overall measles vaccine coverage, we used data from the 100 village clusters followed up since 1990. A civil war occurred in 1998, and in late May, 2006, a 2-week national measles vaccine campaign targeted all children aged between 6 months and 15 years; therefore, we used data from January, 1999, to May, 2006. We assessed measles vaccine coverage by age 12 months, 24 months, and 36 months in the 1999–2006 cohort, which consisted of children who had their vaccination card inspected between Jan 1, 1999, and May 15, 2006, when they were aged 12–23 months, 24–35 months, and 36–47 months, respectively.

To assess the effect of measles vaccine on child mortality, we used data from 1999 to 2006 to compare survival of children who had received measles vaccine with those who had not. Since 2007, BHP has provided vaccines after infancy as part of a vitamin A supplementation trial (NCT00514891); therefore, children who had not been vaccinated against measles received measles vaccine from the BHP team after 12 months of age, even though it was national EPI policy not to vaccinate after this age,[26] hence later cohorts could not contribute to this analysis. Children aged 12–35 months who had their vaccination card inspected between Jan 1, 1999, and May 15, 2006, were included in the survival analysis.

Statistical analyses

In our analyses of vaccination coverage before and after the change in vaccination programme, we compared the 2009 cohort with the 2007 cohort as a reference. We compared the distribution of background variables in the two cohorts by Fisher's exact test, rank-sum test, and the *t* test, analysed vaccination coverage by calculating relative risks using binomial regression adjusted for clustering by robust variance estimates (no convergence problems were encountered), and assessed distribution of age at vaccination by rank-sum test.

In our analyses of survival of children who had been vaccinated against measles compared with those who had not, children from the 1999–2006 cohort entered the analysis when they had their vaccination card inspected (landmark approach).[27] As in a previous study of child survival using data from BHP,[10] follow-up was censored at the subsequent visit, or 6 months later if the subsequent visit occurred more than 6 months after the previous visit. Follow-up was stopped on May 15, 2006, because the national measles vaccine campaign began.

We used a Cox proportional-hazards model with age as underlying time to compare mortality rates between children who had and had not received measles vaccine, stratified for village cluster. Estimates are presented as crude ratios and are also adjusted for ethnicity, maternal age, and schooling. We assessed interaction between measles vaccination status and sex using Wald statistics. We assessed the proportional hazards assumption by

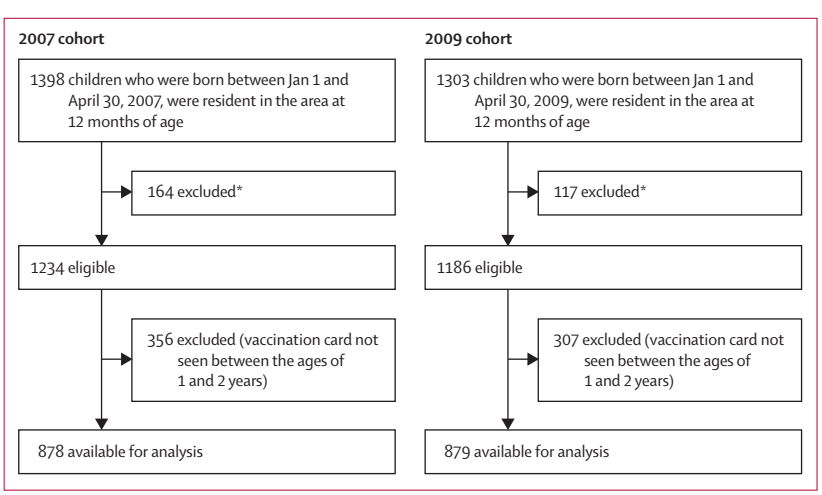

***Figure 1*: Description of the 2007 and 2009 cohorts**
*Children living in villages where a research project provided all health interventions.

log-log plots and Schoenfeld residuals. We analysed data using Stata (version 11.2).

Role of the funding source

The funders had no role in study design, data collection, data analysis, or writing of the report. The corresponding author had full access to all the data in the study, and had final responsibility for the decision to submit for publication.

Results

For assessment of vaccination coverage, we followed up 1398 children born between January and April, 2007, and 1303 born between January and April, 2009. We excluded 164 children from the 2007 cohort and 117 children from the 2009 cohort because they lived in the villages with monthly outreach clinics,[25] leaving 1234 children in the 2007 cohort and 1186 in the 2009 cohort (figure 1). We assessed vaccination coverage in children whose vaccination card was seen between the ages of 12 and 23 months: 878 (71%) of 1234 children in the 2007 cohort had their vaccination card seen during this period compared with 879 (74%) of 1186 children in the 2009 cohort. The main reason for not seeing a vaccination card between the ages of 12 and 23 months was that the mother was not present; at 847 (88%) of the 965 visits made to the 663 children for whom a vaccination card was not seen, the mother was not present.

The distribution of background variables for the two cohorts is shown in table 1. Nutritional status, measured by mid-upper-arm circumference, was slightly lower in the 2009 cohort than in the 2007 cohort; however, the difference of 1 mm is unlikely to be clinically significant.

The proportion of fully vaccinated children was the same in the two cohorts: 53% (468 of 878) in the 2007 cohort and 53% (467 of 879) in the 2009 cohort (table 2). The coverage for individual antigens varied. Coverage of DTP-3 increased significantly from 73% (644 of 878) in the 2007 cohort to 81% (712 of 879) in the 2009 cohort (table 2). The increase in coverage was due to more children beginning their DTP vaccination series and to a lower dropout rate: in the 2007 cohort, 824 (94%) of children received DTP-1, but 234 (27%) did not receive DTP-3, whereas in 2009, 862 (98%) received DTP-1, and 167 (19%) did not receive DTP-3. Hence, the RR for dropout was 0·71 (95% CI 0·59–0·86). In parallel, the median age of children when they received each dose of DTP declined significantly (table 2).

Despite the improvement in DTP-3 coverage, the median age at measles vaccination increased significantly

	2007 cohort (n=878)	2009 cohort (n=879)	p value*
Child characteristics			
Male sex	428 (49%)	436 (51%)	0·74
Age at interview, months	19·5 (16·6–22·0)	19·5 (16·8–21·7)	0·80†
Mid-upper-arm circumference, mm	144 (13)	143 (12)	0·005‡
Maternal characteristics			
Age, years	27·5 (22·5–32·6)	27·0 (22·4–32·1)	0·15†
Any formal education	201 (23%)	221 (26%)	0·29
Ethnicity	..	..	0·12
Balanta	176 (20%)	171 (19%)	..
Fula	200 (23%)	212 (24%)	..
Mandinga	175 (20%)	213 (24%)	..
Pepel	125 (14%)	97 (11%)	..
Manjaco or Mancanha	73 (8%)	61 (7%)	..
Other or unknown	129 (15%)	125 (14%)	..

Data are number (%), median (IQR), or mean (SD). Information was missing for some children: 50 children in the 2007 cohort and 37 in the 2009 cohort did not have their mid-upper-arm circumference measured; for 15 and 11 children, respectively, no information about maternal age could be obtained; and for 18 and 18 children, respectively, no information about maternal education could be obtained. *Distributions compared by Fisher's exact tests unless stated otherwise. †Distributions compared by rank-sum test. ‡Distributions compared by *t* test.

***Table 1*: Background variables in the 2007 and 2009 cohorts for children whose vaccination cards were seen between the ages of 12 and 23 months**

	Vaccination coverage, n (%)			Median age at vaccination, days (IQR)		
	2007 cohort (n=878)	2009 cohort (n=879)	Relative risk (95% CI)*	2007 cohort (n=878)	2009 cohort (n=879)	p value†
Fully vaccinated‡	468 (53%)	467 (53%)	1·00 (0·89–1·11)	NA	NA	NA
BCG	780 (89%)	801 (91%)	1·03 (0·99–1·07)	44 (15–101)	35 (17–65)	0·001
DTP-1 or pentavalent-1	824 (94%)	862 (98%)	1·04 (1·02–1·07)	74 (53–126)	65 (51–90)	<0·0001
DTP-2 or pentavalent-2	753 (86%)	825 (94%)	1·09 (1·04–1·14)	141 (98–201)	116 (93–162)	<0·0001
DTP-3 or pentavalent-3	644 (73%)	712 (81%)	1·10 (1·04–1·17)	192 (141–246)	168 (133–223)	0·001
OPV-1	823 (94%)	865 (98%)	1·05 (1·02–1·08)	76 (53–126)	64 (50–90)	<0·0001
OPV-2	740 (84%)	822 (94%)	1·11 (1·06–1·16)	141 (97–200)	119 (93–167)	<0·0001
OPV-3	596 (68%)	689 (78%)	1·15 (1·08–1·24)	187 (139–246)	176 (135–240)	0·14
Measles vaccine	620 (71%)	577 (66%)	0·93 (0·85–1·01)	290 (275–314)	299 (278–322)	0·002

NA=not applicable. DTP=diphtheria-tetanus-pertussis vaccine. Pentavalent=diphtheria-tetanus-pertussis plus *Haemophilus influenzae* type b plus hepatitis B vaccine. OPV=oral polio vaccine. *2009 *vs* 2007; adjusted for clustering by robust variance estimates. †Analysed by rank-sum test. ‡Received BCG, three doses of DTP or pentavalent vaccine, three doses of OPV, and measles vaccine by 12 months of age.

***Table 2*: Vaccination coverage by 12 months of age and median age at vaccination for children in the 2007 and 2009 cohorts whose vaccination cards were seen between the ages of 12 and 23 months**

between 2007 and 2009 (table 2). The proportion of children who had received measles vaccine by 12 months of age thus declined slightly from 620 (71%) children in the 2007 cohort to 577 (66%) in the 2009 cohort (table 2). The effect of the vaccine programme change on DTP-3 coverage was significantly different from the effect on measles vaccine coverage ($p_{interaction}$=0·002). Coverage of all vaccines did not differ between boys and girls (data not shown).

The BHP teams started providing DTP and measles vaccine in September, 2007. BHP teams vaccinated more children in the 2009 cohort than in the 2007 cohort (appendix). Excluding children vaccinated by the BHP team, more children in the 2009 cohort received DTP-3 than did those in the 2007 cohort (RR 1·07, 95% CI 1·00–1·14), whereas fewer children in the 2009 cohort received measles vaccine than did those in the 2007 cohort (RR 0·91, 0·83–1·00; p=0·002 for the differential effect for DTP-3 and measles vaccine coverage; appendix).

For children with a vaccination card seen only between the ages of 24 and 35 months, 83 (62%) of 133 children had received DTP-3 by 12 months of age in the 2007 cohort compared with 93 (66%) of 140 in the 2009 cohort; measles vaccine coverage by 12 months of age was 47% in both cohorts (62 of 133 children in the 2007 cohort and 66 of 140 children in the 2009 cohort). 1011 (82%) of the 1234 children in the 2007 cohort had a vaccination card that was seen between the ages of 12 and 35 months compared with 1019 (86%) of the 1186 children in the 2009 cohort. In these children, DTP-3 coverage increased from 71% (717 of 1011) in the 2007 cohort to 79% (805 of 1019) in the 2009 cohort (RR 1·10, 95% CI 1·04–1·17) and measles vaccine coverage declined from 67% (682 of 1011) in 2007 to 63% (643 of 1019) in 2009 (RR 0·93, 95% CI 0·85–1·01; p=0·0002 for the differential effect for DTP-3 and measles vaccine coverage).

In the children who had received measles vaccine before age 12 months, the proportion who received measles vaccine on the same day as at least one other child in the same cluster was 48% (298 of 620) in the 2007 cohort and 57% (331 of 577) in the 2009 cohort (RR 1·19, 95% CI 1·03–1·37). By contrast, the frequency of same-day administration of DTP-3 was unchanged: of the children who received DTP-3, 305 (47%) of 644 children in the 2007 cohort and 320 (45%) of 712 in the 2009 cohort received DTP-3 at the same time that another child in the same village received DTP-3 (RR 0·95, 95% CI 0·82–1·09).

Although we noted only a small decline in measles vaccine coverage by 12 months of age between 2007 and 2009 (table 2), total measles vaccine coverage has declined further because measles vaccine also used to be given after 12 months of age. In the 12119 children in the 1999–2009 cohort whose vaccination card was seen when aged 12–35 months, 6479 (53%) had received measles vaccine by 12 months of age. Of the children whose card was seen between the ages of 24 and 35 months, 6359 (83%) of 7642 had received measles vaccine by 24 months of age, and of those whose card was seen between the ages of 36 and 47 months, 4221 (92%) of 4589 had received measles vaccine by 36 months of age (table 3). Hence, of the 5640 (47%) children in the 1999–2006

	Measles vaccine coverage
All children in the 1999–2006 mortality cohort	n=12119
Measles vaccine by age 12 months	6479 (53%)
Measles vaccine after age 12 months*	9231 (76%)
Card seen between 24–35 months	n=7642
Measles vaccine by age 24 months	6359 (83%)
Card seen between 36–47 months	n=4589
Measles vaccine by age 36 months	4221 (92%)
Children with no measles vaccine by age 12 months	n=5640
Card seen between 24–35 months	n=3663
Measles vaccine by age 24 months	2380 (65%)
Card seen between 36–47 months	n=2279
Measles vaccine by age 36 months	1911 (84%)

*Recorded at the first instance that the vaccination card was seen.

***Table 3*: Measles vaccination coverage in 1999–2006 mortality cohort**

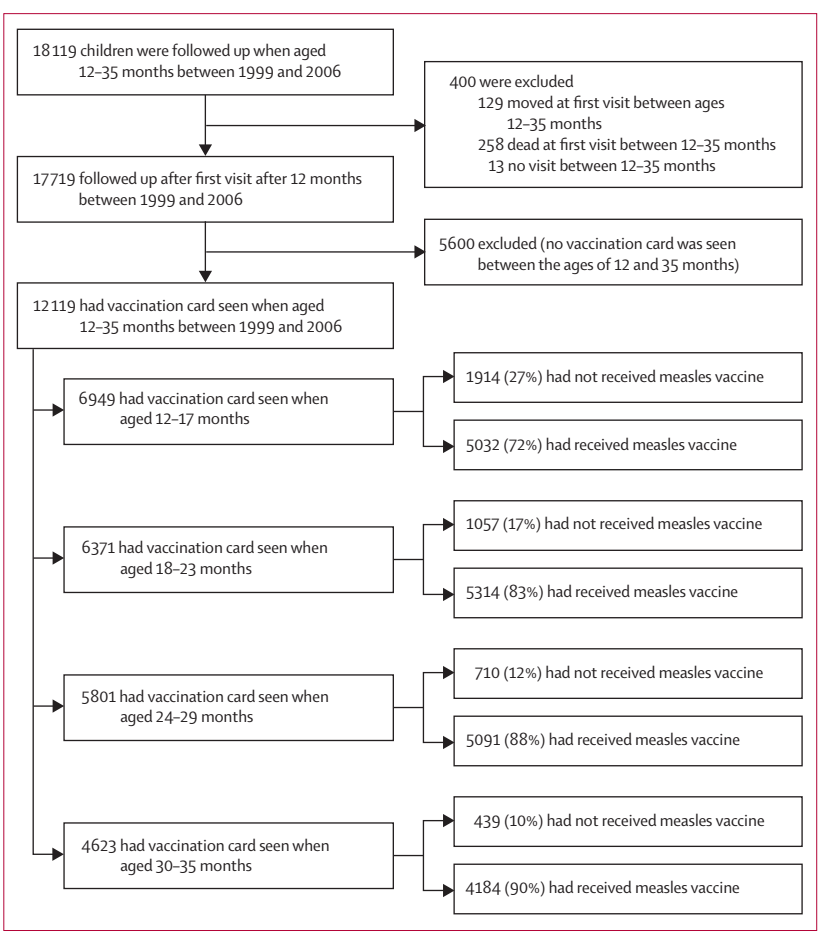

***Figure 2*: Description of the 1999–2006 cohort.**

See Online for appendix

cohort who had not received measles vaccine by 12 months of age, 2279 (40%) had a card inspected between the ages of 36 and 47 months, of whom 1911 (84%) had received measles vaccine by age 36 months.

Of the 18 119 children aged 12–35 months at surveillance rounds in 1999–2006, 12 119 (67%) had had their vaccination card inspected (figure 2). During the 6 months after vaccine card inspection, children who had been vaccinated against measles had significantly lower mortality than did those who were not vaccinated against measles (table 4; figure 3). The lower mortality in the measles-vaccinated group was mainly due to girls who had received measles vaccine having a lower mortality than girls who had not (table 4; $p_{interaction}=0·07$).

Mothers of children who had received measles vaccine were slightly younger and more likely to come from the Muslim ethnic groups Fula and Mandinga than from other ethnic groups; maternal education was not associated with measles vaccination (appendix). Adjusting for these background variables had little effect on the mortality rate ratios [MRRs], the overall adjusted MRR being 0·71 (95% CI 0·56–0·90; table 4). The mid-upper-arm circumference was larger in children who had received measles vaccine than in those had not, possibly owing to a beneficial effect of measles vaccine on morbidity (intermediate variable), but also possibly due to health status before vaccination, which might have affected whether a child was vaccinated or not. When we adjusted for mid-upper-arm circumference as well, the MRR for measles-vaccinated children was 0·73 (95% CI 0·58–0·94) overall, 0·62 (0·45–0·85) in girls, and 0·90 (0·63–1·28) in boys.

Compared with children who had not received measles vaccine, the children who received measles vaccine after 12 months of age had an MRR of 0·67 (95% CI 0·53–0·86); the MRR was 0·56 (0·41–0·78) for girls and 0·83 (0·58–1·19) for boys (data not shown).

11 deaths were reported to be due to measles in five girls (two who had received measles vaccine and three who had not) and six boys (three who had received measles vaccine and three who had not). Censoring these deaths, the MRR was 0·75 (95% CI 0·59–0·95) overall, 0·62 (0·45–0·84) for girls, and 0·95 (0·67–1·36) for boys. Censoring deaths that occurred from May, 2003, to May, 2004, when Guinea-Bissau had a measles epidemic[28] removed 79 deaths (in 11 children who had not received measles vaccine and 68 who had) and resulted in an MRR of 0·72 (95% CI 0·57–0·90).

	Number of observations*	Mortality rate per 1000 PYRS (deaths/PYRS)	Mortality rate ratio (95% CI)†	Adjusted mortality rate ratio (95% CI)‡
All children	..	..	0·72 (0·57–0·90)	0·71 (0·56–0·90)
Measles unvaccinated§	4120 (17%)	56·7 (103/1816)	..	..
Measles vaccinated	19 621 (83%)	41·3 (358/8665)	..	..
Girls	..	..	0·59 (0·44–0·80)	0·59 (0·43–0·80)
Measles-unvaccinated	2060 (18%)	66·3 (60/906)	..	..
Measles-vaccinated	9636 (82%)	40·7 (173/4256)	..	..
Boys	..	..	0·89 (0·63–1·26)	0·87 (0·62–1·23)
Measles-unvaccinated	2060 (17%)	47·3 (43/910)	..	..
Measles-vaccinated	9985 (83%)	42·0 (185/4409)	..	..
Age when vaccination card was inspected				
12–17 months	..	..	0·74 (0·52–1·05)	0·76 (0·53–1·09)
Measles-unvaccinated	1914 (28%)	56·3 (48/852)	..	..
Measles-vaccinated	5032 (72%)	45·8 (102/2229)	..	..
18–23 months	..	..	0·64 (0·42–0·97)	0·61 (0·40–0·93)
Measles-unvaccinated	1057 (17%)	67·1 (31/462)	..	..
Measles-vaccinated	5314 (83%)	43·8 (103/2349)	..	..
24–29 months	..	..	0·72 (0·43–1·21)	0·70 (0·42–1·18)
Measles-unvaccinated	710 (12%)	58·3 (18/309)	..	..
Measles-vaccinated	5091 (88%)	42·4 (95/2241)	..	..
30–35 months	..	..	0·98 (0·41–2·33)	0·96 (0·40–2·28)
Measles-unvaccinated	439 (10%)	31·1 (6/193)	..	..
Measles-vaccinated	4184 (90%)	31·4 (58/1846)	..	..

PYRS=person-years of observation. DTP=diphtheria-tetanus-pertussis vaccine. OPV=oral polio vaccine. *Eg, a child whose vaccination card was inspected two times between 12 and 35 months contributes two non-overlapping periods of observation. †Stratified by village cluster. ‡Stratified by village cluster and adjusted for ethnicity, maternal age in quartiles, and schooling. §At 3563 (86%) of 4120 observations for children who had not received measles vaccine the child had received one or more other vaccines; for 2810 (79%) of these 3563 observations the child had received DTP as the most recent vaccine (2200 [78%] had received DTP or DTP plus OPV as their most recent vaccine and 610 [22%] had received DTP with BCG).

Table 4: **Mortality for children according to whether or not they had received measles vaccine between 1999 and 2006**

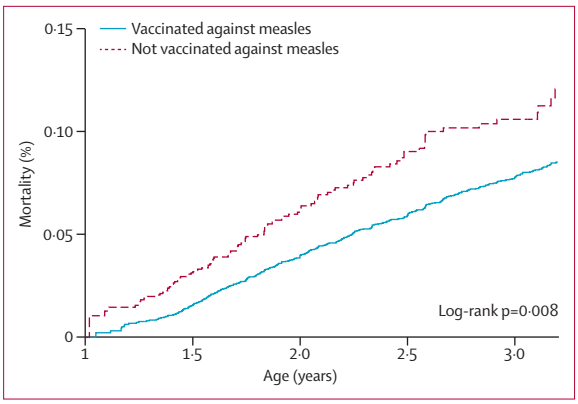

***Figure 3*: Mortality of children according to measles vaccination status**
Includes children who received measles vaccine after 12 months of age; children contributed an observation time after card inspection (at which they were classified as vaccinated or unvaccinated) and stayed in the analysis for the next 6 months.

If no measles vaccines had been given after 12 months of age in the 1999–2006 cohort, 4926 (47%) of 10 481 person-years of observation (appendix) would have been lived without measles vaccination between 12 and 36 months of age, rather than 1816 (17%) person-years of observation. On the basis of the beneficial effect of measles vaccine on all-cause mortality that we have reported, vaccination after 12 months of age saved 40 (8%) of 501 deaths in the cohort in children aged 1–3 years. For girls this reduction in mortality was 11% (30 of 263) and for boys it was 4% (ten of 238).

Discussion

The introduction of new vaccines in Guinea-Bissau in 2008 was accompanied by not only intensified outreach vaccination services, but also reinforced wastage policies, including not offering vaccinations to children older than 12 months and only opening multidose vials with live attenuated vaccines when more than half of the doses could be used. As expected, better outreach services increased coverage and timeliness of pentavalent vaccinations. However, the median age of measles vaccination increased and the measles vaccine coverage at 12 months of age dropped from 71% to 66%. Before the change in the vaccination programme, 84% of unvaccinated children received measles vaccine between the ages of 12 months and 35 months. Had this trend continued, 95% would have received measles vaccine by 36 months of age in the 2009 cohort. Hence, the fact that measles vaccine is no longer provided after 12 months of age might make an increasing proportion of children susceptible to measles infection.

This finding might also have more far-reaching outcomes, because even when measles deaths were censored, children who had received measles vaccine had a lower risk of mortality than did children who had not received the vaccine, and the benefit was particularly marked for girls. Hence, a decline in total measles vaccine coverage might lead to an increase in overall child mortality, particularly for girls.

The continuous demographic surveillance done by the BHP teams is likely to provide better information from a more representative selection of the population than that provided by many EPI vaccination surveys, for which the reliability of techniques used to select random samples of the population has been questioned.[29] However, the 6-monthly inspection of vaccination cards might remind mothers to get their children vaccinated, resulting in higher vaccination coverage than in the source population.

We used vaccination cards seen during routine visits to establish vaccination status. However, by not including children whose vaccination cards were not inspected, we might have overestimated vaccine coverage. Maternal absence (often because they were travelling) was the main reason for not being able to inspect a vaccination card. The children whose vaccination cards were not seen between the ages of 12 and 24 months were travelling more and their vaccination coverage was lower than those whose cards were seen at ages 12–24 months, as shown by the lower coverage in children seen only after 24 months of age. However, the relative changes in DTP-3 coverage and measles vaccine coverage remained the same when children whose vaccination cards were not seen between 12 and 23 months but were seen after 24 months of age were included in the analysis.

To create comparable cohorts, we excluded children who were exposed to extended vaccine shortages. Hence, we only included children born during a 4-month window. BHP started vaccinating during biannual visits after September, 2007. The 2009 cohort therefore had the opportunity to be vaccinated by the BHP team at all visits during their first year of life, whereas the 2007 cohort was not vaccinated by the BHP team at their first visit in 2007. When children vaccinated by the BHP were excluded from our analysis, the rise in DTP-3 coverage between 2007 and 2009 was slightly lower, but the fall in measles vaccine coverage was stronger than when those children were included in the analysis. Hence, the BHP provision of vaccines might have lessened the decline in measles vaccine coverage. Irrespective of whether we used data from all children or only those who had not received vaccines from the BHP team, we noted a significant differential effect for DTP-3 and measles vaccine (appendix). According to WHO figures, Guinea-Bissau reported a 1-month measles vaccine shortage in 2008, and shortages of *H influenzae* type b and hepatitis B vaccines of 1–2 months in 2009.[30] However, these shortages do not explain the differences in the vaccination coverage patterns for DTP-3 and measles vaccine; a shortage of measles vaccine in 2008 would reduce the measles vaccine coverage in the 2007 cohort and a shortage of pentavalent vaccines in 2009 would have lowered the pentavalent coverage in the 2009 cohort, and hence the shortages, if anything, would have reduced the reported differential trends in coverage.

***Panel*: Research in context**

Systematic review

We searched PubMed for publications on "mortality" or "death" in relation to one of the vaccine terms "DTP", "DPT", "diphtheria-tetanus-pertussis", or "pentavalent vaccine" published before Dec 1, 2011, in English, French, Portuguese, Spanish, German, and Scandinavian languages, as described previously.[15] Likewise, as described previously,[14] we examined existing reviews and searched PubMed for studies comparing the mortality of children who had and had not received measles vaccine. The results of studies of diphtheria-tetanus-pertussis (DTP) vaccine suggest that DTP is associated with increased female mortality, as previously reviewed.[15] By contrast, measles vaccination has been shown in many observational studies and randomised trials to have beneficial non-specific effects that are not explained by the prevention of measles infection.[10–14] The performance of vaccination programmes are often assessed by the coverage of three doses of DTP (DTP-3).[33] We postulate that the focus on DTP-3 has diminished the emphasis on measles vaccine, even though this vaccine is associated with improved child survival.

Interpretation

When pentavalent (DTP plus hepatitis B and *Haemophilus influenzae* type b) vaccine was introduced into the vaccination programme in Guinea-Bissau in 2008, the age of vaccination declined and DTP-3 coverage increased, but simultaneously the age of children who received measles vaccine increased and the coverage declined. To ensure that the assessment of vaccination programmes is an indicator of the desired effects on child survival, the coverage of measles vaccine administered after DTP-3 could be considered as the main performance indicator.

The analysis of mortality—comparing children who received measles vaccine with those who did not—is observational, and a self-selection bias and differential access to vaccination might have also contributed to the reported difference. However, by stratifying for cluster, mortality in children who had and had not received measles vaccine was compared within the same village. Furthermore, adjusting for other background variables had little effect on the estimates. We used a landmark approach, in which children did not change vaccination status on the date of vaccination, but on the date that the information was obtained. This method prevents survival bias due to better vaccination information for survivors and provides conservative estimates of the difference in mortality between vaccinated and unvaccinated children.[27]

In line with the observational data presented here, randomised trials have shown major reductions in mortality not related to the prevention of measles infection, and the beneficial effect has been stronger for girls than for boys.[11,12,31] The recent SAGE review concluded that measles vaccine seemed to have stronger beneficial effects for girls than for boys.[8] In our study, the strongest beneficial effect was also observed in girls. The effect was slightly stronger for children who had received their measles vaccine after 12 months of age and thus were closer to starting the observation period. If selection bias had explained the beneficial effect of measles vaccine after 12 months of age, the survival benefit should be strongest for the children vaccinated before 12 months of age.

Despite the huge investments in improving vaccination coverage, few studies have assessed the effects of donor support; all studies have focused on DTP-3 coverage and none have reported measles vaccine coverage.[32–37] Higher DTP-3 than measles vaccine coverage has been described in other cohorts[3,38,39] and, as mentioned, 58% of African countries had higher measles vaccine coverage than DTP-3 coverage in 1985, whereas 68% had a higher coverage for DTP-3 than for measles vaccine in 2009.[1] A report of vaccine coverage from 2008 suggested that 5 million children in Africa had not received DTP-3, but 7·5 million had not received measles vaccine.[38]

Children who receive measles vaccine have consistently had lower mortality than children who have not received the vaccine, when compared with unvaccinated children and when compared with those who have received DTP, both in observational studies[10,13] and randomised trials (panel).[11,12] In 1990–96, when overall child mortality was higher in rural Guinea-Bissau than it was in our present study, we undertook a similar study in children aged 7–13 months, in the same village clusters as we used in this study. During the 6 months after vaccination card inspection, children who had received measles vaccine had a lower risk of mortality than did children who had not received measles vaccine (adjusted mortality ratio 0·48, 95% CI 0·27–0·87).[10]

The increase in DTP-3 coverage from 73% to 81% between 2007 and 2009 and the addition of hepatitis B and *H influenzae* type b antigens are likely to have beneficial effects on the prevalence of the target diseases from all five pathogens. However, despite the additional resources used to introduce new vaccines and achieve higher DTP-3 coverage, the age that children received measles vaccine increased and the coverage declined. Although the increase in the median age that children received measles vaccine might be explained by children being taken for vaccination at an older age, the fact that the increase in the age that children received measles vaccine was associated with a higher proportion of children receiving measles vaccine on the same day does suggest that the increase in age was due to the more restrictive policy of only opening multidose vials on specific days. When only children aged younger than 12 months are eligible, and when opening a vial requires the presence of six to seven children, some opportunities to vaccinate might be missed and the age that children undergo vaccination will increase. It should be emphasised that it is not WHO[24] or GAVI policy to restrict measles vaccination. However, Guinea-Bissau's proposal to GAVI[23] and their multiyear plan for 2010–14 aimed to further reduce vaccine wastage;[40] with a narrow wastage margin, programme officers are likely to implement restrictive policies for multidose vials and vaccinations after 12 months of age.

Hence, encouraging good programme performance (timely vaccinations, reduced wastage) can seemingly conflict with good programme outcomes (higher coverage), which could affect child survival. We reported

that children who received the measles vaccine had a lower risk of mortality after 12 months of age than did children who did not receive the vaccine. In the 1999–2006 cohort, mortality was also lower for children who received measles vaccine after 12 months of age than for those who did not receive it at all. As shown in previous studies,[10,11,13] exclusion of the measles deaths in our study did not change the beneficial effect. Thus the reduction in child mortality associated with measles vaccination as the most recent vaccination cannot be explained by prevention of measles infection alone. By contrast, no prospective study has shown DTP as the most recent vaccination to be associated with a beneficial non-specific effect.[10,15–18,41] The beneficial effect of measles vaccination has been strongest for girls, and results of some studies have suggested that DTP might have a negative effect in girls.[11,15,17,41,42] Hence, girls might be affected more than boys from the declining measles vaccine coverage.

Several ways to increase measles vaccine coverage could be considered. First, wastage might be reduced if two-dose or five-dose vials were used instead of ten-dose vials; however, the measles vaccine is cheap and the per-dose cost is lower for ten-dose vials than for single-dose vials if more than two doses are used from a ten-dose vial.[43] From a public health perspective, donors should monitor local implementation practices to ensure that wastage management does not conflict with WHO recommendations and overrule the right of every child to get timely vaccination. Second, since a 3-month window between 9 and 11 months of age is too narrow to vaccinate all children against measles, especially in remote areas, measles vaccination after 12 months of age should be encouraged—eg, by also assessing coverage of measles vaccine at 18 or 24 months of age. Third, even though measles vaccine coverage is an indicator of progress towards Millennium Development Goal (MDG) 4,[44] changes in measles vaccine coverage have received little attention because DTP-3 coverage has been the main performance indicator for vaccination programmes assessed by the Global Vaccine Action Plan[5] and has been used to establish whether or not a country is eligible for GAVI financial rewards.[21] Programme managers have therefore encouraged activities that increased DTP-3 coverage. Since 2012, GAVI's revised performance-based funding criteria also include measles vaccine: GAVI rewards countries with US$30 per additional child who receives measles vaccine if measles vaccine coverage increases, in addition to US$30 per child for DTP-3 coverage increases.[45] This incentive is clearly an improvement over using only DTP-3 as a performance indicator, but many children might still receive DTP after measles vaccine, which has been associated with higher child mortality than for DTP followed by measles vaccine, particularly for girls.[8,15] A performance indicator, such as the proportion of children who received measles vaccine at least 4 weeks after DTP-3, would be more logical, because this outcome is positively associated with child survival.[11]

Vaccines and vaccination policies are justified, but rarely assessed, in terms of their effect on child survival. Results of our study suggest that the focus on DTP-3 as the main performance indicator and the focus on reducing waste in the vaccination programme might have had unfortunate effects. In Guinea-Bissau, increased funding for outreach services improved the coverage and timeliness of DTP vaccination, but simultaneously the coverage for measles vaccine declined, leaving a third of children susceptible to measles infection, potentially hindering progress towards MDG4.

Contributors

ABF, PA, and CSB planned the study. ABF, LH, AR, IB, and MF supervised data collection, data entry, and data cleaning. ABF had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. ABF wrote the first draft of the paper and has primary responsibility for the final content. All authors contributed to and approved the final manuscript.

Declaration of interests

We declare no competing interests.

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References

1 UNICEF, World Health Organization. Immunzation summary: a statistical reference containing data through 2009 (the 2011 edition). http://www.childinfo.org/files/32775_UNICEF.pdf (accessed July 4, 2014).

2 Burton A, Monasch R, Lautenbach B, et al. WHO and UNICEF estimates of national infant immunization coverage: methods and processes. *Bull World Health Organ* 2009; **87**: 535–41.

3 WHO. Global routine vaccination coverage, 2010. *Wkly Epidemiol Rec* 2011; **86:** 509–13.

4 GAVI Alliance. GAVI Alliance progress report 2010. http://www.gavialliance.org/results/gavi-progress-reports/ (accessed July 3, 2014).

5 WHO. Global vaccine action plan 2011–2020. http://www.who.int/immunization/global_vaccine_action_plan/GVAP_doc_2011_2020/en/index.html (accessed Aug 4, 2013).

6 Arevshatian L, Clements C, Lwanga S, et al. An evaluation of infant immunization in Africa: is a transformation in progress? *Bull World Health Organ* 2007; **85:** 449–57.

7 Benn CS, Netea MG, Selin LK, Aaby P. A small jab—a big effect: nonspecific immunomodulation by vaccines. *Trends Immunol* 2013; **34:** 431–39.

8 Higgins JPT, Soares-Weiser K, Reingold A. Systematic review of the non-specific effects of BCG, DTP and measles containing vaccines. http://www.who.int/immunization/sage/meetings/2014/april/3_NSE_Epidemiology_review_Report_to_SAGE_14_Mar_FINAL.pdf?ua=1 (accessed May 23, 2014).

9 WHO. Meeting of the Strategic Advisory Group of Experts on immunization, April 2014—conclusions and recommendations. *Wkly Epidemiol Rec* 2014; **21:** 221–36.

10 Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *BMJ* 2000; **321:** 1435–38.

11 Aaby P, Martins CL, Garly ML, et al. Non-specific effects of standard measles vaccine at 4·5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ* 2010; **341:** c6495.

12 Aaby P, Garly ML, Bale C, et al. Survival of previously measles-vaccinated and measles-unvaccinated children in an emergency situation: an unplanned study. *Pediatr Infect Dis J* 2003; **22:** 798–805.

13 Aaby P, Samb B, Simondon F, Seck AM, Knudsen K, Whittle H. Non-specific beneficial effect of measles immunisation: analysis of mortality studies from developing countries. *BMJ* 1995; **311:** 481–85.

14 Aaby P, Martins CL, Garly ML, Rodrigues A, Benn CS, Whittle H. The optimal age of measles immunisation in low-income countries: a secondary analysis of the assumptions underlying the current policy. *BMJ Open* 2012; **2:** e000761.

15 Aaby P, Benn C, Nielsen J, Lisse IM, Rodrigues A, Ravn H. Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. *BMJ Open* 2012; **2:** e000707.

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

17 Aaby P, Ravn H, Roth A, et al. Early diphtheria-tetanus-pertussis vaccination associated with higher female mortality and no difference in male mortality in a cohort of low birthweight children: an observational study within a randomised trial. *Arch Dis Child* 2012; **97:** 685–91.

18 Aaby P, Benn CS, Nielsen J, Lisse IM, Rodrigues A, Jensen H. DTP vaccination and child survival in observational studies with incomplete vaccination data. *Trop Med Int Health* 2007; **12:** 15–24.

19 Aaby P, Benn CS. Assessment of childhood immunisation coverage. *Lancet* 2009; **373:** 1428.

20 WHO. GIVS global immunization vision and strategy 2006–2015. http://whqlibdoc.who.int/hq/2005/WHO_IVB_05.05.pdf (accessed July 4, 2014).

21 GAVI Alliance. Immunisation services support . http://www.gavialliance.org/support/iss/ (accessed April 19, 2013).

22 GAVI Alliance. Annual progress report Guinea-Bissau 2009 (in French). http://www.gavialliance.org/country/guinea-bissau/proposals/ (accessed Aug 15, 2013).

23 GAVI Alliance. Proposal for NVS - YF, Penta support: Guinea-Bissau (approved by GAVI on Aug 1, 2007). http://www.gavialliance.org/country/guinea-bissau/documents/#approvedproposal (accessed June 14, 2014).

24 WHO. Measles vaccines: WHO position paper. *Wkly Epidemiol Rec* 2009; **84:** 349–60.

25 Mann V, Fazzio I, King R, et al. The EPICS trial: Enabling Parents to Increase Child Survival through the introduction of community-based health interventions in rural Guinea Bissau. *BMC Public Health* 2009; **9:** 279.

26 Fisker AB, Bale C, Rodrigues A, et al. High-dose vitamin A at vaccination contacts after 6 months of age: a randomized trial. *Pediatrics* (in press).

27 Jensen H, Benn CS, Lisse IM, Rodrigues A, Andersen PK, Aaby P. Survival bias in observational studies of the impact of routine immunizations on childhood survival. *Trop Med Int Health* 2007; **12:** 5–14.

28 Bale C, Garly ML, Martins C, Nielsen J, Whittle H, Aaby P. Risk factors for measles in young infants in an urban African area with high measles vaccination coverage. *Pediatr Infect Dis J* 2011; **30:** 689–93.

29 Luman ET, Worku A, Berhane Y, Martin R, Cairns L. Comparison of two survey methodologies to assess vaccination coverage. *Int J Epidemiol* 2007; **36:** 6330–41.

30 WHO. WHO vaccine-preventable diseases: monitoring system. 2013 global summary.http://apps.who.int/immunization_monitoring/globalsummary/indicators?ir%5Bc%5D%5B%5D=GNB&ir%5Ba%5D=on&commit=Ok+with+the+selection (accessed March 31, 2014).

31 Martins CL, Benn CS, Andersen A, et al. A randomized trial of a standard dose of edmonston-zagreb measles vaccine given at 4.5 months of age: effect on total hospital admissions. *J Infect Dis* 2014; **209:** 1731–38.

32 Banerjee AV, Duflo E, Glennerster R, Kothari D. Improving immunisation coverage in rural India: clustered randomised controlled evaluation of immunisation campaigns with and without incentives. *BMJ* 2010; **340:** c2220.

33 Lim SS, Stein DB, Charrow A, Murray CJL. Tracking progress towards universal childhood immunisation and the impact of global initiatives: a systematic analysis of three-dose diphtheria, tetanus, and pertussis immunisation coverage. *Lancet* 2008; **372:** 2031–46.

34 Lu C, Michaud CM, Gakidou E, Khan K, Murray CJL. Effect of the Global Alliance for Vaccines and Immunisation on diphtheria, tetanus, and pertussis vaccine coverage: an independent assessment. *Lancet* 2006; **368:** 1088–95.

35 Ndiritu M, Cowgill KD, Ismail A, et al. Immunization coverage and risk factors for failure to immunize within the Expanded Programme on Immunization in Kenya after introduction of new *Haemophilus influenzae* type b and hepatitis B virus antigens. *BMC Public Health* 2006; **6:** 132.

36 Chandir S, Khan AJ, Hussain H, et al. Effect of food coupon incentives on timely completion of DTP immunization series in children from a low-income area in Karachi, Pakistan: a longitudinal intervention study. *Vaccine* 2010; **28:** 3473–78.

37 Usman HR, Akhtar S, Habib F, Jehan I. Redesigned immunization card and center-based education to reduce childhood immunization dropouts in urban Pakistan: a randomized controlled trial. *Vaccine* 2009; **27:** 467–72.

38 WHO. Meeting of the Strategic Advisory Group of Experts on immunization, April 2010—conclusions and recommendations. *Wkly Epidemiol Rec* 2010; **85:** 197–212.

39 Mutua MK, Kimani-Murage E, Ettarh RR. Childhood vaccination in informal urban settlements in Nairobi, Kenya: who gets vaccinated? *BMC Public Health* 2011; **11:** 6.

40 Ministry of Public Health, Republic of Guinea-Bissau. Comprehensive multi-year plan for the expanded program on immunization: Guinea-Bissau, 2010–2014. Guinea-Bissau: Ministry of Public Health, 2009.

41 Veirum JE, Sodemann M, Biai S, et al. Routine vaccinations associated with divergent effects on female and male mortality at the paediatric ward in Bissau, Guinea-Bissau. *Vaccine* 2005; **23:** 1197–204.

42 Fine PE, Smith PG. 'Non-specific effects of vaccines'—an important analytical insight, and call for a workshop. *Trop Med Int Health* 2007; **12:** 1–4.

43 Lee BY, Norman BA, Assi TM, et al. Single versus multi-dose vaccine vials: an economic computational model. *Vaccine* 2010; **28:** 5292–300.

44 UN Statistics Division. Millennium Development Goals indicators. The official United Nations site for the MDG indicators. http://mdgs.un.org/unsd/mdg/Host.aspx?Content=Indicators/OfficialList.htm (accessed Sept 12, 2013).

45 GAVI Alliance. Information sheet on Performance Based Funding (PBF). http://www.gavialliance.org/support/apply/ (accessed Nov 9, 2013).