Supplementary Materials to:

The mortality effects of disregarding the strategy to save doses of measles vaccine – a cluster-randomized trial in Guinea-Bissau

Stine Byberg, Peter Aaby, Amabelia Rodrigues, Christine Stabell Benn, Ane Bærent Fisker

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Contents

Supplementary methods:

Setting

The Bandim Health Project (BHP) collects information aimed at assessing health of all children below five years of age[1]. At every visit, information on vaccinations is obtained by inspecting the child's health card and the dates of the received vaccinations are noted. If a child has died or been admitted to hospital since the previous visit, this is registered. A BHP nurse accompanies each of the mobile teams and offers routine vaccines to children who are missing vaccinations and provides essential drugs (anti-malarials, antibiotics) to sick children when indicated.

Trial design and randomization: The MVEPI trial

The 182 rural clusters under BHP surveillance were randomized to either "MV-for-all-policy" or "Restrictive-MV-policy" stratified in the following manner: Within each region the clusters were divided into two strata based on mortality rates of children born in 2007-2010. Clusters with a mortality rate above the regional median mortality rate were assigned one block, clusters with a mortality rate below the median to another block. Within each block the clusters were randomized to MV-for-all-policy or Restrictive-MV-policy based on computer generated random numbers.

Our initial sample size calculations were based on a 50% reduction in child mortality and follow-up to 3 years of age. In March 2011, after we had only enrolled for a few weeks, this was revised to 30% and follow-up was extended to 5 years of age. Consequently, the planned number of enrolments in the age group 12-35 months was increased to from 3500 to 3676 children.

Enrolment

The children underwent a clinical examination and were weighed, and major risk factors were documented. According to local practice, children who are overtly sick are not vaccinated. In addition, children suffering from severe malnutrition (mid-upper-arm circumference (MUAC)<110mm), the most common manifestation of AIDS, were not vaccinated but referred to the health centre.

Ill children and children who were not at home during the first visit were eligible for enrolment during a subsequent visit provided they still fulfilled the enrolment criteria.

In July 2012 we initiated a randomised trial of an additional early MV in three of the regions[2]. This meant that we made MV available to all children below 12 months of age regardless of the number of measles unvaccinated children present, and we therefore paused enrolments in these regions, while continuing in the rest of the country. We gradually resumed enrolment in the affected regions after 25 September 2013, but in these regions we only offered enrolment to children above 12 months in 2013-15.

Statistical methods

We assessed the impact of the MV-for-all-policy on the risk of hospital admission and MUAC measured at the first visit after enrolment. The effect of MV-for-all on admission to hospital was estimated in Cox proportional hazards models analogous to the mortality analyses. The impact of the MV-for-all-policy on MUAC was assessed in linear regression analyses, adjusted for MUAC at baseline.

Ethical considerations

The BHP HDSS surveillance was initiated in 1990 at the request of the Ministry of Health. Surveyed women provide oral consent at the time of registration. All mothers/guardians provided written informed consent if their child was vaccinated after 12 months of age. The protocol was approved by the National Ethics Committee in Guinea-Bissau and the Central Ethical Committee in Denmark gave consultative approval.

Supplementary results:

Mortality among not enrolled children

Among the 12,287 already measles vaccinated children (See Figure 1 "Not eligible: -Already measles vaccinated") there were 335 deaths between eligibility assessment and 5 years of age/censoring: 173 in 16,292 person years in the MV-for-all clusters and 162 in 17,116 person years in the Restrictive-MV-policy clusters. In a Cox proportional hazards model stratified for the randomization variables (region and pre-trial mortality strata), the hazard ratio (HR) indicated no difference by trial arm among children aged 12-35 months at eligibility assessment (HR=0.98 (0.75-1.28)) but tended to be lower in the intervention villages among the youngest children (15%, n=1791, HR=0.67 (0.40-1.14)).

Community level effects

Among the 22,047 children aged 9-35 when attempted visited (See Figure 1 "Community level analysis"), there were 646 deaths between the first visit and 5 years of age/censoring: 321 in 30,522 person years in the MV-for-all clusters and 325 in 29,775 person years in the Restrictive-MV-policy clusters. The HR from a Cox proportional hazards model stratified for the randomization variables was 0.96 (0.81-1.13).

Sex-differential effects and non-proportional hazards

There was some evidence of non-proportional hazards in the analysis allowing the effects to differ by sex: p=0.13 for children aged 12-35 months and p=0.03 for all children. To define time windows with proportional hazards, we split the follow-up time at 2.5 years of age. The HRs before 2.5 years of age for children aged 12-35 months at eligibility assessment were 1.28 (0.69-2.37) for boys and 1.31 (0.77-2.21) for girls, while the HRs after 2.5 years were 0.45 (0.24-0.84) for boys and 1.73 (0.70-4.29) for girls (p=0.02 for a differential effect in boys and girls after 2.5 years, test of interaction between age-band, sex and MV-policy arm, p=0.05). Changing the timescale to time since eligibility assessment, the proportional hazards tests yielded test statistics of p=0.45 for children aged 12-35 months and p=0.20 for all children. The sex-differential effects were similar: HR=0.77 (0.42-1.42) for boys and HR=1.21 (0.64-2.29) for girls (p=0.34 for interaction) in children 12-35 months and HR=0.78 (0.49-1.24) for boys and 1.45 (0.89-2.38) for girls (p=0.09 for interaction) in all children.

Effects on hospital admission

In line with the results observed for mortality, children eligible for enrolment in the rainy season living in MV-for-all clusters tended to have higher rates of hospital admissions HR=1.36 (0.65-2.85)) while children enrolled in the dry season tended to have lower rates, HR=0.88 (0.44-1.77). This was similar for children aged 9-11 months (Supplementary Table 3).

Changing effects over calendar time

Moving the cut-off date 3 months for- or backwards from the actual date of initiating the early MV trial yielded similar results (Supplementary figure 2).

Supplementary discussion

Strengths and limitations

While we are confident that we can classify whether the individual child experience an event during follow-up, arriving at information on the exact date of both deaths and admissions is more challenging. Although the field assistants ask for evidence concerning the health facility contacts (e.g. a paper prescription or clinical notes of the admission in the child's health card), this is not always available, and dates may therefore be recorded with less precisions (i.e. with the month or season of event). However, as enrolment took place during the HDSS routine visits, we are still able to place episodes before or after the entry into the trial.

Although we followed 4767 children in the study, we only included 2778 children aged 12-35 months and therefore only 76% of the planned 3676 children. More importantly, mortality was less than half of what we had anticipated. Nonetheless, we saw no indication that had we would have seen a difference in overall mortality if we had had the planned power.

Consistency with previous studies

We saw no beneficial effect of living in MV-for-all clusters on MUAC. To our knowledge, few studies have previously assessed the effect of MV on MUAC. In one trial of an additional early dose of MV, MV was associated with higher MUAC at 24 months of age[3], while another trial observed no effect at 9 months[4].

Supplementary Tables

Supplementary Table 1: Vaccination campaigns targeting children aged 0-59 months of age

Year	Dates	Vaccine and target age group	Other Interventions
2009	03/07-07/07	MV (9-59 months)	VAS/MbZ
2010	06/03-09/03	OPV (0-59 months)	
	23/04-26/04	OPV (0-59 months)	
	28/05-02/06	OPV (0-59 months)	VAS/MbZ
	14/10-18/10	H1N1 (6-59 months)	
2011	25/03-29/03	OPV (0-59 months)	
	29/04-05/05	OPV (0-59 months)	VAS/MbZ
	25/11-29/11	OPV (0-59 months)	VAS/MbZ
2012	23/03-26/03	OPV (0-59 months)	
	02/12-06/12	MV (9-59 months)	VAS/MbZ
2013	24/05-27/05	OPV (0-59 months)	VAS/MbZ
	03/11-06/11	OPV (0-59 months)	VAS/MbZ
2014	14/10-17/10	OPV (0-59 months)	
	29/11-03/12	OPV (0-59 months)	VAS/MbZ
2015	02/10-05/10	OPV (0-59 months)	VAS/MbZ
	04/12-09/12	MV (9-59 months)	VAS/MbZ
2016	17/06-30/06	MenA (1-29 years)	VAS/MbZ

H1N1: H1N1-Influenza vaccination; OPV: Oral Polio Vaccine, MV: Measles vaccine; MenA: Meningitis A vaccine
VAS/Mbz: Co-administered with vitamin A for children 6-59 months (100,000 IU for children 6-11 months; 200,000 IU for children 12-59 months) and Mebendazole (500 mg, for children 12-59 months)

Supplementary Table 2: Mortality and hazard ratios for death of children eligible for enrolment in the MVEPI trial (Per-protocol analyses). Overall and stratified by sex.

	N	Deaths/person years (PYRS)	Mortality rate (per 1000 PYRS)	HR 95%CI*	P-value test of interaction
Children 12-35 months at eligibility assessment					
-Restrictive-MV-policy	1254	40/3,456	11.6	1.00 (ref)	
-MV-for-all	1280	42/3,458	12.1	0.98 (0.65-1.49)	
All Children					
-Restrictive-MV-policy	2174	73/6,410	11.4	1.00 (ref)	
-MV-for-all	2263	86/6,609	13.0	1.11 (0.80-1.52)	
Children 12-35 months at eligibility assessment					
Boys					
-Restrictive-MV-policy	620	25/1,740	14.4	1.00 (ref)	
-MV-for-all	623	20/1,724	11.6	0.74 (0.40-1.37)	
Girls					
-Restrictive-MV-policy	634	15/1,716	8.7	1.00 (ref)	
-MV-for-all	657	22/1,734	12.7	1.38 (0.69-2.75)	0.23
All Children					
Boys					
-Restrictive-MV-policy	1079	44/3,245	13.6	1.00 (ref)	
-MV-for-all	1098	37/3,248	11.4	0.80 (0.50-1.29)	
Girls					
-Restrictive-MV-policy	1095	29/3,166	9.2	1.00 (ref)	
-MV-for-all	1165	49/3,361	14.6	1.57 (0.92-2.69)	0.09

*Estimated in a Cox proportional hazards model stratified for region and pretrial mortality level. 95% CI estimated using a robust standard error accounting for intra-cluster correlation

Supplementary Table 3: Rates of first hospital admission and hazard ratios for admission among all children aged 9-35 months eligible for enrolment in the MVEPI trial. Overall and stratified by sex and season of eligibility assessment

	N	Deaths/person years (PYRS)	Mortality rate (per 1000 PYRS)	HR 95%CI*	P-value test of interaction
All Children					
-Restrictive-MV-policy	2339	65/6,682	9.7	1.00 (ref)	
-MV-for-all	2428	68/6,883	9.9	0.95 (0.67-1.36)	
Boys					
-Restrictive-MV-policy	1157	32/3,395	9.4	1.00 (ref)	
-MV-for-all	1187	31/3,398	9.1	0.89 (0.49-1.60)	
Girls					
-Restrictive-MV-policy	1182	33/3,287	10.0	1.00 (ref)	
-MV-for-all	1241	37/3,485	10.6	1.02 (0.64-1.62)	0.74
Dry season					
-Restrictive-MV-policy	1211	40/3,445	11.6	1.00 (ref)	
-MV-for-all	1242	33/3,539	9.3	0.76 (0.45-1.29)	
Rainy season					
-Restrictive-MV-policy	1128	25/3,237	7.7	1.00 (ref)	
-MV-for-all	1186	35/3,344	10.5	1.26 (0.77-2.08)	0.18

*****Estimated in a Cox proportional hazards model stratified for region and pretrial mortality level. 95% CI estimated using a robust standard error accounting for intra-cluster correlation

Supplementary Table 4: Differences in MUAC among the restrictive vial policy and the MV-for-all policy at first visit after enrolment, adjusted for baseline MUAC and stratified by age and sex

	N	Restrictive-MV-policy (MUAC mm (SD))	MV-for-all policy (MUAC mm (SD))	Difference (95%CI)*
Children aged 12-35 months at eligibility assessment	2287	145 (12)	146 (12)	0.77 (-0.20-1.74)
Children aged 9-11 months at eligibility assessment	1805	143 (13)	142 (12)	-1.08 (-2.46-0.30)
All children	4092	144 (12)	144 (12)	-0.07 (-0.82-0.67)
Boys 12-35 months	1133	146 (12)	147 (13)	0.80 (-0.61-2.21)
Girls 12-35 months	1154	145 (12)	146 (11)	0.75 (-0.58-2.08)
Boys 9-11 months	891	145 (13)	143 (13)	-1.87 (-3.64 - -0.10)
Girls 9-11 months	914	142 (12)	142 (11)	-0.35 (-1.87 – 1.18)
All Boys	2024	146 (12)	145 (13)	-0.35 (-1.47-0.76)
All Girls	2068	143 (12)	144 (12)	0.20 (-0.81-1.21)

*Adjusted for baseline MUAC and village cluster

Supplementary Table 5: Mortality and hazard ratios for death among children 9-35 months eligible for enrolment in the MVEPI trial. Follow-up time split at campaigns during follow-up.

	N	Deaths/person years (PYRS)	Mortality rate (per 1000 PYRS)	HR 95%CI*	P-value test of interaction
Before eligibility for MV campaign					
-Restrictive MV policy	2339	44/2,880	15.3	1.00 (ref)	
-MV for all	2428	53/2,885	18.4	1.16 (0.79-1.71)	
After eligibility for MV campaign[¤]					
-Restrictive MV policy	2143	37/3,895	9.5	1.00 (ref)	
-MV for all	2208	39/4,098	9.5	0.95 (0.61-1.49)	0.50
Before eligibility for OPV campaign					
-Restrictive MV policy	2339	20/959	20.9	1.00 (ref)	
-MV for all	2428	16/992	16.1	0.76 (0.40-1.45)	
After eligibility for OPV campaign[&]					
-Restrictive MV policy	2235	61/5,816	10.5	1.00 (ref)	
-MV for all	2314	76/5,990	12.7	1.16 (0.82-1.63)	0.24
Before eligibility for MenAfriVac					
-Restrictive MV policy	2339	76/6,091	12.5	1.00 (ref)	
-MV for all	2428	79/6,266	12.6	0.97 (0.70-1.34)	
After eligibility for MenAfriVac[^]					
-Restrictive MV policy	1074	5/684	7.3	1.00 (ref)	
-MV for all	1139	13/717	18.1	2.42 (0.89-6.60)[#]	0.09

*Estimated in a Cox proportional hazards model stratified for region and pretrial mortality level. 95% CI estimated using a robust standard error accounting for intra-cluster correlation

[¤]The HR was 0.77 (0.53-1.13) for after-vs-before exposure to MV campaigns

[&]The HR was 0.83 (0.55-1.27) for after-vs-before exposure to OPV campaigns

^The HR was 1.24 (0.73-2.12) for after-vs-before exposure to MenAfriVac campaign

[#]HR in girls=5.77 (0.68-48.39); HR in boys=1.58 (0.43-5.79)

Supplementary Table 6: Mortality and hazard ratios for death among children eligible for enrolment in the MVEPI trial by season of enrolment.

	N	Deaths/person years (PYRS)	Mortality rate (per 1000 PYRS)	HR 95%CI*	P-value test of interaction
Children 12-35 months at eligibility assessment					
Dry season					
-Restrictive-MV-policy	698	26/1,890	13.8	1.00 (ref)	
-MV-for-all	730	20/1,947	10.3	0.69 (0.40-1.21)	
Rainy season					
Restrictive-MV-policy	675	18/1,807	10.0	1.00 (ref)	
-MV-for-all	675	25/1,776	14.1	1.35 (0.72-2.53)	0.13
All Children					
Dry season					
-Restrictive-MV-policy	1211	44/3,500	12.6	1.00 (ref)	
-MV-for-all	1242	43/3,586	12.0	0.91 (0.62-1.33)	
Rainy season					
-Restrictive-MV-policy	1128	37/3,275	11.3	1.00 (ref)	
-MV-for-all	1186	49/3,397	14.4	1.24 (0.78-1.98)	0.30

*Estimated in a Cox proportional hazards model stratified for region and pretrial mortality level. 95% CI estimated using a robust standard error accounting for intra-cluster correlation

Supplementary Table 7: Number of exposures to OPV campaigns prior to eligibility assessment. By age group, overall and during different phases of the trial.

	Period								
	Entire			Period 1: Early (<18/07/2012)			Period 2: Late (>=18/07/2012)		
No. of doses*	12-35 mo.	9-11 mo.	All	12-35 mo.	9-11 mo.	All	12-35 mo.	9-11 mo.	All
0	15 (1%)	173 (9%)	188 (4%)	0 (0%)	23 (2%)	23 (1%)	15 (1%)	150 (15%)	165 (6%)
1	278 (10%)	540 (27%)	818 (17%)	0 (0%)	131 (14%)	131 (6%)	278 (18%)	409 (40%)	687 (27%)
2	884 (32%)	1132 (57%)	2016 (42%)	175 (14%)	669 (69%)	844 (38%)	709 (46%)	463 (45%)	1172 (46%)
3	683 (25%)	144 (7%)	827 (17%)	424 (35%)	144 (15%)	568 (26%)	259 (17%)	0 (0%)	259 (10%)
4	450 (16%)	0 (0%)	450 (9%)	233 (19%)	0 (0%)	233 (11%)	217 (14%)	0 (0%)	217 (8%)
5	353 (13%)	0 (0%)	353 (7%)	304 (25%)	0 (0%)	304 (14%)	49 (3%)	0 (0%)	49 (2%)
6	83 (3%)	0 (0%)	83 (2%)	68 (6%)	0 (0%)	68 (3%)	15 (1%)	0 (0%)	15 (1%)
7	32 (1%)	0 (0%)	32 (1%)	22 (2%)	0 (0%)	22 (1%)	10 (1%)	0 (0%)	10 (0%)

Supplementary Table 8: Mortality and hazard ratios for death among children eligible for enrolment when aged 9-35 in the MVEPI trial by number of exposures to OPV campaigns prior to eligibility assessment. Observation time split by subsequent exposure to OPV campaigns.

	N	Deaths/person years (PYRS)	Mortality rate (per 1000 PYRS)	HR 95%CI*	P-value test of interaction
0-2 doses, No/not yet OPV after enrolment					
-Restrictive MV policy	1474	13/629	20.7	1.00 (ref)	
-MV for all	1548	12/672	17.8	0.85 (0.39-1.88)	
3+ doses, No/not yet OPV after enrolment					
Restrictive MV policy	865	8/341	23.5	1.00 (ref)	
-MV for all	880	5/366	13.7	0.57 (0.20-1.64)	0.55
0-2 doses, after OPV after enrolment					
-Restrictive MV policy	1418	36/3,842	9.4	1.00 (ref)	
-MV for all	1483	60/3,964	15.1	1.58 (1.09-2.27)	
3+ doses, after OPV after enrolment					
Restrictive MV policy	812	24/1,963	12.2	1.00 (ref)	
-MV for all	814	15/1,980	7.6	0.57 (0.30-1.07)	0.004

*Estimated in a Cox proportional hazards model stratified for region and pretrial mortality level. 95% CI estimated using a robust standard error accounting for intra-cluster correlation

Supplementary Table 9. Mortality and hazard ratios for death among children eligible for enrolment in the MVEPI trial by reception of pentavalent vaccine at eligibility assessment.

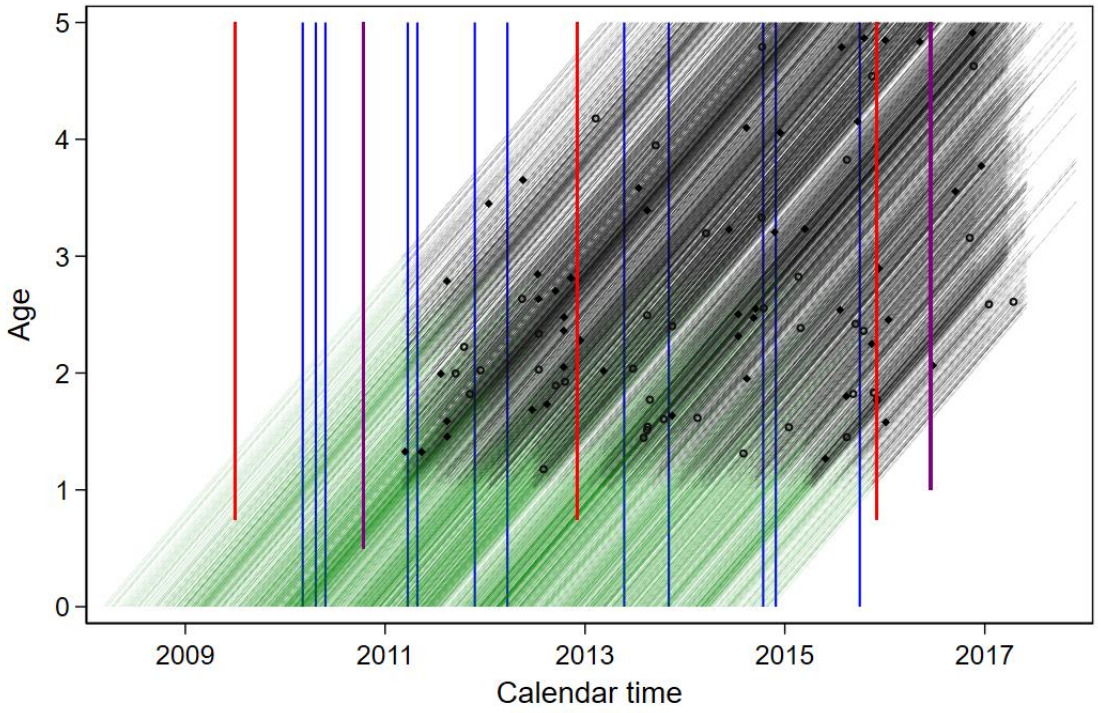
	N	Deaths/person years (PYRS)	Mortality rate (per 1000 PYRS)	HR 95%CI*	P-value, test of interaction
Received pentavalent vaccine at the time of assessment of eligibility into MVEPI study (Children aged 9-11 months¤)					0.88
-Restrictive-MV-policy	271	9/839	10.7	1.00 (ref)	
-MV-for-all	339	15/1060	14.2	1.21 (0.53-2.81)	
Received no pentavalent vaccine at the time of assessment of eligibility into MVEPI study (Children aged 9-11 months)					
-Restrictive-MV-policy	695	28/2238	12.5	1.00 (ref)	
-MV-for-all	684	32/2200	14.5	1.13 (0.69-1.85)	

*Estimated in a Cox proportional hazards model stratified for region and pretrial mortality level. 95% CI estimated using a robust standard error accounting for intra-cluster correlation

¤: Not estimated among children aged 12-35 months at eligibility assessment as few of the older children were vaccinated at the date of eligibility assessment.

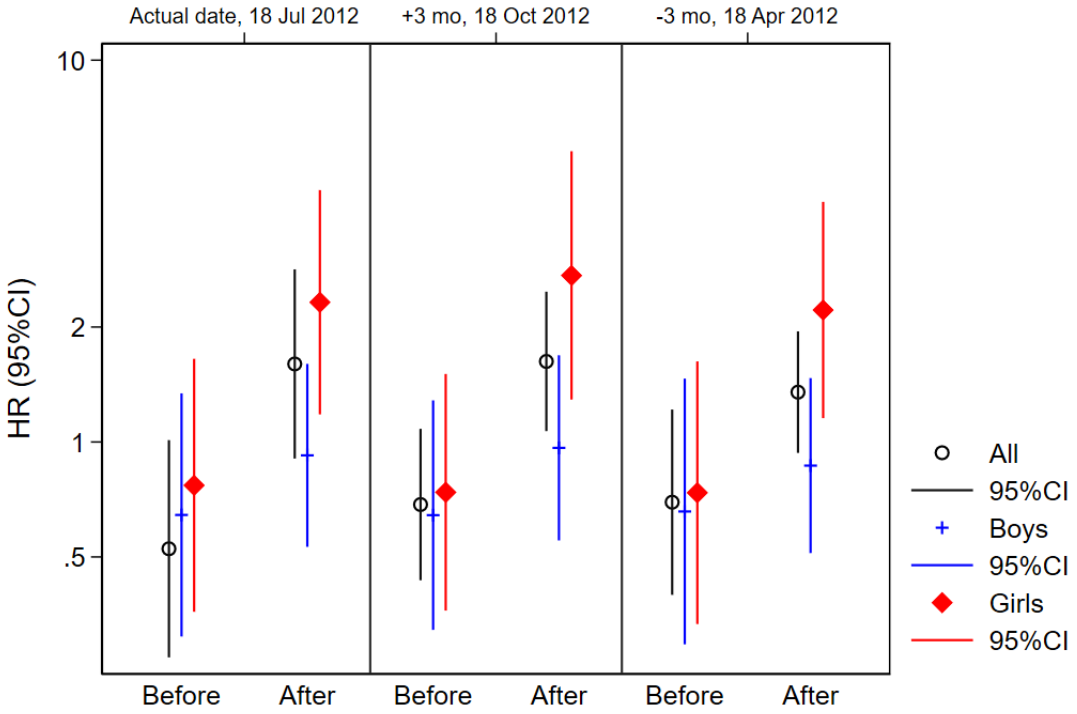
Supplementary Figures

Supplementary figure 1: Lexis diagram displaying the time before (green lines) and under study (black lines) of children followed after eligibility assessment at 12-36 months.



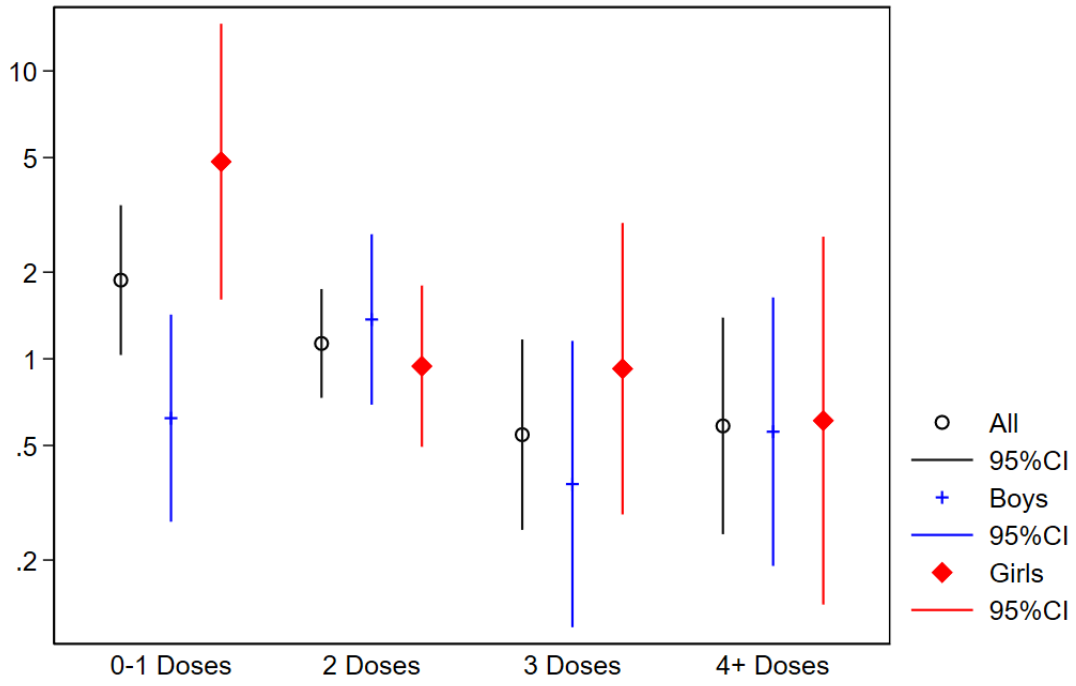
Note: Lexis diagram illustrating lifelines of study children pre-enrolment (green) and after enrolment visits (black). Deaths indicated with filled diamonds (boys) and circles (girls). Vertical lines indicate national vaccination campaigns: three measles vaccination campaigns in red (target age group 9-59 months), 12 campaigns with oral polio vaccine in blue (target age group 0-59 months), one H1N1-influenza vaccination campaign in purple in 2010 (target age group 6-59 months) and one meningitis A vaccination campaign in purple in 2016 (target age group 12 months-29 years).

Supplementary figure 2: Hazard Ratios when splitting the dataset at the date of initiating enrolment in a concurrent trial of early measles vaccination.



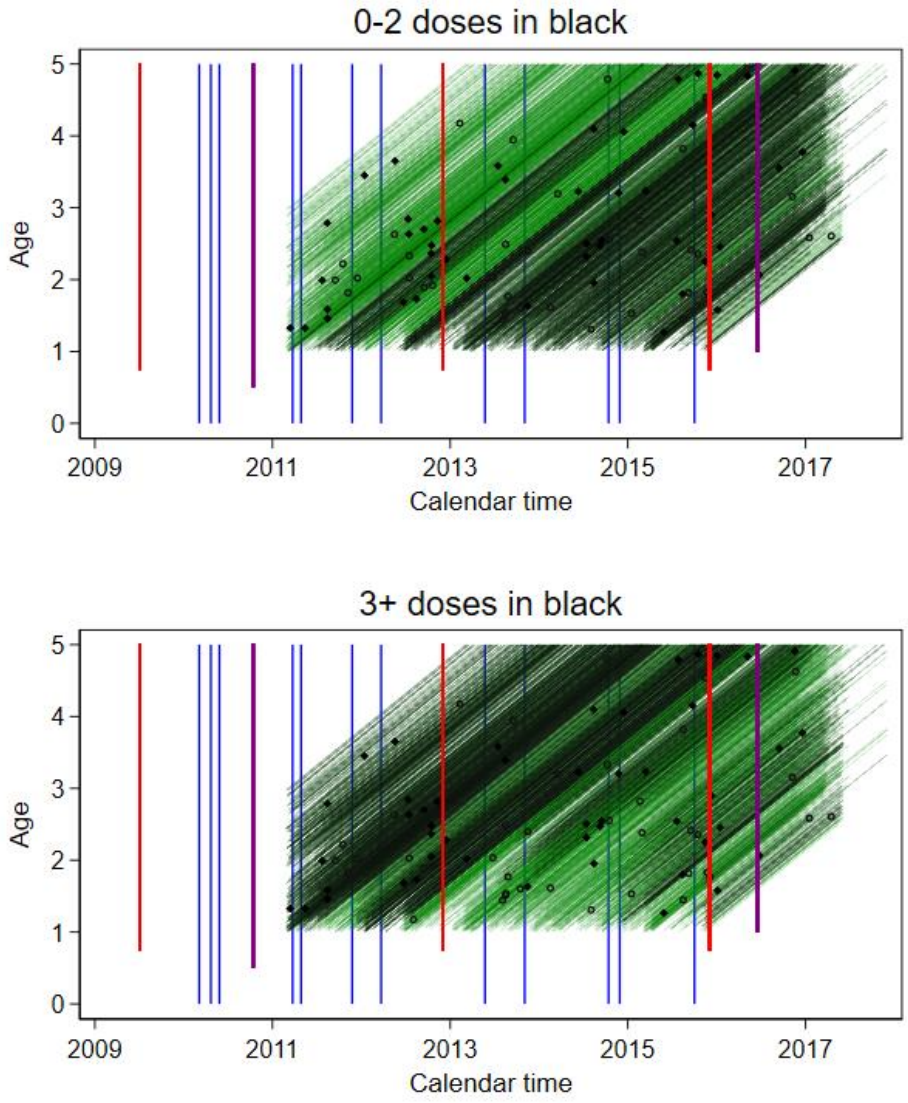
Note: Among children aged 9-35 months at eligibility assessment, follow-up to 5 years.

Supplementary figure 3: Hazard ratios overall and by sex by exposure to different number of OPV campaigns prior to assessment for eligibilty for enrolment in the MVEPI trial



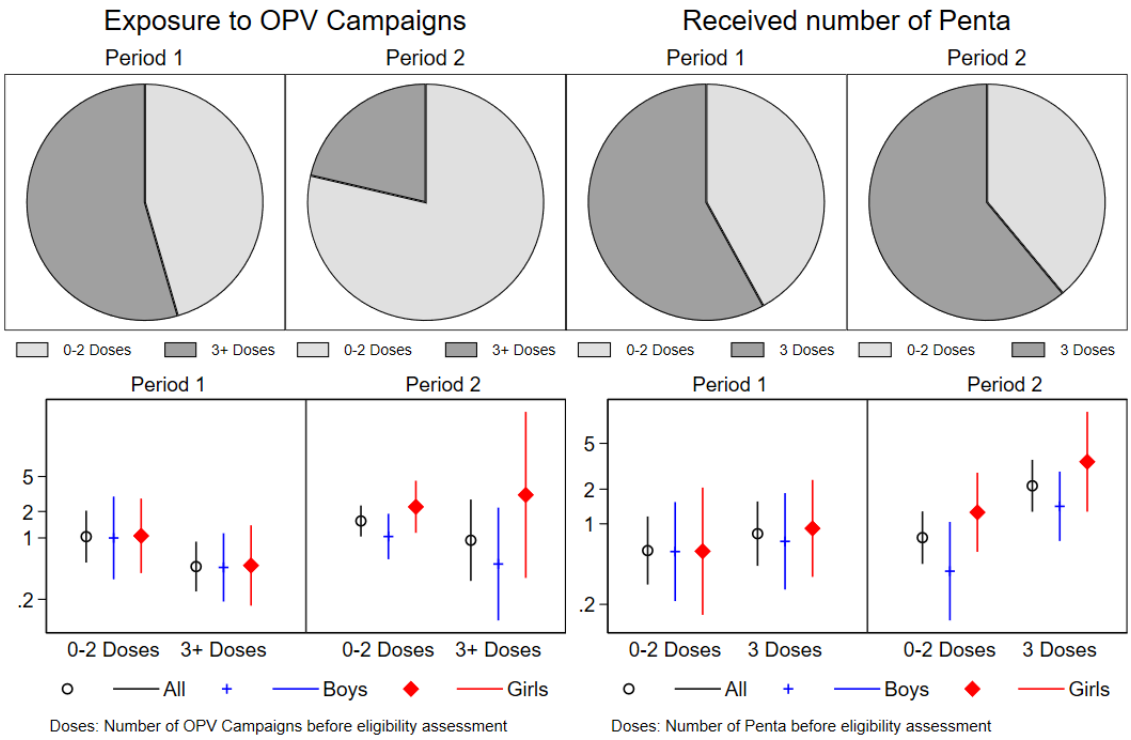
Note: Among children aged 9-35 months at eligibility assessment, follow-up to 5 years.

Supplementary figure 4: Lexis diagrams displaying the time under study of children followed after eligibility assessment at 12-36 months, by number of OPV doses prior to eligibility assessment.



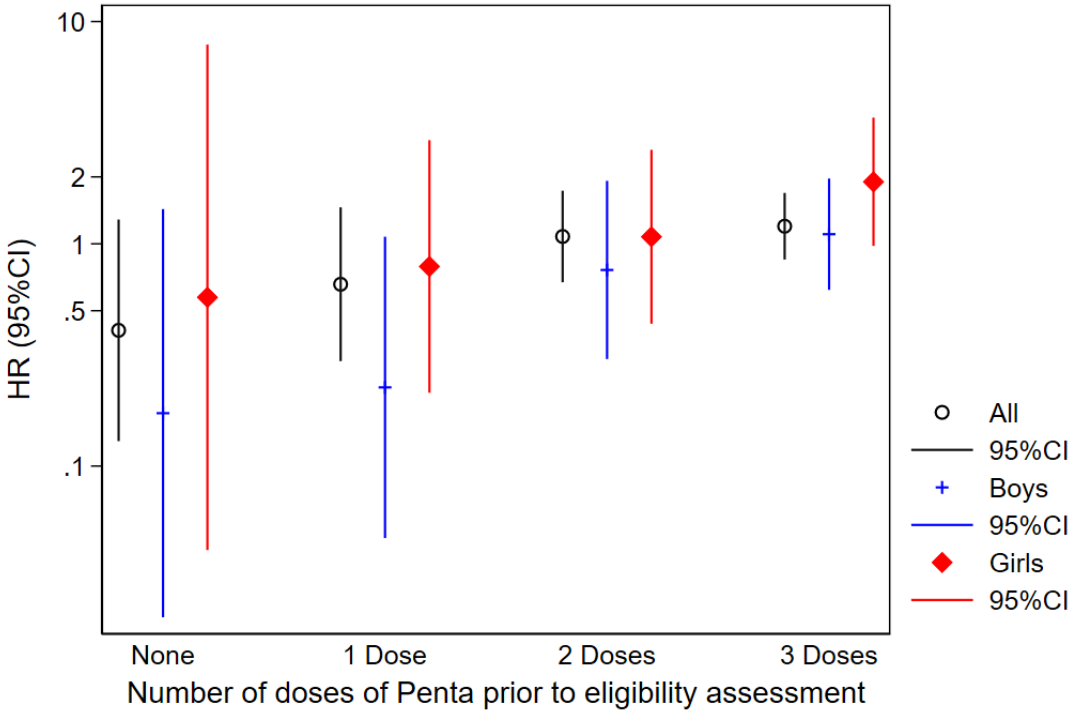
Note: Lexis diagram illustrating lifelines of children under study. Black lines for selected number of OPV campaigns prior to enrolment. Deaths indicated with filled diamonds (boys) and circles (girls). Vertical lines indicate national vaccination campaigns: 3 measles vaccination campaigns in red (target age group 9-59 months), 12 campaigns with oral polio vaccine in blue (target age group 0-59 months), 1 H1N1-influenza vaccination campaign in purple in 2010 (target age group 6-59 months) and 1 meningitis A vaccination campaign in purple in 2016 (target age group 12 months-29 years).

Supplementary figure 5: Distribution of exposure to OPV campaigns and doses of pentavalent vaccine prior to eligibility assessment and hazard ratios comparing the mortality rates by trial arm in the strata defined by period, number of doses and sex.



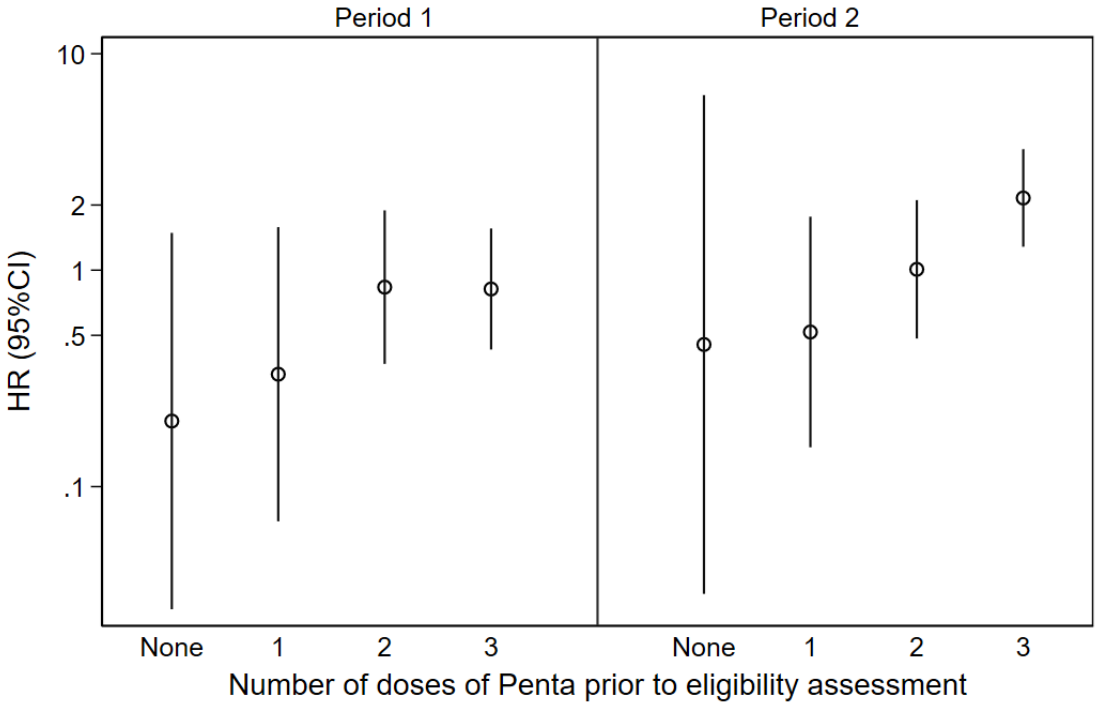
Note: Among children aged 9-35 months at eligibility assessment, follow-up to 5 years.

Supplementary Figure 6. Hazard ratios overall and by sex for children who had received 0-3 doses of Pentavalent vaccine (Penta) prior to enrolment into the MVEPI trial



Note: Among children aged 9-35 months at eligibility assessment, follow-up to 5 years.
Number of doses of Penta modelled linear variable: p=0.18 overall; p=0.86 for boys, p=0.08 for girls.

Supplementary figure 7: Hazard ratios for children who had received 0-3 doses of Pentavalent vaccine (Penta) prior to enrolment into the MVEPI trial by period of eligibility assessment (before and after 18 July 2012).



Note: Among children aged 9-35 months at eligibility assessment, follow-up to 5 years.

References

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