



University of Southern Denmark

Contrasting female-male mortality ratios after routine vaccinations with pentavalent vaccine versus measles and yellow fever vaccine. A cohort study from urban Guinea-Bissau

Fisker, Ane B; Biering-Sørensen, Sofie; Lund, Najaaraq; Djana, Queba; Rodrigues, Amabelia; Martins, Cesario L; Benn, Christine S

Published in:
Vaccine

DOI:
[10.1016/j.vaccine.2016.07.034](https://doi.org/10.1016/j.vaccine.2016.07.034)

Publication date:
2016

Document version
Final published version

Document license
CC BY-NC-ND

Citation for pulished version (APA):

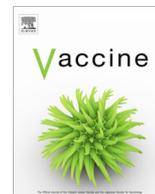
Fisker, A. B., Biering-Sørensen, S., Lund, N., Djana, Q., Rodrigues, A., Martins, C. L., & Benn, C. S. (2016). Contrasting female-male mortality ratios after routine vaccinations with pentavalent vaccine versus measles and yellow fever vaccine. A cohort study from urban Guinea-Bissau. *Vaccine*, 34(38), 4551-4557. <https://doi.org/10.1016/j.vaccine.2016.07.034>

Terms of use

This work is brought to you by the University of Southern Denmark through the SDU Research Portal. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk



Contrasting female-male mortality ratios after routine vaccinations with pentavalent vaccine versus measles and yellow fever vaccine. A cohort study from urban Guinea-Bissau



Ane B. Fisker^{a,b,c,*}, Sofie Biering-Sørensen^{a,b}, Najaaraq Lund^{a,b}, Queba Djana^a, Amabelia Rodrigues^a, Cesario L. Martins^a, Christine S. Benn^{b,c}

^a Bandim Health Project, INDEPTH Network, Apartado 861, Bissau, Guinea-Bissau

^b Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark

^c OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, 5000 Odense C, Denmark

ARTICLE INFO

Article history:

Received 25 April 2016

Received in revised form 16 July 2016

Accepted 18 July 2016

Available online 27 July 2016

Keywords:

Non-specific/heterologous effects of vaccines

Mortality

Sex-difference

Pentavalent vaccine

Measles vaccine

ABSTRACT

Background: In addition to protection against the target diseases, vaccines may have non-specific effects (NSEs). Measles vaccine (MV) has beneficial NSEs, providing protection against non-measles deaths, most so for girls. By contrast, though protecting against diphtheria, tetanus and pertussis, DTP vaccine is associated with increased female mortality relative to male mortality. In 2008, Guinea-Bissau replaced DTP with the DTP-containing pentavalent vaccine (Penta; DTP-H. influenza type B-Hepatitis B) at 6, 10 and 14 weeks and yellow fever vaccine (YF) was to be given with MV. We investigated possible sex-differential mortality rates following Penta and MV+YF vaccination.

Methods: Bandim Health Project (BHP) registers vaccines given by the three government health centres in the study area and vital status through demographic surveillance. We assessed the association between sex and mortality by vaccination status in Cox proportional hazards models with age as underlying timescale. Follow-up was censored at a subsequent vaccination contact or after 6 months of follow-up.

Results: Between September 2008 and April 2011, we registered 23,448 vaccination contacts for children aged 42–365 days; 17,313 were for Penta and 3028 for MV (2907 co-administered with YF). During follow-up 112 children died. The female/male mortality rate ratio was 1.73 (1.11–2.70) following Penta and 0.38 (0.12–1.19) after MV ($p = 0.02$ for same effect). Adjusting for maternal education or weight-for-age at the time of vaccination did not change the estimates.

Conclusion: Penta appears to have the same negative effects on mortality as those seen for DTP. Assessing post-vaccination mortality for boys and girls is necessary to improve the vaccination programme.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Vaccines are given to prevent specific infections but there is increasing evidence that vaccines may have much broader effects on overall child health [1]. The effects which are not explained by specific disease prevention have been named “non-specific” or “heterologous”. While disease-specific immune memory may not be altered by administration of subsequent vaccines, the

non-specific effects (NSEs) are shaped most strongly by the most recent vaccination [2–4].

The NSEs can be beneficial but also harmful. The live *Bacillus Calmette-Guérin* (BCG) and measles vaccine (MV) are associated with beneficial NSEs improving overall child survival [5–12]. However, though protecting against the targeted infections, the inactivated diphtheria-tetanus pertussis vaccines (DTP) is associated with higher child mortality [3,5,12–17]. NSEs are often sex-differential; while MV has been associated with stronger beneficial effects for girls than for boys, DTP has been linked to higher female mortality [2,3,6,13,18,19]. In low-income countries, vaccines are recommended sequentially during infancy: BCG vaccine at birth, three doses of DTP-containing vaccine at 6, 10 and 14 weeks of age and a MV at 9 months of age. If DTP and MV have strong effects

* Corresponding author at: Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, 2300 Copenhagen S, Denmark.

E-mail addresses: a.fisker@bandim.org (A.B. Fisker), s.biering@bandim.org (S. Biering-Sørensen), najaaraq.lund@gmail.com (N. Lund), q.djana@gmail.com (Q. Djana), a.rodriques@bandim.org (A. Rodrigues), c.martins@bandim.org (C.L. Martins), cb@ssi.dk (C.S. Benn).

on child survival, changing from having DTP to having MV as the most recent vaccine should lower the female/male mortality rate ratio (F/M MRR). In 2007 this led to the formulation of the hypothesis, that the transition from DTP to MV should result in an inversion of the F/M MMRs [20].

During the last 10 years the DTP-containing pentavalent vaccine (“Penta”: DTP-H. influenza type B-Hepatitis B) has replaced DTP [21] and yellow fever vaccine (YF) has been introduced to be co-administered with MV [22] in many low-income countries. Hence, we investigated whether the F/M MRR after Penta and after MV+YF were similar to the effects previously reported for DTP and MV.

2. Methods

2.1. Setting and population

Bandim Health Project (BHP) runs a Health and Demographic Surveillance system (HDSS) in six suburban districts in Bissau, the capital of Guinea-Bissau. The HDSS covers a population of 100,000 individuals. All households are visited monthly to enquire about pregnancies and deaths. At the first visit after birth information on socioeconomic status is collected. The indicators include maternal characteristics (education and ethnicity) and household characteristics (type of roofing, availability of bathroom and electricity). Children below the age of 3 years are visited every 3 months to collect information on vital status as well as vaccinations, hospitalisations and nutritional status.

2.2. Assessment of exposure

Since the end of August 2008, the national Expanded Programme on Immunizations (EPI) vaccination schedule in Guinea-Bissau is BCG and OPV at birth, Penta with OPV at 6, 10 and 14 weeks and MV and YF vaccine at 9 months of age [23]. Vaccinations are administered at the three health centres in the study area and during outreach campaigns with occasional vaccination posts outside the health centres. A BHP-assistant records all vaccinations and weighs all children presenting for vaccination using an electronic scale (SECA 835/SECA 336).

2.3. Assessment of outcome

Mortality was assessed at all home visits. After registration of a death a verbal autopsy [24] was conducted by a trained field assistant. A medical doctor subsequently reviewed the collected information to assess the cause of death.

2.4. Interventions taking place during the study period

A full list of campaigns with vitamin A and vaccinations which were conducted during the study period is provided in Supplementary Table 1. In July 2009, children aged 9 months–5 years received MV; OPV was given to children 0–5 years in March, April and May 2010 and in March and April 2011. In October 2010 an H1N1 influenza vaccination campaign targeted children aged 6 months to 5 years.

Three randomised trials (RCTs) could have affected the study cohort. One RCT compared OPV+BCG vs BCG-only among normal birth weight children (enrolled neonates between July 2008 and October 2011) [25]; another RCT compared delayed BCG (current practice) vs. BCG at birth to low birth weight children (enrolled neonates between February 2008–September 2013) [26]. A third RCT compared vitamin A supplementation (VAS) versus placebo

at vaccination contacts after 6 months (enrolled children aged 6–23 months between August 2008–December 2009) [27].

2.5. Epidemiological and statistical methods

Our main interest was the F/M-MRR after Penta (which was defined as a Penta ±OPV) and MV+YF, respectively. A child resident in the BHP HDSS and aged 42 and 365 days could enter the analysis on the date of vaccination at a health centre/outreach post in the study area. We excluded vaccination contacts at which VAS was given, as it was pre-specified that the hypothesis regarding female-male differences in mortality after DTP and MV should be tested in settings where no other interventions were given [20] and we have shown in many previous studies that vitamin A and vaccines interact [28–30].

Baseline characteristics were compared by sex within vaccine groups. We calculated a weight for age-Z-score (WAZ) for the vaccinated children using the 2006-WHO child growth standard [31] and the WHO Anthro version 3.2.2 macro for Stata [32].

We compared the survival of girls and boys in Cox-proportional Hazards models with age as the underlying timescale. Children entered the survival analysis at dates of vaccination between 1 September 2008 and 18 April 2011. The study was terminated at 18 April 2011 because children receiving Penta after this date were likely to enter a RCT of early MV at 4.5 months of age (clinicaltrials.gov, NCT 01486355). Analyses were conducted using Stata 13.0.

As discussed above, the NSEs are strongest for the most recent vaccine. Therefore a child contributed survival time with a given vaccine until the reception of a subsequent vaccine or for a maximum of 6 months, as pre-specified in the hypothesis [20]. Hence, follow-up was censored when children received a different vaccine. The children could re-enter the analysis in a new vaccination group according to a subsequently received vaccine. All analyses of Penta were additionally censored at 9 months of age, since children become eligible for MV at 9 months of age, and prolonging follow-up beyond that date would inevitably accumulate more follow-up time in children who were delayed for MV compared with children who received MV, creating the possibility for selection bias. Furthermore, we additionally censored at the first vaccination campaign occurring during the follow-up period. We stratified the analysis for participation in one of the neonatal trials. Previous studies have suggested, that early administration increases the negative effect of DTP [3,33]. In the 1980s, DTP was only given from 3 months of age [14]; we assessed whether sex-differential mortality patterns after Penta depended on age at vaccination. Finally, we assessed whether excluding the first 4 months after the introduction of Penta and YF, where outreach campaigns were frequent and children received vaccines out of sequence, altered the results.

These analyses are based on children who presented for vaccination at the health centres/outreach posts in the study area. To obtain an estimate of the F/M MRR among children who were unlikely to have been Penta-vaccinated, we compared mortality by sex among children who had not (yet) presented for any vaccination after 42 days. This was done by linking the routine surveillance database with the health centre vaccination records, allowing all children under BHP surveillance between 1st September 2008 to 18th April 2011 to contribute to the analysis. In this analysis, children contributed time at risk between whichever came last: date of registration, date of birth and 1st September 2008, and until whichever came first: any registered vaccination contact after 42 days, migration or death. We compared mortality of girls to the mortality of boys in a Cox-proportional hazards model with age as underlying timescale. The F/M MRR was estimated in three age intervals: birth to 41 days, 42 days to 8 months and 9 (274 days) to 18 months. In the age group “birth to 41 days” the

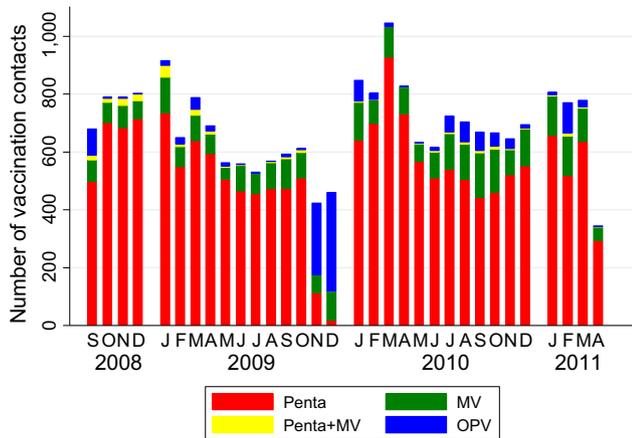


Fig. 1. Number of vaccination contacts by vaccine and calendar time. Number of registered vaccination contacts with children aged 42–365 days registered in the Bandim Health Projects urban HDSS, 1st September 2008 – 18th April 2011. Penta: Pentavalent vaccine: diphtheria-tetanus-pertussis, H. influenza type B and Hepatitis B. MV: Measles vaccine. OPV: Oral Polio Vaccine. Category Penta includes Penta ± OPV; Category MV includes MV ± yellow fever vaccine.

children were likely to have received BCG and OPV0. Among the older children they are likely to be Penta-(and measles-) unvaccinated because we censor for vaccination contacts after 42 days; however not all children are vaccinated at the health

centres and some may therefore have been vaccinated with Penta and MV elsewhere.

2.6. Role of the funding source

The funders had no role in study design, data collection and analysis, preparation of the manuscript, or decision to publish.

3. Results

Between September 1, 2008 and April 18, 2011, 23,248 vaccination contacts for 8730 children aged 42–365 days were registered by BHP assistants in the urban study area. Eight hundred children received high-dose VAS at enrolment in an RCT [27] and were excluded from the present study. At the remaining 22,448 contacts, 77% (17,313) received a Penta (85% (14,747) with OPV), 13% (3028) MV and YF vaccines, 6% (1379) OPV-only and 1% (271) Penta with MV and YF (Fig. 1). Smaller groups of children received other combinations of vaccines (Supplementary Table 2) and were not considered further.

Background characteristics for the two main groups, Penta and MV+YF, are shown in Table 1. The distribution of children in the two vaccination groups did not differ by sex ($p = 0.60$). Within the vaccination groups, age at vaccination and place of residence did not differ by sex. There were no systematic differences by sex in other background factors (Table 1). Information on weight was missing for 2% (368/20,341) of the vaccination contacts. The

Table 1
Background variables by sex among children vaccinated with pentavalent or measles and yellow fever vaccines.

	Penta ± OPV		P-value ^a	MV ± YF		P-value ^a
	Boys	Girls		Boys	Girls	
Number	8790	8523		1553	1475	
Median age (IQR)	96 (61–133)	96 (61–133)	0.86	287 (278–303)	288 (278–303)	0.32
<i>Place of residence</i>						
Bandim1+2	4046 (46)	3803 (45)	0.12	646 (42)	612 (41)	0.99
Belem+Mindara	1283 (15)	1316 (15)		231 (15)	221 (15)	
Cuntum1+2	3459 (39)	3401 (40)		673 (43)	642 (44)	
<i>Ethnicity</i>						
Pepel	2510 (29)	2460 (29)	0.02	463 (30)	424 (29)	0.39
Fula/Mandinga	2445 (28)	2365 (28)		334 (22)	339 (23)	
Manjaco/Mancanha	1600 (18)	1423 (17)		318 (21)	274 (19)	
Other	2188 (25)	2248 (26)		432 (28)	432 (29)	
Missing information	47	27		6	6	
<i>Maternal schooling</i>						
None	2018 (24)	2105 (26)	0.01	295 (20)	319 (23)	0.14
Primary (1–4 years)	1212 (15)	1149 (14)		214 (14)	198 (14)	
Secondary (>5 years)	5096 (61)	4717 (59)		980 (66)	882 (63)	
Missing information	464	552		64	76	
<i>Housing</i>						
Type of roof						
Straw	189 (2)	205 (2)	0.26	38 (2)	38 (3)	0.82
Hard	8530 (98)	8240 (98)		1504 (98)	1425 (97)	
Missing information	71	78		11	12	
<i>Toilet facilities</i>						
None	2 (0)	8 (0)	0.09	1 (0)	3 (0)	0.56
Latrine	7407 (85)	7206 (85)		1286 (84)	1215 (83)	
Inside	1307 (15)	1220 (14)		253 (16)	244 (17)	
Missing information	74	89		13	13	
<i>Electricity</i>						
Yes	2457 (28)	2369 (28)	0.83	459 (30)	423 (29)	0.58
No	6254 (72)	6073 (72)		1079 (70)	1040 (71)	
Missing information	79	81		15	12	
<i>Weight for age (WAZ)</i>						
Mean WAZ (SD)	−0.33 (1.17)	−0.36 (1.10)	0.13	−0.41 (1.18)	−0.31 (1.11)	0.03
Missing information	114	137		65	52	

^a P-value for test of no difference in background factors between boys and girls.

Table 2
Mortality following vaccination contacts in urban Guinea-Bissau, 2008–11.

Vaccine	Sex	Rate per 1000 PYRS (Deaths/PYRS)	Female/male mortality rate ratio (95% CI)	P-value ^a	Female/male mortality rate ratio (95% CI) ^b	P-value ^a
Penta	Boys	15.5 (31/1,999)	1.73 (1.11–2.70)	0.02	1.86 (1.16–2.98)	0.01
	Girls	26.8 (52/1,939)				
MV+YF	Boys	14.8 (11/745)	0.38 (0.12–1.19)		0.38 (0.12–1.21)	
	Girls	5.6 (4/716)				
Penta+MV+YF	Boys	19.4 (1/52)	0.99 (0.06–15.83)		1.07 (0.07–17.19)	
	Girls	18.7 (1/54)				
OPV	Boys	33.1 (5/151)	1.16 (0.33–3.99)		1.17 (0.34–4.03)	
	Girls	38.3 (5/130)				
<i>Censoring at 9 months for Penta</i>						
Penta	Boys	16.1 (28/1,738)	1.55 (0.96–2.49)	0.03	1.69 (1.02–2.82)	0.02
	Girls	24.9 (42/1,686)				
MV+YF	Boys	14.8 (11/745)	0.38 (0.12–1.19)		0.38 (0.12–1.20)	
	Girls	5.6 (4/716)				
<i>Censoring at 9 months for Penta + at vaccination campaigns</i>						
Penta	Boys	11.9 (13/1,089)	1.66 (0.95–2.91)	0.12	1.78 (0.98–3.24)	0.11
	Girls	25.7 (27/1,049)				
MV+YF	Boys	6.5 (2/307)	0.27 (0.03–2.42)		0.27 (0.03–2.45)	
	Girls	3.5 (1/283)				

^a P-value for test of same effect of sex in Penta and MV+YF groups.

^b Adjusting for maternal education reduced the number of deaths by 8, all in the Penta group (4 boys and 4 girls).

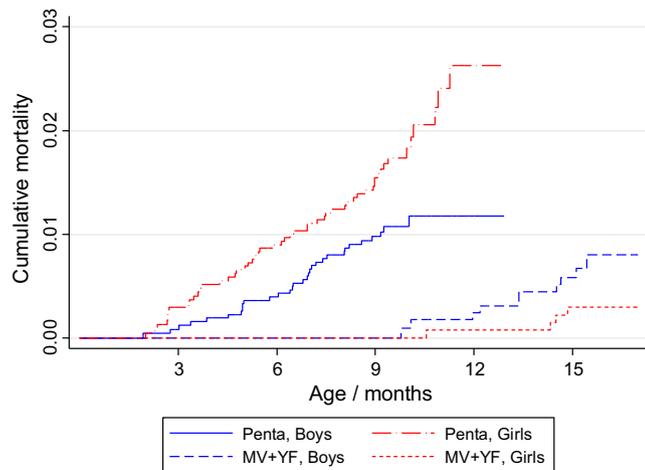


Fig. 2. Cumulative mortality according to sex and reception measles and yellow fever or Pentavalent vaccines. Cumulative mortality for children presenting for vaccination with Pentavalent vaccine (Penta: diphtheria-tetanus-pertussis-H. influenza type B, Hepatitis B with or without oral polio vaccine) or measles vaccine (with or without yellow fever vaccine; MV+YF) at age 42–365 days. Follow up for up to 6 months or until reception of a subsequent vaccine.

WAZ was 0.09 (0.01–0.18) higher for girls than for boys in the MV+YF-group, while there were no other significant differences by sex. The median age of vaccination did not differ by sex for Penta1, Penta2 or Penta3 (Supplementary Table 3).

During 6 months of follow-up 112 deaths occurred and verbal autopsies were conducted for 101; 11 families had moved away before a verbal autopsy could be conducted. Two deaths were due to injuries/accidents and were censored on the date of death (1 boy, Penta; 1 boy, MV+YF). The main causes of death were malaria (35%), gastro-intestinal infections (22%) and respiratory infections (15%) (Supplementary Table 4).

There were 83 non-accident deaths following Penta, 52 girls and 31 boys resulting in a F/M MRR of 1.73 (1.11–2.70). Among recipients of MV and YF 4 girls and 11 boys died, the F/M MRR being 0.38 (0.12–1.19). The inversion in the F/M MRR was statistically significant, $p = 0.02$ (Table 2, Fig. 2). Adjusting for maternal education had little effect (F/M MRR = 1.86 (1.16–2.98) after Penta

Table 3
Mortality following vaccination contacts in urban Guinea-Bissau according to number of Penta vaccines received.

Vaccine	Sex	Rate per 1000 PYRS (Deaths/PYRS)	Female/male mortality rate ratio (95% CI)	Adjusted female/male mortality rate ratio (95% CI) ^a
Penta1	Boys	18.9 (10/529)	1.40 (0.61–3.20)	1.41 (0.62–3.23)
	Girls	26.2 (13/497)		
Penta2	Boys	21.0 (10/475)	2.16 (1.02–4.57)	3.20 (1.28–8.02)
	Girls	46.1 (22/477)		
Penta3	Boys	11.1 (11/994)	1.59 (0.74–3.39)	1.52 (0.71–3.28)
	Girls	17.6 (17/966)		
<i>Censoring at 9 months</i>				
Penta1	Boys	19.2 (10/520)	1.28 (0.55–2.97)	1.31 (0.57–3.04)
	Girls	24.4 (12/492)		
Penta2	Boys	23.9 (10/419)	1.72 (0.79–3.73)	2.43 (0.94–6.27)
	Girls	41.5 (18/434)		
Penta3	Boys	10.0 (8/799)	1.57 (0.64–3.84)	1.59 (0.65–3.90)
	Girls	15.8 (12/760)		
<i>Censoring at 9 months for Penta + at vaccination campaigns</i>				
Penta1	Boys	19.5 (8/410)	1.22 (0.47–3.15)	1.23 (0.48–3.19)
	Girls	23.4 (9/384)		
Penta2	Boys	18.0 (6/333)	2.34 (0.90–6.08)	3.74 (1.04–13.41)
	Girls	42.2 (14/332)		
Penta3	Boys	10.8 (6/558)	1.55 (0.55–4.37)	1.58 (0.56–4.43)
	Girls	16.8 (9/535)		

^a Adjusting for maternal education reduced the number of deaths by 8, all in the Penta group (3 boys and 1 girl after Penta2, 1 girl after Penta3).

and 0.38 (0.12–1.21) after MV+YF ($p = 0.01$ for interaction between vaccine type and sex)). Adjusting for WAZ and for participation in one of the neonatal trials also made no difference (data not shown). Censoring follow up at 9 months of age or at the first campaign revealed the same patterns (Table 2).

The F/M MRR after Penta was 1.03 (0.48–2.22) for deaths classified as due to malaria, but 2.24 (1.28–3.92) for other non-accident deaths; 2.82 (0.90–8.86) for gastrointestinal deaths; 1.55 (0.44–5.51) for respiratory infections and 7.22 (0.89–58.6) for septicaemia.

Though not statistically significant, the sex-differential mortality may have been slightly stronger after Penta2 (F/M MRR = 2.16 (1.02–4.57)) and Penta3 (F/M MRR = 1.59 (0.74–3.39)) than after penta1 (F/M MRR = 1.40 (0.61–3.20)) (Table 3). The F/M MRR was

1.85 (1.08–3.15) for children Penta-vaccinated before 3 months of age, and 1.49 (0.66–3.36) for those vaccinated later.

Most Penta vaccines (85%, 14,747/17,313) were co-administered with OPV and we observed no sex-difference in the small group which received Penta only with 6 months of follow-up (Supplementary Table 5). Limiting the analysis to the children vaccinated in 2009–11, the F/M MRR was 2.00 (1.23–3.28) (47 girls, 24 boys) after Penta. In the MV+YF group the F/M MRR was 0.46 (0.14–1.50) (4 girls, 9 boys) ($p = 0.02$ for interaction between vaccine and sex) (Supplementary Table 6).

A total of 12,580 children were under surveillance between 0–18 months in the BHP HDSS between 1st September 2008 and 18th April 2011. Mortality was higher for boys than for girls between birth and 41 days (F/M MRR = 0.69 (0.53–0.90)). Among children for whom no vaccination contact after 42 days had yet been registered, mortality did not differ by sex between 6 weeks and 8 months (F/M MRR = 1.07 (0.66–1.75)) nor between 9 months and 18 months: 1.18 (0.55–2.55) (Supplementary Table 7).

4. Discussion

4.1. Main results

Female mortality was higher than male mortality after Penta and mortality declined much more for girls than for boys after MV+YF. Hence, the addition of HiB and hepatitis B has not altered the sex-differential mortality effect of DTP. Thus, the hypothesis put forward in 2007, that the transition from DTP to MV should result in an inversion of the F/M MRRs [20], was confirmed also for Penta and MV+YF.

4.2. Strengths and weaknesses

We have compared survival of girls and boys after vaccination with Penta and MV+YF. Since the NSEs are more pronounced for girls than boys and work in opposite directions for the two vaccine groups assessed [13,34], this provides a method to examine the NSEs in an unbiased way. It is clearly not random who is vaccinated and unvaccinated, but by comparing mortality according to sex, we avoid comparing two groups who are differently selected at baseline. Of course the study design would be flawed if there were differences in how early boys and girls were vaccinated but we have found no indication that age of vaccination differed by sex (Table 1, Supplementary Table 3). Adjusting for the background factors which differed slightly between males and females had no impact on the estimates.

The present study used vaccination data collected at health centres and outreach posts in urban Guinea-Bissau, thus obtaining the information on vaccine exposure from the date of administration. The BHP staff registering vaccinations was carefully trained and intensively supervised, thus misclassification of vaccination status is unlikely. Only children who could be followed through the BHP HDSS were included in the analyses. In a supplementary analysis, we compared mortality of girls and boys for whom no vaccination contact had yet been registered after 42 days. While this analysis includes unvaccinated children, it is important that the F/M MRR in this group is not interpreted as the F/M MRR among unvaccinated children. BCG and OPV is recommended at birth and most children will have received these vaccines. By censoring at first registered vaccination contact after 42 days, we censor most children as they receive Penta (and OPV and/or MV). However, vaccines are available elsewhere, and some children for whom no vaccination was registered may have been vaccinated elsewhere. The difference in the F/M MRR in Penta vaccinated children and predominantly Penta-unvaccinated children is likely to reflect the

effect of Penta on the relative mortality of females and males. However, a comparison of the mortality rates in the two cohorts is unlikely to reflect the effect of Penta vaccination. The age of entry in the cohorts differed: Children in the predominantly Penta-unvaccinated cohort entered on 42 days of age, while children entered the main analysis of Penta-vaccinated children at 96 days (Table 1). In addition, we would expect children who enter the main analysis to have a lower mortality risk as it is the healthier and better off children who are vaccinated first [3].

4.3. Consistency with previous studies

The hypothesis that transition from DTP to MV should result in an inversion of the F/M MRR [20] has been tested and confirmed in a number of studies [13,35]. The present study is the first to test whether the pattern also holds true, when two additional antigens have been added to DTP and when YF is co-administered with MV. We found that Penta was associated with a F/M MRR of 1.86 (1.16–2.98) when adjustment was made for background factors. We have recently reviewed the 16 studies reporting the F/M MRR after DTP [35] and the meta-estimate was 1.50 (1.21–1.85) times higher mortality for females, quite similar to the present estimate. A recent review prepared for WHO's Strategic Advisory Group of Experts on Immunization (SAGE) [36] found limited evidence for sex-differences in the mortality response to DTP. However, the SAGE review tested the hypothesis of higher female than male mortality indirectly by contrasting the effect of DTP versus no DTP in girls and boys, rather than comparing the F/M MRRs among DTP vaccinated children as the hypothesis had specified [20]. By limiting the review to studies which also had information on mortality in DTP-unvaccinated children, many studies were excluded. Furthermore, even though it had been specified that studies with survival bias should not be used [20], the SAGE review assumed that biases for girls and boys would cancel out [36] and therefore included studies with survival bias and frailty bias [35,37]. Hence, the SAGE review included uncertain data points and did not include all relevant estimates of sex-differences in mortality among DTP-vaccinated children [35].

The mortality rate for MV+YF vaccinated girls was reduced to almost a 5th of the rate observed after Penta, while boys had only a slight reduction in mortality rate (Table 2). The limited difference in mortality rate between the two age groups for boys is similar to observations from the pre-vaccination era [13,38], but the markedly higher mortality rate for girls in the age group for Penta suggests that the vaccine increases female mortality relative to male mortality. After 9 months of age the effect may be counteracted by vaccination with MV+YF. Lower female than male mortality after MV+YF has also been observed in prior studies from the Gambia and Guinea-Bissau [19,39].

4.4. Interpretation

For several reasons the sex-differential effect of Penta is not likely to be due to differences in mortality rates for boys and girls at different ages. First, in the present study, the F/M MRR after Penta did not vary by the age of vaccination. Second, mortality for children who received OPV only did not differ by sex, while there was marked higher mortality in girls who also received Penta. Third, among children who had not been registered at a vaccination session after 42 days of age, girls did not have higher mortality than boys between 6 weeks and 8 months of age. Data from other studies have also supported that the increased female versus male mortality rate ratio is not an age effect: In an RCT of early MV 4 weeks after DTP3, mortality was higher in girls than in boys in the control group, who had DTP3 as their most recent vaccine, but lower in girls in the intervention group which received MV

[6], and in the pre-vaccination era, the mortality of boys and girls did not differ in the age groups where children now receive Penta and MV [13,38]. Fourth, when the sequence of vaccines is altered and DTP is given after MV, girls again have higher mortality [40–42].

4.5. Implications

The effect of DTP on all cause-mortality was not tested prior to the vaccine being rolled out in low-income countries in the 1970s. The present study adds to the large body of evidence indicating that female mortality is higher than male mortality after vaccination with inactivated DTP-containing vaccines. Since the female/male MRR did not differ in the pre-vaccination era and did not differ among children who had not been registered at a vaccination contact for Penta after 42 days of age, this supports that DTP-containing vaccines are associated with increased mortality for females relative to males.

Our study has important implications for the vaccination programme. First, immunisation programmes cannot be evaluated merely by coverage and incidence of targeted diseases. The impact on overall mortality of both girls and boys should be assessed. The coverage of DTP3 currently serves as the main indicator for monitoring the immunisation programme [43] and globally more children are missing MV than DTP3 [44]. This does not favour the health of girls. To change the vaccination programme, SAGE has called for RCTs [45]. While randomised trials are certainly justified, it has been 15 years since the first concerns about increased mortality following DTP vaccination were raised [5] and observational studies are at present the best evidence we have. Similar observational studies could be undertaken in other HDSS settings where linkage to the vaccination registries exist [46,47] or can be established. Provided consistent findings, such data would emphasise the urgency of improving the vaccination programme.

Second, it is imperative, that new vaccines intended for introduction in the vaccination programme are evaluated for their effect on all cause child mortality overall and separately for boys and girls. For example, the new RTS,S malaria vaccine was recently linked to excess female mortality [48,49].

Future vaccination programmes should seek to abrogate negative NSEs of Penta. The negative effect of DTP-containing vaccines for girls can apparently be reversed by providing a live MV shortly after the last dose of DTP. However, there may be more to gain if the negative effect of Penta in girls could be prevented also during the first months of life. A strategy which might look promising, could be to co-administer a second dose of BCG with the second or third dose of Penta since studies have indicated that co-administered BCG+DTP is associated with lower female mortality than DTP-only [12,50]. An alternative could be to delay vaccination with DTP-containing vaccines in girls. The same vaccination schedule may not be optimal for boys and girls, and sex-differential vaccination programmes should be considered.

4.6. Conclusion

While the shift from DTP to Penta added protection against two further pathogens, the problem of increased mortality for females relative to males following DTP-containing vaccinations has not been solved. To improve child health, we need to shift from monitoring the immunisation programme by DTP3 coverage to assessing the impact of vaccines on mortality of boys and girls.

Conflicts of interest

None.

Author contributions

ABF: designed the study. CB: provided input to the study design and analyses; ABF, SBS, NL, QD, AR, CLM: supervised data collection, data entry and/or data cleaning; ABF: analysed the data, wrote the first manuscript draft and had primary responsibility for its final content. All authors contributed to and approved the final manuscript.

Funding

This work was supported by the Danish Council of Independent Research [DFR-1333-00192], European Union FP7 support for OPTI-MUNISE [Health-F3-2011-261375] and DANIDA [104.Dan.8-920]. The Bandim Health Project received support from the Danish National Research Foundation via CVIVA [DNRF108].

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2016.07.034>.

References

- [1] Benn CS, Netea MG, Selin LK, Aaby P. A small jab – a big effect: nonspecific immunomodulation by vaccines. *Trends Immunol* 2013;34:431–9.
- [2] Aaby P, Ibrahim SA, Libman MD, Jensen H. The sequence of vaccinations and increased female mortality after high-titre measles vaccine: trials from rural Sudan and Kinshasa. *Vaccine* 2006;24:2764–71.
- [3] Aaby P, Ravn H, Roth A, Rodrigues A, Lisse IM, Diness BR, 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.
- [4] Aaby P, Biai S, Veirum JE, Sodemann M, Lisse I, Garly ML, et al. DTP with or after measles vaccination is associated with increased in-hospital mortality in Guinea-Bissau. *Vaccine* 2007;25:1265–9.
- [5] Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *BMJ* 2000;321:1435–8.
- [6] Aaby P, Martins CL, Garly ML, Bale C, Andersen A, Rodrigues A, 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.
- [7] Aaby P, Garly ML, Bale C, Martins C, Jensen H, Lisse I, 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.
- [8] 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;311:481–5.
- [9] 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.
- [10] Biering-Sorensen S, Aaby P, Napirna BM, Roth A, Ravn H, Rodrigues A, et al. Small randomized trial among low-birth-weight children receiving bacillus calmette-guereir vaccination at first health center contact. *Pediatr Infect Dis J* 2012;31:306–8.
- [11] Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? *J Infect Dis* 2011;204:245–52.
- [12] Hirve S, Bavdekar A, Juvekar S, Benn CS, Nielsen J, Aaby P. Non-specific and sex-differential effects of vaccinations on child survival in rural western India. *Vaccine* 2012;30:7300–8.
- [13] 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.
- [14] 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.
- [15] 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.
- [16] Velema JP, Alihonou EM, Gandaho T, Hounye FH. Childhood mortality among users and non-users of primary health care in a rural west African community. *Int J Epidemiol* 1991;20:474–9.
- [17] Welaga P, Nielsen J, Adjuik M, Debpuur C, Ross DA, Ravn H, et al. Non-specific effects of diphtheria-tetanus-pertussis and measles vaccinations? An analysis

- of surveillance data from Navrongo, Ghana. *Trop Med Int Health* 2012;17:1492–505.
- [18] Aaby P, Vessari H, Nielsen J, Maleta K, Benn CS, Jensen H, et al. Sex differential effects of routine immunizations and childhood survival in rural Malawi. *Pediatr Infect Dis J* 2006;25:721–7.
- [19] Aaby P, Jensen H, Walraven G. Age-specific changes in the female-male mortality ratio related to the pattern of vaccinations: an observational study from rural Gambia. *Vaccine* 2006;24:4701–8.
- [20] 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.
- [21] GAVI. GAVI Alliance Progress Report 2010 Available at: Available from: <<http://www.gavi.org/results/gavi-progress-reports/>>2011.
- [22] GAVI the Vaccine Alliance. Yellow fever vaccine support. 2015. Available at: <<http://www.gavi.org/support/nvs/yellow-fever/>>.
- [23] Fisker AB, Hornshøj L, Rodrigues A, Balde I, Fernandes M, Benn CS, et al. Effects of the introduction of new vaccines in Guinea-Bissau on vaccine coverage, vaccine timeliness, and child survival: an observational study. *Lancet Global Health* 2014;2:e478–87.
- [24] INDEPTH network. Indepth Verbal Autopsy. Available at: <http://www.indepth-network.org/index.php?option=com_content&task=view&id=96&Itemid=184>.
- [25] Lund N, Andersen A, Hansen AS, Jepsen FS, Barbosa A, Biering-Sorensen S, et al. The effect of oral polio vaccine at birth on infant mortality: a randomized trial. *Clin Infectious Diseases: Off Publ Infectious Diseases Soc Am* 2015;61:1504–11.
- [26] Biering-Sorensen S, Aaby P, Lund N, Monteiro I, Jensen KJ, Eriksen HB, et al. Early BCG and neonatal mortality among low-birth-weight infants: A randomised controlled trial, submitted for publication.
- [27] Fisker AB, Bale C, Rodrigues A, Balde I, Fernandes M, Jorgensen MJ, et al. High-dose vitamin A with vaccination after 6 months of age: a randomized trial. *Pediatrics* 2014;134:e739–48.
- [28] Benn CS, Martins C, Rodrigues A, Ravn H, Fisker AB, Christoffersen D, et al. The effect of vitamin A supplementation administered with missing vaccines during national immunization days in Guinea-Bissau. *Int J Epidemiol* 2009;38:304–11.
- [29] Benn CS, Aaby P, Nielsen J, Binka FN, Ross DA. Does vitamin A supplementation interact with routine vaccinations? An analysis of the Ghana vitamin A supplementation trial. *Am J Clin Nutr* 2009;90:629–39.
- [30] Benn CS, Rodrigues A, Yazdanbakhsh M, Fisker AB, Ravn H, Whittle H, et al. The effect of high-dose vitamin A supplementation administered with BCG vaccine at birth may be modified by subsequent DTP vaccination. *Vaccine* 2009;27:2891–8.
- [31] World Health Organization. Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organisation; 2006.
- [32] World Health Organization. WHO Anthro (version 3.2.2, January 2011) and macros. 2011. Available at: <<http://www.who.int/childgrowth/software/en/>>.
- [33] Valentiner-Branth P, Perch M, Nielsen J, Steinsland H, Garly ML, Fischer TK, et al. Community cohort study of *Cryptosporidium parvum* infections: sex-differential incidences associated with BCG and diphtheria-tetanus-pertussis vaccinations. *Vaccine* 2007;25:2733–41.
- [34] Aaby P, Jensen H, Samb B, Cisse B, Sodemann M, Jakobsen M, et al. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. *Lancet* 2003;361:2183–8.
- [35] Aaby P, Ravn H, Fisker AB, Rodrigues A, Benn CS. Is DTP associated with increased female mortality? Testing the Tropical Medicine and International Health-hypotheses of the non-specific effects of vaccines, submitted.
- [36] Higgins JPT, Soares-Weiser K, Reingold A. Systematic review of the non-specific effects of BCG, DTP and measles containing vaccines Available at: Available from: <http://www.who.int/immunization/sage/meetings/2014/april/3_NSE_Epidemiology_review_Report_to_SAGE_14_Mar_FINAL.pdf?ua=1>2014.
- [37] Aaby P, Ravn H, Benn CS. The WHO Review of the Possible Non-Specific Effects of Diphtheria-Tetanus-Pertussis Vaccine. *Pediatr Infect Dis J*. 2016.
- [38] Aaby P, Jensen H, Rodrigues A, Garly ML, Benn CS, Lisse IM, et al. Divergent female-male mortality ratios associated with different routine vaccinations among female-male twin pairs. *Int J Epidemiol* 2004;33:367–73.
- [39] Fisker AB, Ravn H, Rodrigues A, Ostergaard MD, Bale C, Benn CS, et al. Co-administration of live measles and yellow fever vaccines and inactivated pentavalent vaccines is associated with increased mortality compared with measles and yellow fever vaccines only. An observational study from Guinea-Bissau. *Vaccine* 2014;32:598–605.
- [40] Aaby P, Garly ML, Nielsen J, Ravn H, Martins C, Bale C, et al. Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines: observations from vaccination trials in Guinea-Bissau. *Pediatr Infect Dis J* 2007;26:247–52.
- [41] Benn CS, Aaby P. Diphtheria-tetanus-pertussis vaccination administered after measles vaccine: increased female mortality? *Pediatr Infect Dis J* 2012;31:1095–7.
- [42] Krishnan A, Srivastava R, Dwivedi P, Ng N, Byass P, Pandav CS. Non-specific sex-differential effect of DTP vaccination may partially explain the excess girl child mortality in Ballabgarh, India. *Trop Med Int Health* 2013;18:1329–37.
- [43] World Health Organization. Global Vaccine Action Plan 2011–2020; Available from: <http://www.who.int/immunization/global_vaccine_action_plan/GVAP_doc_2011_2020/en/index.html>. In: World Health Organization, editor. Geneva 2013.
- [44] Global routine vaccination coverage, 2014. *Wkly Epidemiol Rec.* vol. 90, 2015. p. 617–23.
- [45] World Health Organization. Summary of the SAGE April 2014 meeting, 2014.
- [46] Berhane Y, Worku A, Demissie M, Tesfaye N, Asefa N, Aniemaw W, et al. Children who received PCV-10 vaccine from a two-dose vial without preservative are not more likely to develop injection site abscess compared with those who received pentavalent (DPT-HepB-Hib) vaccine: a longitudinal multi-site study. *PLoS ONE* 2014;9:e97376.
- [47] Burton DC, Bigogo GM, Audi AO, Williamson J, Munge K, Wafula J, et al. Risk of injection-site abscess among infants receiving a preservative-free, two-dose vial formulation of pneumococcal conjugate vaccine in Kenya. *PLoS ONE* 2015;10:e0141896.
- [48] Malaria vaccine: WHO position paper-January 2016. *Wkly Epidemiol Rec.* vol. 91, 2016. p. 33–51.
- [49] Klein SL, Shann F, Moss WJ, Benn CS, Aaby P. RTS, S malaria vaccine and increased mortality in girls. *mBio* 2016;7.
- [50] Aaby P, Nielsen J, Benn CS, Trape JF. Sex-differential and non-specific effects of routine vaccinations in a rural area with low vaccination coverage: an observational study from Senegal. *Trans R Soc Trop Med Hyg* 2015;109:77–84.