

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010;362:289-98.

## Methods and Statistical Analysis

### Randomization

A randomization list was generated at GSK Biologicals, Rixensart, using a standard SAS® (Statistical Analysis System) program and this was used to number the vaccines. A randomization blocking scheme (1:1:1 ratio) was used to ensure that balance between treatments was maintained throughout the study. The vaccine doses were distributed to each study centre while respecting the randomization block size.

### HIV Testing and Treatment

Infant blood samples were screened for HIV antibody by enzyme-linked immunosorbent assay (ELISA) and HIV infection was confirmed by polymerase chain reaction (PCR) amplification of HIV pro-viral DNA. All HIV-infected mothers and children were counseled and referred to HIV clinics where they were managed for their HIV, using national guidelines for HIV treatment in Malawi and South Africa. Medical care and treatment were provided by the governments for free as part of standard of care.

### Statistical Analysis

Vaccine efficacy (VE) was calculated using the formula,  $VE = (1 - \text{relative risk}) \times 100$ , where  $\text{relative risk} = \frac{\text{cumulative incidence of severe rotavirus gastroenteritis in the placebo group}}{\text{cumulative incidence of severe rotavirus gastroenteritis in the vaccinated group}}$ . The 95% confidence interval for vaccine efficacy for the primary outcome was derived from the exact confidence interval for the Poisson rate ratio.<sup>1</sup> A p-value was also calculated using a two-sided Fisher's exact test. To account for an interim analysis run by an independent analysis center,

declaration of statistically significant vaccine efficacy at the final analysis required a two-sided p-value of less than 0.038, this value representing an O'Brien & Fleming type I error adjusted for the 35 subjects with severe rotavirus gastroenteritis included in the interim analysis (which had nominal alpha of 0.0155) and the 126 severe rotavirus gastroenteritis outcomes included in the final analysis.

Vaccine efficacy estimates were similarly calculated, with corresponding 95% confidence intervals and p-values, for severe rotavirus gastroenteritis by dose-regimen, by country, and by circulating wild-type strain (G1 and non-G1) and for hospitalization and for all cause gastroenteritis.

The incidence rate in a group was computed as the number of infants reporting at least one event of severe rotavirus gastroenteritis divided by the total follow-up time until one year of age with corresponding 95% confidence intervals.<sup>ii</sup> Number of severe rotavirus gastroenteritis events prevented (per 100 infants per year) was obtained as 100 times the difference in incidence rate between the group that received placebo and the group that received *Rotarix*. This associated confidence interval was derived using the method conceptualized by Zou and Donner.<sup>iii</sup>

The percentages of infants reporting serious adverse events during the entire study period and classified, based on Medical Dictionary for Regulatory Activities, was tabulated with corresponding 95% confidence intervals<sup>iv</sup> by group.

The seropositivity rates (anti-rotavirus immunoglobulin A concentration  $\geq 20$  U/ml), and seroconversion rates (anti-rotavirus immunoglobulin A  $\geq 20$  U/ml in initially seronegative infants) were calculated with corresponding 95% confidence intervals<sup>iv</sup> one month post-last dose of *Rotarix*/placebo.

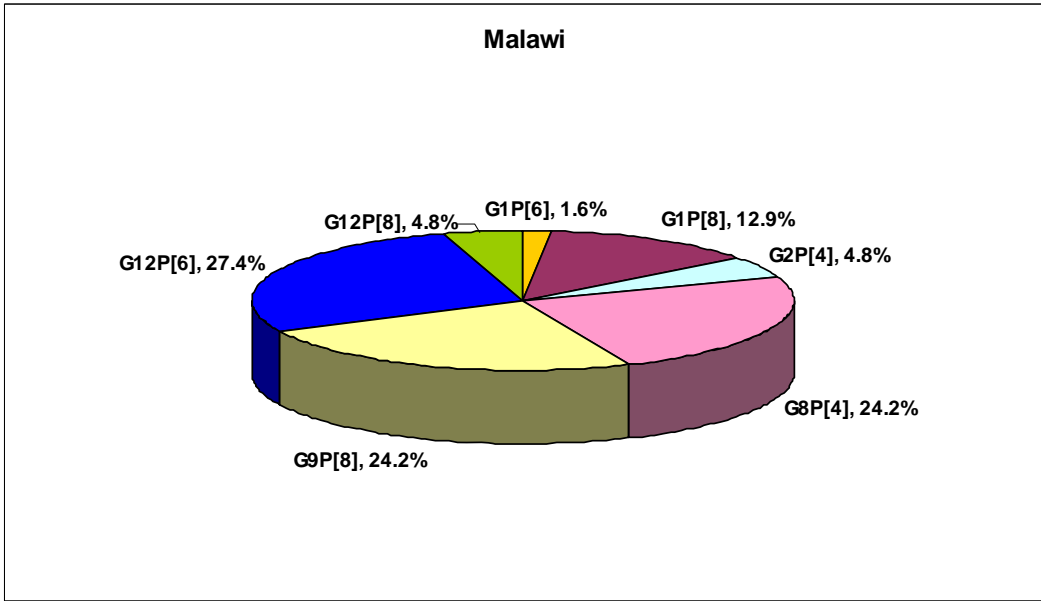
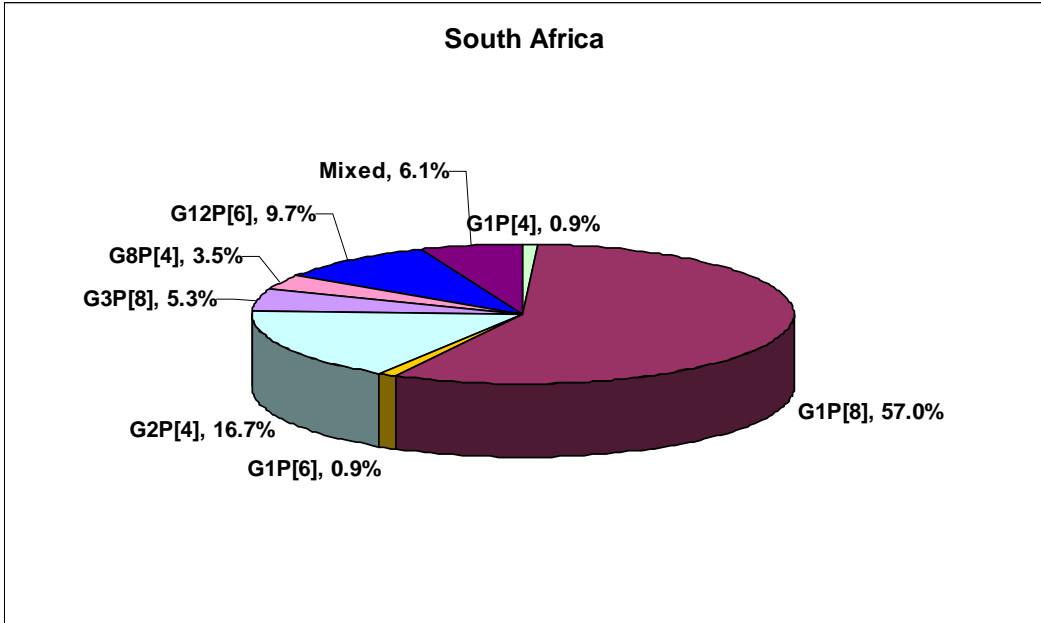
All statistical analyses were performed using SAS v9.1 and 95% confidence intervals were calculated using Proc StatXact-7. All reported p-values are 2-sided and not adjusted for multiple testing.

#### Study Power

Assuming an underlying incidence of severe rotavirus gastroenteritis of 2%, 3960 evaluable infants would provide 84% power to detect a statistically significant difference in rates of severe rotavirus gastroenteritis between the *Rotarix*-pooled group and the placebo group if vaccine efficacy was 60%. Assuming 20% of infants would not be evaluable for the primary analysis, a total sample size of 4950 was planned.

- <sup>i</sup>. Tang ML, Ng HK. Comment on: confidence limits for the ratio of two rates based on likelihood scores: non-iterative method. *Stat Med* 2004;23:685-92.
- <sup>ii</sup>. Böhning D. Confidence interval estimation of a rate and the choice of sample size. *Stat Med* 1988;7:865-75.
- <sup>iii</sup>. Zou GY, Donner A. Construction of confidence limits about effect measures: a general approach. *Stat Med* 2008;27:1693-702.
- <sup>iv</sup>. Clopper CJ, Pearson E. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934;26:404-13.

**Figure 1. Distribution of major rotavirus strains in placebo groups for South Africa and Malawi**



**Mixed:** G1+G2+G3+P[4]+P[8], G1+G2+P[4], G1+G2+P[4]+P[8], G1+G3+P[8], G2+G8+P[4], G3+G8+P[8]

**Table 1** Characteristics of study population (Total vaccinated cohort)

Characteristics	Malawi		South Africa		Total	
	<i>Rotarix</i> pooled group	Placebo group	<i>Rotarix</i> pooled group	Placebo group	<i>Rotarix</i> pooled group	Placebo group
<b>Infants (No.)</b>	1182	591	2116	1050	3298	1641
<b>Mean Age (weeks)</b>						
At Dose 1	6.5±0.92	6.5±0.97	6.3±0.96	6.3±0.96	6.4±0.95	6.4±0.97
At Dose 2	11.7±1.21	11.6±1.16	11.1±1.23	11.1±1.28	11.3±1.26	11.3±1.26
At Dose 3	16.8±1.29	16.8±1.34	16.0±1.48	16.0±1.53	16.2±1.47	16.3±1.51
<b>Mean duration of efficacy follow-up (months)</b>	7.56	7.44	7.56	7.56	7.56	7.56
<b>Gender (%)</b>						
Male	51.2	50.4	49.3	51.7	50.0	51.2
Female	48.8	49.6	50.7	48.3	50.0	48.8
<b>Race (%)</b>						
African	100.0	100.0	95.6	95.3	97.1	97.0
White - Caucasian / European heritage	-	-	0.2	0.1	0.2	0.1
Other - colored / mixed	-	-	4.2	4.6	2.7	2.9
<b>OPV co-administered (%)</b>						
At Dose 1	99.8	100.0	99.6	99.7	99.7	99.8
At Dose 2	99.4	99.1	99.2	99.4	99.3	99.3
At Dose 3	99.2	98.8	99.3	99.8	99.3	99.5
<b>Growth parameters</b>						
Mean Height at Dose 1 (cm)	52.8±2.91	52.8±3.01	55.2±2.78	55.2±2.94	54.3±3.05	54.3±3.17
Mean Weight at Dose 1 (kg)	5.0±0.71	5.0±0.70	4.8±0.68	4.8±0.68	4.9±0.70	4.9±0.70
Mean BMI at Dose 1 (kg/m <sup>2</sup> )	18.1±2.30	18.0±2.19	15.8±1.98	15.9±2.22	16.6±2.37	16.7±2.44
<b>HIV testing</b>						
*Percentage of infants with consent given for HIV testing	46.6	46.5	22.9	23.0	31.4	31.4
Positive (PCR)	4.5	3.3	6.4	5.8	5.4	4.5

\*HIV tests were performed only for those infants whose parents gave consent

No. = Number of infants in each group; ± = mean ± standard deviation

**Table 2 Efficacy of *Rotarix* against rotavirus gastroenteritis of any severity (according to protocol cohort for efficacy)**

Parameter	<i>Rotarix</i>		Placebo		Vaccine efficacy % (95% CI)	p-value
	N (n)	% (95%CI)	N (n)	% (95%CI)		
<b>Rotavirus gastroenteritis of any severity (Pooled)</b>						
Pooled	2974 (167)	5.6 (4.8–6.5)	1443 (174)	12.1 (10.4–13.9)	53.4 (42.1–62.6)	p<0.001
Two-Dose	1496 (93)	6.2 (5.0–7.6)	-	-	48.4 (33.3–60.4)	p<0.001
Three-Dose	1478 (74)	5.0 (4.0–6.2)	-	-	58.5 (45.2–68.8)	p<0.001
<b>By country (Pooled)</b>						
Malawi	1030 (85)	8.3 (6.6–10.1)	483 (61)	12.6 (9.8–15.9)	34.7 (7.7–53.5)	p=0.009
South Africa	1944 (82)	4.2 (3.4–5.2)	960 (113)	11.8 (9.8–14.0)	64.2 (52.0–73.4)	p<0.001

N = number of infants in each group; n = number of infants reporting at least one event

% = percentage of infants reporting at least one event; 95% CI = 95% Confidence Interval

p-value = Two-sided Fisher Exact test; p-value < 0.05 indicates statistically significant difference

**Table 3 Efficacy of *Rotarix* against severe rotavirus gastroenteritis (Vesikari score  $\geq 11$ ) and hospitalization due to rotavirus gastroenteritis (Total vaccinated cohort)**

Type of gastroenteritis	<i>Rotarix</i>		Placebo		Vaccine efficacy % (95% CI)	p-value
	N (n)	% (95% CI)	N (n)	% (95% CI)		
<b>Severe rotavirus gastroenteritis</b>						
<b>Overall</b>						
Pooled	3298 (68)	2.1 (1.6–2.6)	1641 (83)	5.1 (4.0–6.2)	59.2 (43.1–70.9)	p<0.001
Two-Dose	1647 (37)	2.2 (1.6–3.1)	-	-	55.6 (33.8–70.7)	p<0.001
Three-Dose	1651 (31)	1.9 (1.3–2.7)	-	-	62.9 (43.3–76.3)	p<0.001
<b>Malawi</b>						
Pooled	1182 (52)	4.4 (3.3–5.7)	591 (47)	8.0 (5.9–10.4)	44.7 (16.1–63.4)	p=0.003
Two-Dose	590 (27)	4.6 (3.0–6.6)	-	-	42.5 (5.7–65.5)	p=0.022
Three-Dose	592 (27)	4.2 (2.8–6.2)	-	-	46.9 (12.0–68.7)	p=0.007
<b>South Africa</b>						
Pooled	2116 (16)	0.8 (0.4–1.2)	1050 (36)	3.4 (2.4–4.7)	77.9 (59.2–88.6)	p<0.001
Two-Dose	1057 (10)	0.9 (0.5–1.7)	-	-	72.4 (43.2–87.8)	p<0.001
Three-Dose	1059 (6)	0.6 (0.2–1.2)	-	-	83.5 (60.4–94.3)	p<0.001
<b>Hospitalization for rotavirus gastroenteritis (pooled)</b>						
	3298 (20)	0.6 (0.4–0.9)	1641 (19)	1.2 (0.7–1.8)	47.6 (-3.7–73.5)	p=0.06

N = number of infants in each group; n = number of infants reporting at least one event

% = percentage of infants reporting at least one event; 95% CI = 95% Confidence Interval

p-value = Two-sided Fisher Exact test; p-value < 0.05 indicates statistically significant difference

**Table 4 Risk difference per 100 infant per year by dose and rotavirus type (Total vaccinated cohort)**

Type of gastroenteritis	<i>Rotarix</i>			Placebo			*Rate Difference per 100 infants per year (95% CI)
	N	Incidence rate (episodes per 100 infants per year)	95% CI	N	Incidence rate (episodes per 100 infants per year)	95% CI	
<b>Severe rotavirus gastroenteritis</b>							
Pooled	3298	2.6	2.1–3.3	1641	6.5	5.3–8.1	4.0 (2.1-5.1)
Two-Dose	1647	2.8	2.0–3.9	-	-	-	3.7 (1.8-5.1)
Three-Dose	1	2.4	1.7–3.4	-	-	-	4.2 (2.2-5.4)
	6						
	5						
	1						
<b>By Country (Pooled)</b>							
Malawi	1182	5.7	4.1-7.2	591	10.9	7.2-13.2	5.1 (1.2-8.0)
South Africa	2116	0.9	0.5-1.5	1050	4.3	2.9-5.7	3.4 (1.9-4.8)
<b>By rotavirus type (pooled)</b>							
G1	3298	0.7	0.4–1.1	1641	2.1	1.4–3.0	1.4 (0.5-2.2)
Non-G1	3298	1.9	1.4–2.5	1641	4.4	3.4–5.7	2.5 (1.0-3.5)
<b>Malawi</b>							
G1	1182	0.6	0.3-1.4	591	1.1	0.5-2.7	0.4 (-0.6-2.1)
Non-G1	1182	5.1	3.5-6.4	591	9.7	6.2-11.8	4.6 (0.9-7.2)
<b>South Africa</b>							
G1	2116	0.7	0.4-1.2	1050	2.6	1.5-3.7	1.9 (0.7-3.0)
Non-G1	2116	0.2	0.1-0.6	1050	1.7	1.0-2.8	1.4 (0.6-2.6)

95% CI = 95% Confidence Interval; N = Number of infants in each group (without missing values);

\*Risk Difference = Incidence rate in Placebo – Incidence rate in *Rotarix*

**Table 5 Efficacy of *Rotarix* against rotavirus gastroenteritis of any severity (Total vaccinated cohort)**

Parameter	<i>Rotarix</i>		Placebo		Vaccine efficacy % (95% CI)	p-value
	N (n)	% (95%CI)	N (n)	% (95%CI)		
<b>Rotavirus gastroenteritis of any severity (Pooled)</b>						
Pooled	3298 (200)	6.1 (5.3–6.9)	1641 (213)	13.0 (11.4–14.7)	53.3 (43.1–61.7)	p<0.001
Two-Dose	1647 (113)	6.9 (5.7–8.2)	-	-	47.1 (33.3–58.3)	p<0.001
Three-Dose	1651 (87)	5.3 (4.2–6.5)	-	-	59.4 (47.7–68.7)	p<0.001
<b>By country (Pooled)</b>						
Malawi	1182 (109)	9.2 (7.6–11.0)	591 (85)	14.4 (11.7–17.5)	35.9 (13.8–52.2)	p<0.001
South Africa	2116 (91)	4.3 (3.5–5.3)	1050 (128)	12.2 (10.3–14.3)	64.7 (53.5–73.3)	p<0.001

N = number of infants in each group; n = number of infants reporting at least one event

% = percentage of infants reporting at least one event; 95% CI = 95% Confidence Interval

p-value = Two-sided Fisher Exact test; p-value < 0.05 indicates statistically significant difference

**Table 6 Efficacy of *Rotarix* against all cause severe gastroenteritis (Total vaccinated cohort)**

Parameter	<i>Rotarix</i>		Placebo		Vaccine efficacy % (95% CI)	p-value
	N (n)	% (95%CI)	N (n)	% (95%CI)		
<b>All cause severe gastroenteritis (Pooled)</b>						
Overall	3298 (313)	9.5 (8.5–10.5)	1641 (225)	13.7 (12.1–15.5)	30.8 (17.5–41.9)	p<0.001
Malawi	1182 (221)	18.7 (16.5–21.0)	591 (139)	23.5 (20.2–27.2)	20.5 (1.0–36.0)	p<0.001
South Africa	2116 (92)	4.3 (3.5–5.3)	1050 (86)	8.2 (6.6–10.0)	46.9 (27.9–60.9)	p<0.001

N = number of infants in each group; n = number of infants reporting at least one event

% = percentage of infants reporting at least one event; 95% CI = 95% Confidence Interval

p-value = Two-sided Fisher Exact test; p-value < 0.05 indicates statistically significant difference

**Table 7 Serious Adverse Events**

Adverse event	Rotarix pooled (N = 3928)		Placebo (N = 1641)		Difference between Placebo-Rotarix pooled % (95% CI)*	p-value
	n	% (95% CI)	n	% (95% CI)		
<b>Overall</b>	319	9.7 (8.7–10.7)	189	11.5 (10.0–13.2)	1.84 (0.05–3.74)	0.044
Gastroenteritis	125	3.8 (3.2–4.5)	79	4.8 (3.8–6.0)	1.02 (-0.15–2.32)	0.088
Pneumonia	76	2.3 (1.8–2.9)	46	2.8 (2.1–3.7)	0.50 (-0.40–1.52)	0.287
Sepsis	47	1.4 (1.0–1.9)	19	1.2 (0.7–1.8)	-0.27 (-0.90–0.46)	0.441
Bronchopneumonia	46	1.4 (1.0–1.9)	23	1.4 (0.9–2.1)	0.01 (-0.65–0.78)	0.985
Bronchiolitis	33	1.0 (0.7–1.4)	16	1.0 (0.6–1.6)	-0.03 (-0.58–0.64)	0.932
<b>Vaccine-related AEs</b>	3	0.1 (0.0–0.3)	0	0.0 (0.0–0.2)	-0.09 (-0.27–0.14)	0.222
<b>Deaths</b>	83	2.5 (2.0–3.1)	43	2.6 (1.9–3.5)	0.10 (-0.79–1.11)	0.828

N = number of infants with at least one administered dose; n = number of infants reporting at least once the specified SAEs

% = percentage of infants reporting at least once the specified SAEs; 95% CI = Exact 95% Confidence Interval

95% CI\* = 95% confidence interval for difference in proportions (Standardized asymptotic)

p-value = 2-sided P-value (Standardized asymptotic); p-value < 0.05 will be used as an aid to highlight potential difference worth further attention.

However care must be taken when interpreting putative statistically significant findings since there is no multiplicity adjustment and clinical significance must be taken into account.