

FOURTEENTH INTERNATIONAL  
**ROTAVIRUS SYMPOSIUM**

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# Profiles and influence of maternal and infant histo-blood group antigens (HBGA) on oral rotavirus vaccine (ROTARIX<sup>®</sup>) immunogenicity in Zambia

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14<sup>th</sup> International Rotavirus  
Symposium



**CIDRZ**



# Background

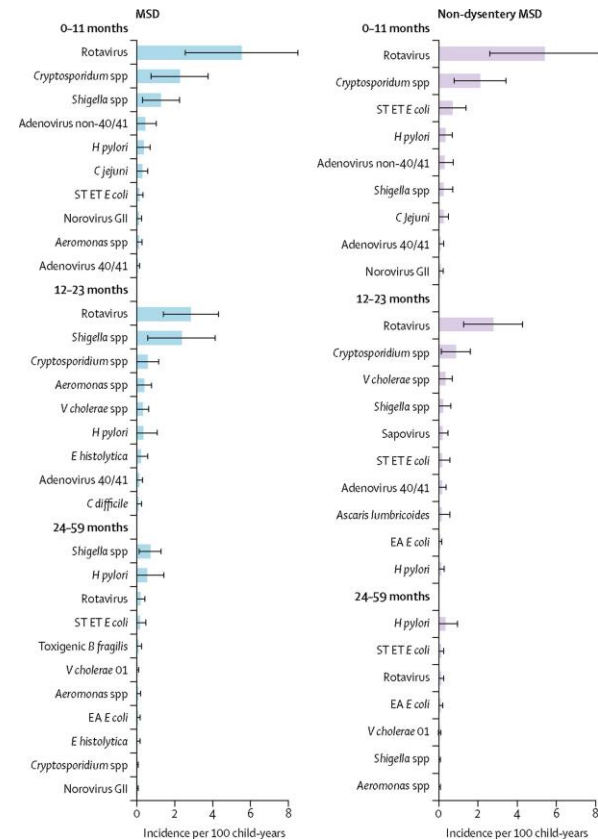
## Global Burden of Disease study (GBD 2019)

### B 0-9 years

1 Neonatal disorders	23.0 (22.0 to 24.1)	1 Neonatal disorders	32.4 (30.7 to 34.1)	-36.2 (-45.4 to -24.7)	-35.4 (-44.8 to -23.8)
2 Lower respiratory infections	17.0 (14.9 to 19.7)	2 Lower respiratory infections	11.6 (10.5 to 12.6)	-69.1 (-75.9 to -60.9)	-69.6 (-76.3 to -61.6)
3 Diarrhoeal diseases	13.1 (10.7 to 15.1)	3 Diarrhoeal diseases	9.3 (7.9 to 10.8)	-67.8 (-75.3 to -57.2)	-68.5 (-75.9 to -58.4)
4 Congenital birth defects	6.6 (4.6 to 10.0)	4 Congenital birth defects	8.6 (7.4 to 10.7)	-41.6 (-54.6 to -17.4)	-40.1 (-51.1 to -17.9)
5 Measles	5.7 (2.0 to 11.8)	5 Malaria	6.4 (3.3 to 10.8)	-36.9 (-61.4 to -2.2)	-38.5 (-63.1 to -6.5)
6 Malaria	4.6 (2.5 to 7.5)	6 Meningitis	2.1 (1.8 to 2.5)	-59.7 (-68.1 to -49.3)	-61.0 (-69.2 to -51.1)
7 Protein-energy malnutrition	4.1 (3.1 to 5.5)	7 Dietary iron deficiency	2.0 (1.3 to 2.9)	-0.8 (-5.3 to 3.6)	-8.2 (-12.3 to -4.1)
8 Meningitis	2.3 (2.0 to 2.7)	8 Protein-energy malnutrition	2.0 (1.7 to 2.3)	-78.1 (-85.0 to -68.9)	-78.3 (-85.5 to -69.9)
9 Whooping cough	1.9 (0.8 to 3.8)	9 Whooping cough	1.9 (0.9 to 3.3)	-54.7 (-74.7 to -17.3)	-53.2 (-75.6 to -20.4)
10 Drowning	1.8 (1.5 to 2.1)	10 STIs	1.4 (0.5 to 2.8)	-16.3 (-30.7 to 1.7)	-14.9 (-30.1 to 2.5)
11 Tuberculosis	1.8 (1.5 to 2.1)	11 Measles	1.3 (0.4 to 2.7)	-90.0 (-92.6 to -86.9)	-90.5 (-92.9 to -87.6)
12 Tetanus	1.7 (1.4 to 1.9)	12 Road injuries	1.1 (1.0 to 1.4)	-61.5 (-68.7 to -45.0)	-63.7 (-70.8 to -48.8)
13 Road injuries	1.3 (1.1 to 1.5)	13 Tuberculosis	1.0 (0.9 to 1.2)	-74.5 (-79.8 to -67.8)	-75.5 (-80.6 to -69.2)
14 Dietary iron deficiency	0.9 (0.6 to 1.3)	14 HIV/AIDS	1.0 (0.9 to 1.2)	-18.6 (-35.6 to 3.6)	-25.0 (-35.3 to -13.6)
15 STIs	0.7 (0.2 to 1.5)	15 INTS	1.0 (0.6 to 1.5)	68.3 (27.4 to 121.2)	61.4 (20.6 to 109.3)
16 Typhoid and paratyphoid	0.7 (0.3 to 1.3)	16 Drowning	0.9 (0.8 to 1.1)	-77.6 (-81.3 to -70.1)	-79.0 (-82.6 to -72.2)
17 Foreign body	0.6 (0.5 to 0.7)	17 Haemoglobinopathies	0.9 (0.7 to 1.0)	-10.3 (-30.3 to 22.5)	-13.7 (-34.3 to 14.7)
18 HIV/AIDS	0.6 (0.5 to 0.7)	18 Typhoid and paratyphoid	0.8 (0.4 to 1.5)	-46.7 (-59.1 to -31.1)	-50.7 (-62.5 to -36.9)
19 Encephalitis	0.5 (0.4 to 0.7)	19 Asthma	0.5 (0.4 to 0.8)	-32.2 (-46.2 to -14.5)	-37.5 (-50.0 to -21.5)
20 Acute hepatitis	0.5 (0.4 to 0.5)	20 Foreign body	0.5 (0.4 to 0.5)	-62.9 (-69.6 to -56.2)	-63.6 (-70.2 to -57.1)
21 Haemoglobinopathies	0.4 (0.3 to 0.6)	21 EMBID	0.5 (0.4 to 0.6)	-18.9 (-33.3 to -0.9)	-22.1 (-36.1 to -6.0)
22 Leukaemia	0.4 (0.3 to 0.6)	22 Sudden infant death	0.5 (0.2 to 1.0)	-50.6 (-61.6 to -29.8)	-46.9 (-61.7 to -30.0)
23 Sudden infant death	0.4 (0.2 to 0.9)	23 Idiopathic epilepsy	0.5 (0.3 to 0.6)	-30.7 (-45.8 to 3.6)	-34.0 (-49.1 to -3.8)
24 Asthma	0.4 (0.3 to 0.5)	24 Other unspecified infectious	0.4 (0.3 to 0.6)	-28.4 (-48.3 to 7.8)	-29.3 (-50.3 to 3.3)
25 Falls	0.4 (0.3 to 0.5)	25 Dermatitis	0.4 (0.2 to 0.7)	2.7 (1.7 to 3.7)	-6.0 (-6.9 to -5.1)
26 Leukaemia	0.4 (0.4 to 0.5)	26 Leukaemia	0.4 (0.4 to 0.5)	-54.8 (-67.7 to -32.9)	-55.3 (-69.5 to -37.0)
30 Other unspecified infectious	0.3 (0.2 to 0.4)	27 Falls	0.4 (0.3 to 0.5)	-47.2 (-67.0 to -18.0)	-48.3 (-68.7 to -22.6)
33 INTS	0.3 (0.1 to 0.4)	28 Encephalitis	0.4 (0.3 to 0.5)	-67.6 (-76.7 to -47.6)	-68.5 (-77.9 to -50.2)
34 EMBID	0.3 (0.2 to 0.3)	32 Tetanus	0.3 (0.3 to 0.5)	-91.3 (-93.8 to -85.6)	-91.2 (-93.8 to -85.6)
44 Dermatitis	0.2 (0.1 to 0.3)	39 Acute hepatitis	0.3 (0.2 to 0.3)	-73.1 (-81.7 to -59.1)	-74.1 (-82.6 to -61.1)

■ Communicable, maternal, neonatal, and nutritional diseases  
■ Non-communicable diseases  
■ Injuries

## Global Enteric Multicenter study (GEMS 2019)

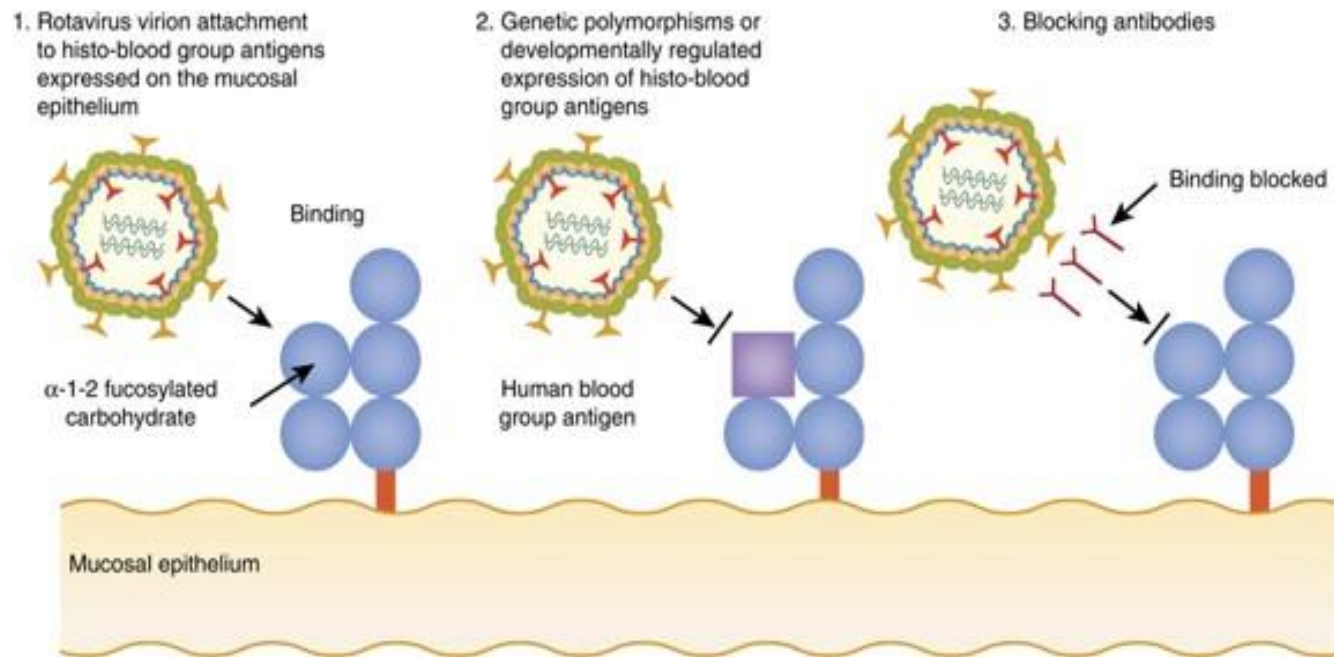


# Background Cont'd

- Oral live-attenuated RV vaccines (e.g. Rotarix™) are available life-saving tools but exhibit low immunogenicity in LMIC children.  
(Church et al., 2017)
- Variations in host genetic susceptibility to RV via histo-blood group antigens (HBGA) has been proposed as plausible explanation  
(Lee et al., 2018)
- FUT2 gene (secretor gene) regulates expression and ability to secrete these HBGA (e.g. on mucosal epithelial cells, in breast milk & saliva)  
(Cooling, 2015)



# Rationale: Polymorphisms in HBGA gene and secretor status may influence susceptibility to RV infection and live oral RV vaccines



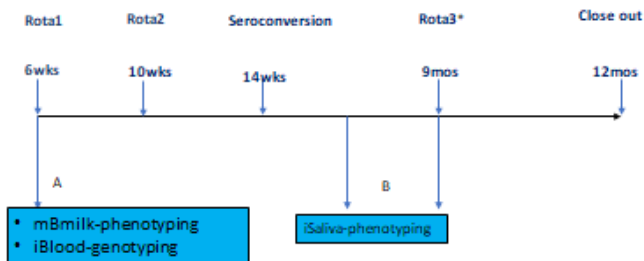
RV can use HBGA as receptors during infection

Figure 1. (Gozalbo-Rovira *et al.*, 2019; Huang *et al.*, 2012)



# Aim: To profile HBGA genotypes and phenotypes in a mother-infant pair vaccination cohort and assess influence on Rotarix™ vaccine immunogenicity

## Study sample collection



- A.1. Profile of maternal HBGA and association with (i) RV-IgA titre (ii) seroconversion at 14 weeks  
 2. Profile of infant genotype and association with (i) RV-IgA titre (ii) seroconversion at 14 weeks
- B. 1. Profile of infant HBGA phenotype and association with RV-IgA titre post dose 3 at 12 months

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**Evaluation of ROTARIX® Booster Dose Vaccination at 9 Months for Safety and Enhanced Anti-Rotavirus Immunity in Zambian Children: A Randomised Controlled Trial**

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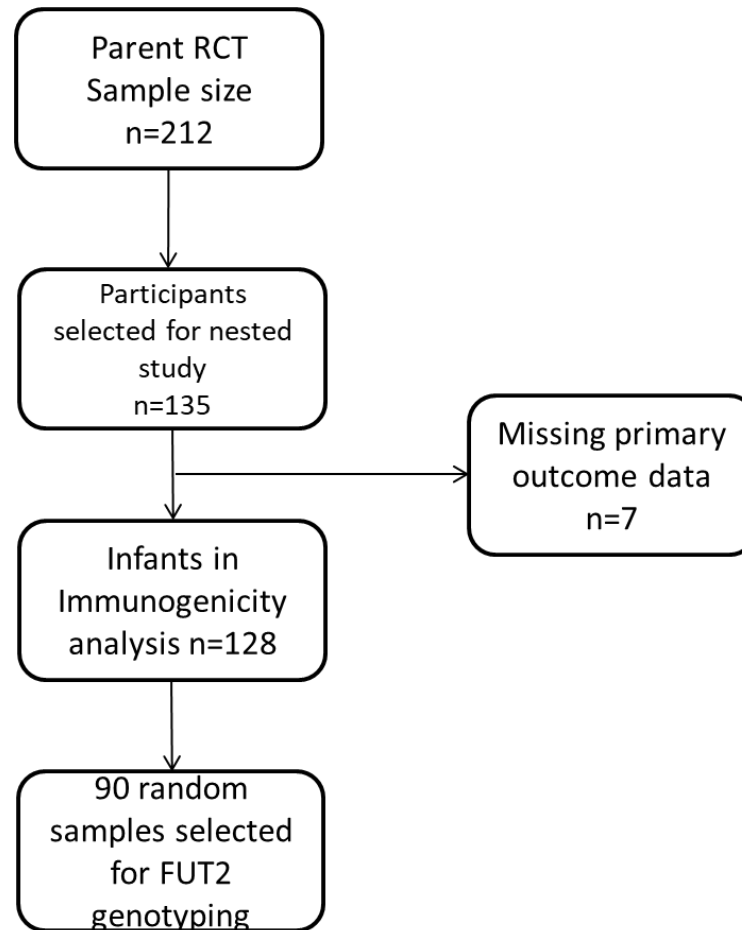
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# Figure 2. Analysis Flow Chart



# Laboratory Materials & Methods

## Assays

### HGBA Phenotyping

Lewis A, B antibodies (ELISA)

Blood Group A, B, H antibodies (ELISA)

Lectin (UEA-1) (ELISA)

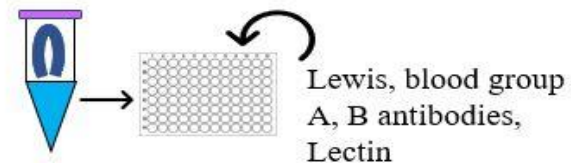
### FUT2 Genotyping

RFLP\_PCR

### Serology

Rotavirus-IgA antibody (ELISA)

HGBA Phenotyping using infant saliva and breastmilk using ELISA



~1ml saliva/breastmilk

HGBA genotyping on buffy coat using RFLP-PCR





# Table 1. Baseline Characteristics and Infant Seroconversion



	Seroconverted			P-value
	Total Population (N=128)	No (n = 91, 71.1%)	Yes (n = 37, 28.9%)	
	n (%)	n (%)	n (%)	
<b>Infant Characteristics</b>				
Age (Weeks)				
Median (IQR)	6 (6-6)	6 (6-6)	6 (6-6)	0.442
Mean (Std.Dev)	6 (0.6)	6 (0.6)	5.9 (0.7)	
<b>Sex</b>				
Male	69 (53.9)	51 (73.9)	18 (26)	0.447
Female	59 (46.1)	40 (67.7)	19 (32.2)	
<b>Treatment</b>				
Control	57 (44.5)	41 (71.9)	16 (28)	0.852
Intervention	71 (55.5)	50 (70.4)	21 (29.5)	
<b>Feeding</b>				
Exclusive Breastfeeding	122 (95.3)	86 (70.4)	36 (29.5)	0.672
Mixed Feeding	6 (4.7)	5 (83.3)	1 (16.6)	
<b>Birthweight (kg)</b>				
< 2.5	5 (3.9)	3 (60)	2 (40)	0.626
≥ 2.5	123 (96.1)	88 (71.5)	35 (28.4)	
<b>HIV Exposure</b>				
Not Exposed	89 (69.5)	62 (69.6)	27 (30.3)	0.590
Exposed	39 (30.5)	28 (73.6)	10 (26.3)	
<b>Nutritional Status</b>				
<b>Malnourished</b>				
No (WHZ ≥ -2)	126 (98.4)	89 (70.6)	37 (29.3)	1.000
Yes (WHZ < -2)	2 (1.6)	2 (100)	0 (0)	
<b>Stunted</b>				
No (HAZ ≥ -2)	107 (83.6)	78 (72.8)	29 (27.1)	0.310
Yes (HAZ < -2)	21 (16.4)	13 (61.9)	8 (38)	
<b>Wasted</b>				
No (WAZ ≥ -2)	119 (93.0)	86 (72.2)	33 (27.7)	0.281
Yes (WAZ < -2)	9 (7.0)	5 (55.5)	4 (44.4)	

		<b>Seroconverted</b>			
<b>Mother's Characteristics</b>					
<b>Age</b>					
<20	20 (15.6)	15 (75)	5 (25)	0.080	
20-24	45 (35.2)	37 (82.2)	8 (17.7)		
25-29	34 (26.6)	19 (55.8)	15 (44.1)		
≥30	29 (22.7)	20 (68.9)	9 (31)		
<b>Highest Education Level</b>					
None	6 (4.7)	4 (66.7)	2 (33.3)	0.470	
Primary	40 (31.3)	25 (62.5)	15 (37.5)		
Secondary	81 (63.3)	61 (75.3)	20 (24.6)		
Tertiary	1 (0.8)	1 (100)	0 (0)		
<b>Water Source</b>					
Piped into house/yard	45 (35.2)	33 (75)	12 (25)	0.882	
Protected well	5 (3.9)	4 (80)	1 (20)		
Public borehole/tap and pipe	78 (60.9)	54 (80)	24 (20)		
<b>Shared Toilet Facility</b>					
No	24 (18.8)	17 (70.8)	7 (29.1)	0.975	
Yes	104 (81.3)	74 (71.1)	30 (28.8)		
<b>Type of Toilet Facility</b>					
Flushing toilet	26 (20.3)	17 (65.4)	9 (34.6)	0.476	
Pit latrine	102 (79.7)	74 (72.6)	28 (27.5)		

# Figure 3. Maternal and Infant HBGA Frequency Distribution

Figure 3(a). Maternal Lewis and Secretor profiles

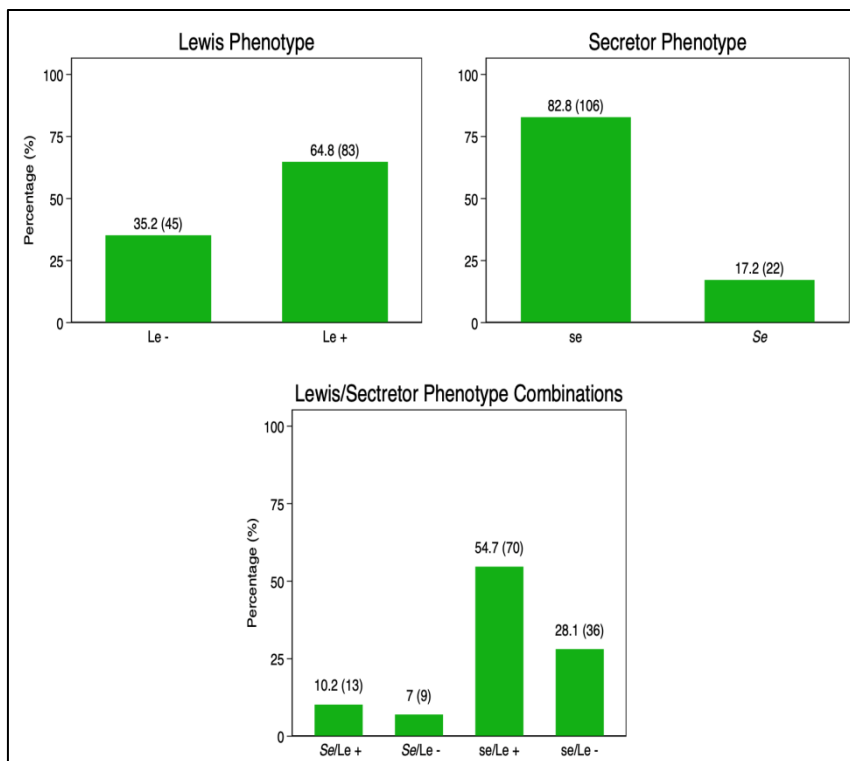
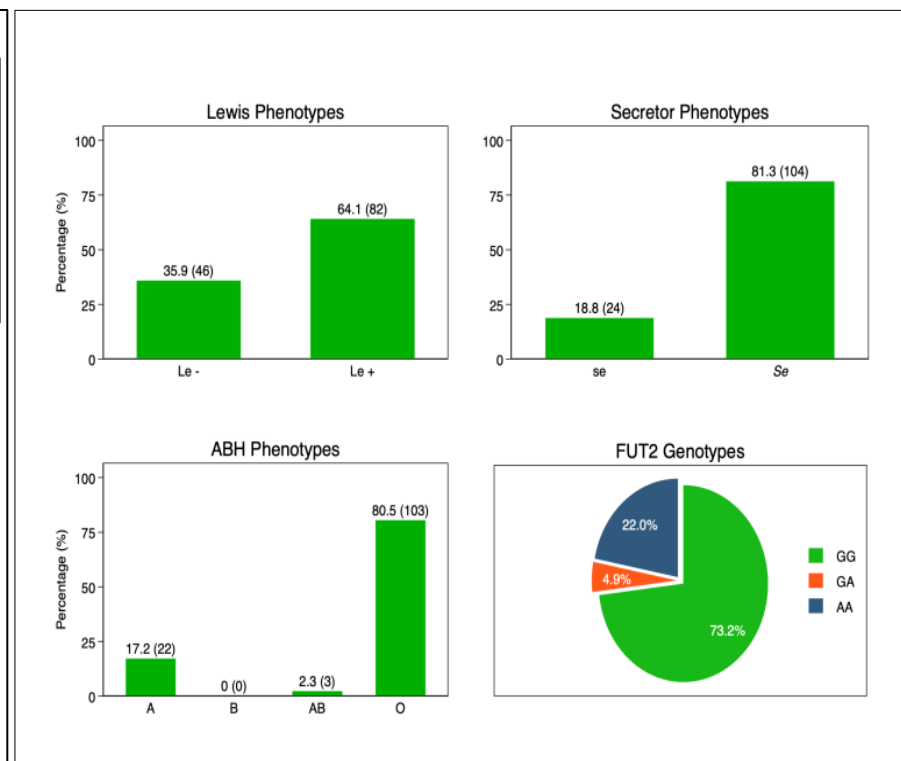


Figure 3(b). Infant ABO, Lewis and Secretor HBGA profiles



# Figure 4. Maternal and Infant HBGA Frequency Distribution

Figure 4(a). Two-sided t-test Trend plot for infant RV-IgA titre by infant ABO phenotype

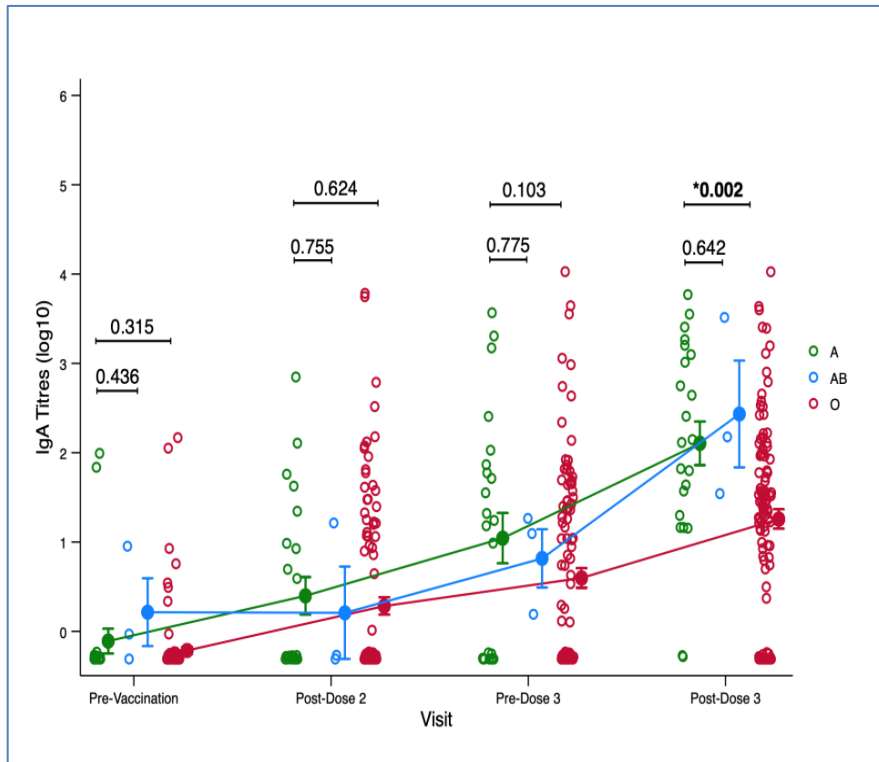
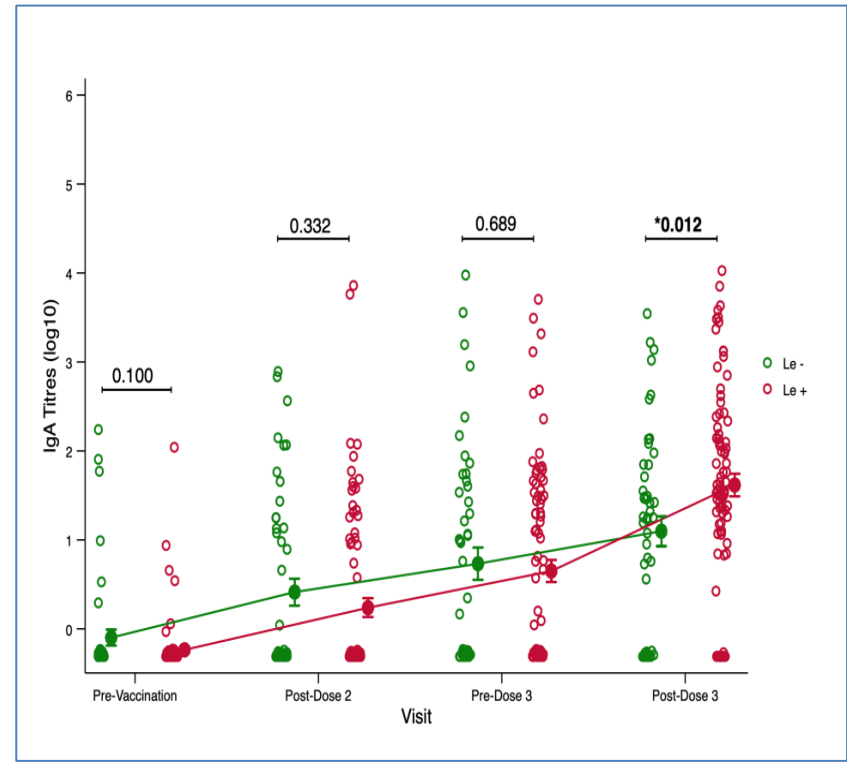


Figure 4(b). Two-sided t-test Trend plot for infant RV-IgA titre by Lewis phenotype



# Table 2. Maternal and infant HBGA profiles and anti-rotavirus IgA titres 1- month post ROTARIX<sup>®</sup> dose 2

(N = 128) (Overall)

Characteristics	Number of mother-infant pairs (% of total)	GMTs		ANOVA P-value	GMT Ratio		ANOVA P-value	Seroconversion (n = 37, 28.9%) n (%)	Chi-Square P-value
		GMT	95% CI		GMR	95% CI			
<b>Infant</b>									
<b>Infant HBGA Phenotype</b>									
A	22 (17.2)	2.5 (0.9, 6.8)	0.874	ref				7 (31.8)	0.929
AB	3 (2.3)	1.6 (0, 270.6)		0.6 (0.1, 5.6)	0.690			1 (33.3)	
O	103 (80.5)	1.9 (1.2, 3)		0.8 (0.3, 2.2)	0.620			29 (28.2)	
<b>Infant Lewis Phenotype</b>									
Le- (Le a-b-)	46 (35.9)	2.6 (1.3, 5.2)	0.332	ref				14 (30.4)	0.775
Le+ (Le a+b-, Le a-b+, or Le a+b+)	82 (64.1)	1.7 (1.1, 2.8)		0.7 (0.3, 1.5)	0.341			23 (28.2)	
<b>Secretor Phenotype</b>									
Non-secretor (se)	24 (18.8)	1.3 (0.6, 2.8)	0.279	ref				5 (20.8)	0.24
Secretor Phenotype (Se)	104 (81.3)	2.2 (1.4, 3.5)		1.7 (0.7, 4.2)	0.213			32 (30.8)	
<b>Infant FUT2 Genotype*</b>									
Homozygous secretor (GG)	60 (46.9)	1.4 (0.8, 2.5)	0.093	ref				15 (25.0)	0.289
Heterozygous secretor (GA)	4 (3.1)	5.6 (0, 1426.5)		3.9 (0.2, 85.1)	0.385			2 (50.0)	
Non-secretor (AA)	18 (14.1)	4.9 (1.5, 16.3)		3.4 (1.0, 11.9)	0.050			7 (38.9)	
Missing	46 (35.9)	2 (1, 3.8)		1.4 (0.6, 3.2)	0.447			13 (28.3)	
<b>Mother</b>									
<b>Lewis Phenotype</b>									
Le- (Le a-b-)	45 (35.2)	1.6 (0.9, 2.8)	0.358	ref				13 (28.9)	0.997
Le+ (Le a+b-, Le a-b+, or Le a+b+)	83 (64.8)	2.3 (1.4, 3.9)		1.5 (0.7, 3.2)	0.330			24 (28.9)	
<b>Secretor Phenotype</b>									
Non-secretor (se)	106 (82.8)	2 (1.3, 3.1)	0.850	ref				32 (30.2)	0.336
Secretor Phenotype (Se)	22 (17.2)	1.8 (0.7, 5.2)		0.9 (0.3, 2.6)	0.852			5 (22.7)	

# Table 3. Maternal and infant HBGA profiles and seroconversion 1- month post ROTARIX<sup>®</sup> dose 2

Characteristics	Crude Odds Ratio (95% CI)	P-value
<b>Infant HBGA Phenotype</b>		
A	ref	
AB	1.1 (0.1, 13.9)	0.958
O	0.8 (0.3, 2.3)	0.731
<b>Infant Lewis Phenotype</b>		
Le- (Le a-b-)	ref	
Le+ (Le a+b-,Le a-b+, or Le a+b+)	0.9 (0.4, 2)	0.775
<b>Infant Secretor Phenotype</b>		
Non-secretor (se)	ref	
Secretor Phenotype (Se)	1.7 (0.6, 4.9)	0.337
<b>Infant FUT2 Genotype</b>		
Homozygous secretor (GG)	ref	
Heterozygous secretor (GA)	3 (0.4, 23.2)	0.292
Non-secretor (G428A)	1.9 (0.6, 5.8)	0.255
<b>Mother Lewis Phenotype</b>		
Le- (Le a-b-)	ref	
Le+ (Le a+b-,Le a-b+, or Le a+b+)	1.0 (0.4, 2.2)	0.997
<b>Mother Secretor Phenotype</b>		
Non-secretor (se)	ref	
Secretor Phenotype (Se)	0.7 (0.2, 2.0)	0.484
<b>Treatment Arm</b>		
Control (MR)	ref	
Intervention (ROTARIX <sup>®</sup> +MR)	1.1 (0.5, 2.3)	0.852

# Table 4. Maternal and Infant HBGA profiles and anti-rotavirus IgA titres at 12-months

Characteristics	V12 GMTs GMT (95% CI)	ANOVA, P-value	GMT Ratio (95% CI)	P-value
<b>Infant</b>				
<b>Infant ABO Phenotype</b>				
A	5.02 (4.14, 6.07)	<b>0.002</b>	ref	
AB	5.28 (1.86, 15)		0.59 (0.10, 3.47)	0.560
O	3.7 (3.35, 4.08)		0.36 (0.09, 1.41)	0.140
<b>Infant Lewis Phenotype</b>				
Le- (Le a-b-)	3.57 (3.03, 4.22)	<b>0.015</b>	ref	
Le+ (Le a+b-,Le a-b+, or Le a+b+)	4.17 (3.75, 4.63)		0.83 (0.31, 2.23)	0.705
<b>Secretor Phenotype</b>				
Non-secretor (se)	2.89 (2.26, 3.71)	<b>&lt; 0.001</b>	ref	
Secretor Phenotype (Se)	4.14 (3.78, 4.54)		1.94 (0.59, 6.4)	0.276
<b>Infant FUT2 Genotype</b>				
Secretor (GG)/(GA)	3.95 (3.45, 4.52)	0.063	ref	
Non-secretor (AA)	3.24 (2.44, 4.31)		1.66 (0.96, 2.83)	0.543
<b>Mother</b>				
<b>Lewis Phenotype</b>				
Le- (Le a-b-)	4.02 (3.52, 4.58)	0.521	ref	
Le+ (Le a+b-,Le a-b+, or Le a+b+)	3.95 (3.51, 4.44)		1.09 (0.41, 2.88)	0.863
<b>Secretor Phenotype</b>				
Non-secretor (se)	4.08 (3.72, 4.48)	0.368	ref	
Secretor Phenotype (Se)	3.45 (2.64, 4.51)		0.83 (0.25, 2.70)	0.751
<b>Treatment Arm</b>				
Control (MR)	4.08 (3.56, 4.67)	0.260	ref	
Intervention (ROTARIX®+MR)	3.88 (3.44, 4.37)		1.39 (0.55, 3.49)	0.479





# Discussion

- Infant ABO phenotypes showed a higher frequency of group O, followed by A, AB
- A higher frequency of secretors than non-secretor infants (both phenotype and FUT2 genotype)
- Infant Lewis profile showed higher frequency of Lewis(+) than Lewis-null phenotype
- Maternal HBGA profiles showed a higher frequency of non-secretors than secretors and more Lewis(+) than Lewis-null phenotype



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# Discussion Cont'd

- Infant blood group AB showed the highest increase in RV-IgA titres between 9 and 12-months
- Infant Lewis(+) phenotype also showed a significantly higher increase in RV-IgA titres at 12-months compared to Lewis-null phenotype
- Infant secretor phenotype also showed a statistically higher increase in GMTs at 12-months compared to non-secretors



# Conclusion

- Maternal and infant HBGAs were not associated with Rotarix® immunogenicity in early infant life.
- Infant HBGAs antigens seem to influence rotavirus-IgA antibody titres much later in infant life.
- *Increase in titres most likely as a result of natural infection*
- Further robust studies are needed to comprehensively establish reasons for low Rotarix® immunogenicity in early infant life
- Next study approaches to focus on alternative RV vaccines, OR improvements on currently existing oral, live-attenuated vaccines



# Acknowledgements

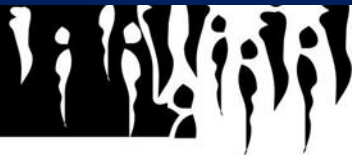
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Thank You!



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