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Pranjal Muthe Date 04/28/2021

Evaluating the Use of Immunoglobulins A and G Independently As Correlates of Protection Against Rotavirus Gastroenteritis

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Evaluating the Use of Immunoglobulins A and G Independently As Correlates of Protection Against Rotavirus Gastroenteritis

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

Evaluating the Use of Immunoglobulins A and G Independently As Correlates of Protection Against Rotavirus Gastroenteritis

By Pranjal Muthe

Background: Rotavirus is the leading cause of acute gastroenteritis in infants worldwide. Use of correlates of protection for rotavirus gastroenteritis would facilitate development of next generation rotavirus vaccines. We aimed to assess the thresholds of antirotavirus Immunoglobulin A and Immunoglobulin G units as correlates of protection that best predict the reduced risk of rotavirus gastroenteritis.

Methods: We used data from a total of 1304 infants collected in the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development, (MAL-ED) study. Logistic regression analyses were employed to estimate the odds ratios (ORs) describing the relationship between IgA and IgG thresholds and occurrence of rotavirus gastroenteritis.

Results: For IgA, odds ratios (ORs) ranged from 0.63 (95% CI =0.46, 0.86) for participants with IgA antibodies ≥ 20 U/mL to 0.467 (95% CI=0.174, 1.048) for participants with IgA antibodies ≥640 U/mL. Taken together, these results show that the increase in IgA threshold resulted in increased protection from rotavirus gastroenteritis. Similarly, for IgG, ORs ranged from 0.68 (95% CI= 0.423, 1.270) for participants with IgG antibodies ≥ 20 U/mL to 0.522 (95% CI=0.31, 0.88) for participants with IgG antibodies ≥640 U/mL, showing the increase in IgG threshold resulted in increased protection from rotavirus gastroenteritis.

Discussion: Although no clear pattern of protection was identified for IgA and IgG threshold models in a non-stratified analysis, the lowest OR was associated with the highest threshold (≥640) of IgA and IgG both in our overall (non-stratified) and stratified analyses. Our results highlight that higher antirotavirus IgA and IgG levels provided better protection against the occurrence of rotavirus gastroenteritis in unvaccinated children of the MAL-ED cohort.

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Introduction:

Rotaviruses are a leading cause of acute gastroenteritis, severe diarrhea, hospitalization, and diarrhea-related deaths in infants and children worldwide. As of 2016, rotavirus infection is reported to cause 128500 deaths annually among children younger than 5 years throughout the world.[1] The rotavirus-associated deaths have been highest in countries in sub-Saharan Africa, Southeast Asia, and South Asia. [1] The introduction of rotavirus vaccines has contributed to a substantial decrease in rotavirus burden over the past decade. However, rotavirus vaccines have reduced performance in low-income countries compared to high-income countries.[1-3] A metaregression study showed only 44% rotavirus vaccine effectiveness (95% credibility interval 27–59) after 12 months in low income, high mortality settings compared with 94% (87–98) effective in high-income and low-mortality settings.[4]

In low-income countries, some factors that may interfere with vaccine immunogenicity in infants are maternal exposure to pathogen, maternal antibodies acquired passively via placenta and/or breast milk, onset of malnutrition, onset of environmental enteric dysfunction, exposure to other enteric pathogens, and low socioeconomic status.[3, 5] There is a need to address this disease burden and challenges in vaccine performance in low-income countries. There are currently next generation vaccines being designed and are targeting children in low-and-middle-income countries, where large field studies are difficult to perform. Furthermore, evaluating the new vaccine candidates and vaccination strategies using placebo-controlled efficacy trials has now become difficult to justify ethically and is impractical. Under these conditions, one potential approach to design strategies to manufacture next generation vaccines and facilitate their rapid evaluation would be to use an established correlate of protection against rotavirus gastroenteritis.

Correlates of protection are immunological markers of protection indicating that that a person is immune to a specific pathogen. Correlates of protection are measurable with immunological assays, such as pathogen-specific antibodies. They can be used to assess vaccine performance against clinical outcomes, and to predict the likelihood of clinical diseases. For rotavirus, Immunoglobulin A (IgA) antibodies are most studied and are considered as possible markers of protection. Studies have concluded that higher serum immunoglobulin A (IgA) antibody levels appeared to confer greater protection against rotavirus gastroenteritis .[6, 7] There are some studies that also explored other correlates including antirotavirus immunoglobulin G (IgG) antibodies that correlated with reductions in both rotavirus infection and diarrhea.[7, 8] Additionally, there are a few studies that looked at both IgA and IgG as correlates of protection. However, some showed higher IgG and IgA were protective, some showed higher IgA but not IgG were protective, while some showed neither were protective. [8-10] There are inconsistencies with the results of these studies with most being single-country studies with small sample sizes.

The observations from longitudinal studies assessing protection against rotavirus gastroenteritis conferred by a natural rotavirus infection appear to be conflicting, showing a range of results from no protection to complete protection mirroring the poor vaccine performance in low and middle income countries. [7, 11, 12] A few studies carried out in Mexico and Guinea-Bissau showed that recurrent episodes of rotavirus gastroenteritis were less severe than the first episode. [7, 11] In contrast, a study recently published results on a cohort of young children in Vellore (India), in which the severity of diarrhea did not significantly decrease between the first and second infections, but did between the second and third infections; protection after one episode of rotavirus infection was 43% against rotavirus gastroenteritis, and it took three infections to induce 79% protection against moderate or severe rotavirus gastroenteritis.[13]

There is a need to address the gap in literature of evaluation of IgG and IgA both as correlates of protection against rotavirus, given the lack of availability of data on both IgA and IgG measures.

Our research aim is to compare IgA and IgG as correlates of protection against rotavirus gastroenteritis in low and middle income countries. We aimed to assess the thresholds of IgA and IgG antibodies units that best predict reduced risk of rotavirus gastroenteritis among infants with and without a stratified approach based on infant's vaccination status

Methods:

Data collection: We used data collected from the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development, (MAL-ED) study. The MAL-ED study was conducted in eight countries with historically high prevalence of childhood diarrhea and malnutrition. [14]The study focused on birth cohorts in each of the eight study sites in low- and middle-income countries including: Bangladesh, Brazil, India, Nepal, Peru, Pakistan, South Africa and Tanzania. The study offered a unique opportunity to be able to evaluate both antirotavirus IgG and IgA which was a key intent of our analyses. Details of the study design, surveillance, stool and blood collection, and microbiology analyses methods have been published previously. [14]

Briefly, infants were recruited by 17 days of age and followed to 24 months with collection of monthly surveillance and diarrheal stools. Stool samples were collected monthly for the first 12 months and at 15, 18, 21, and 24 months, and during all diarrheal episodes till 24 months of age. Stools were tested for rotavirus using enzyme immunoassays. About 4–5 mL of blood was

collected at 7 and 15 months of age. Immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies against rotavirus were measured by enzyme immunoassay using standardized protocols. [13] Vaccination history and anthropometric measurements were collected on all children monthly using standardized procedures and the length-for-age (LAZ) z scores were calculated using the World Health Organization (WHO) Multicenter Growth Reference Study Group program. Individual nutritional status (child feeding practices) in the study population was assessed monthly quantitative and qualitative assessments of the food consumed during the first 2 years of life. The socioeconomic status (SES) of families was assessed at 6 months of age.

Statistical Analysis: We used logistic regression to evaluate IgA and IgG levels at 7 months as potential correlates of protection against rotavirus gastroenteritis between 8 and 24 months of age. We limited our analysis to infants who had both IgA and IgG levels collected at month 7 of age. Our outcome of interest was the first rotavirus gastroenteritis episode determined by positive rotavirus immunoassay and presence of diarrhea, occurred between the time interval 8 month to 24 months of age.

For our exposure variables, we adapted six standard antirotavirus IgA and IgG thresholds doubling the titer with each threshold (in U/mL: ≥ 20, ≥ 40, ≥ 80, ≥ 160, ≥ 320, ≥ 640). Infants under antirotavirus IgA and IgG measure of <20 were used as the reference seronegative group. For each threshold, the odds of rotavirus gastroenteritis for infants above the threshold were compared to the odds for seronegative infants. For example, the≥640 threshold compared infants with antirotavirus IgA titers ≥ 640 to those with titers < 20. This allowed us to observe and compare the protection for rotavirus gastroenteritis across IgA and IgG thresholds in a consistent manner.

We included sex, rotavirus vaccination status, breastfeeding status, length for age Z score (LAZ), Water/Sanitation, Assets, Maternal Education and Income (WAMI) index (specific index developed for MAL-ED study) as potential confounders in the model. We selected these covariates based on previous evidence of confounding from our literature review. We included rotavirus vaccination because it directly affects IgA/IgG titers and risk of rotavirus gastroenteritis and was categorized as yes if 1 or more doses of any rotavirus vaccine prior to 7 months was given and no if no vaccine was given. We included breastfeeding status to control for maternal antibodies acquired passively via breastmilk and participants were assigned one of the three categories- *ever*: if participants were ever exclusively breastfed, *never* : if participants were never breastfed, *current*: if participants are currently being breastfed at 7 months of age. However, due to the uneven/ skewed distribution of the population in the categories of this variable (see table 1), we excluded the breastfeeding status variable from the final model. We included LAZ as a proxy of infant's nutritional status. WAMI index was included as a proxy of socio-economic status of infant's household.

Since there was 60%-40% distribution of infants in the vaccination status categories (vaccinated, N= 528 (40.5%)), we performed a stratified analysis based on infant's vaccination status to measure the effect between Ig levels and rotavirus gastroenteritis by the vaccination status.

All analyses were performed using RStudio Version 1.4 (2020).

Results:

A total of 1304 children were included in analysis. Of the 1304 children, 243(23.0%) had at least one episode of diarrhea associated with rotavirus between 7 to 24 months of age. Serology data

showed that 36% of the study cohort had detectable IgA and 92.6% of the study cohort had IgG antibodies >20 units by 7 months. (see Table 1)

Of the1304 children, 49.5% were male, and 59.5% had at least one dose of any rotavirus vaccine. High levels of breastfeeding were reported with 95.7% currently breastfeeding at 7 months ("*current"* category of breastfeeding status), followed by 4.1% having ever exclusively breastfed ("*ever*" category of breastfeeding) and only 0.2 % were never breastfed ("*never*" category of breastfeeding).

Figure 1 shows the general distribution of IgA and IgG titers in the study population. In IgA and IgG threshold models, the number of participants included in the model decreased as the threshold examined increased. For IgA, odds ratios (ORs) ranged from 0.63 (95% CI =0.462, 0.857) for participants with IgA antibodies ≥ 20 U/mL to 0.467 (95% CI=0.174, 1.048) for participants with IgA antibodies ≥640 U/mL. (figure 3A) Taken together, these results show that the increase in IgA threshold resulted in increased protection from rotavirus gastroenteritis. Similarly, for IgG, ORs ranged from 0.68 (95% CI= 0.423, 1.270) for participants with IgG antibodies ≥ 20 U/mL to 0.522 (95% CI=0.31, 0.88) for participants with IgG antibodies ≥640 U/mL, showing the increase in IgG threshold resulted in increased protection from rotavirus gastroenteritis (figure 2B). For both IgA and IgG, the lowest OR was associated with the highest threshold (≥640). However, the overall trends for IgG and IgA differ from each other. Overall, as the IgA threshold increased, the odds ratios were relatively stable for the most part (starting at threshold ≥ 20 U/mL up to ≥ 320 U/mL)but, is closer to zero as the highest threshold (>=640) is reached. Example, odds of infants with IgA levels with the threshold of ≥ 20 U/mL are 0.63 times less likely to get rotavirus gastroenteritis than the odds of those with threshold of < 20 U/mL IgA levels.

In the IgG model, overall, as the IgG threshold increased, the odds ratios were relatively stable but leaning towards zero, (Figure2B) Example, the odds of infants with IgG levels with the threshold of 20 U/mL and above in model are 0.68 times less likely to get the rotavirus gastroenteritis than the odds of those with threshold of lower than 20 U/mL IgG levels.

For IgA and IgG models, it is important to note that only small differences were observed in the ORs with rather wide confidence intervals, thefore, we cannot conclude there is any sort of pattern of increased protection with increased threshold of Ig levels.

Table 4 and 5 and figures 3A, 3B & 4A, 4B show the results of the stratified analyses we performed for vaccinated infants (N=528) and unvaccinated infants (N=776) In IgA threshold model for vaccinated infants, the OR was 0.87 (95% CI= 0.49, 1.55) for infants with IgA levels ≥ 20 U/mL, and OR was 0.56 (95% CI=0.06,4.73) for infants with igA levels ≥ 640 U/mL. This means that the odds of vaccinated infants with IgA levels with the threshold of ≥ 20 U/mL are 0.87 times less likely to get rotavirus gastroenteritis than the odds of those with threshold of < 20 U/mL IgA levels. In IgG threshold model for vaccinated infants, the OR was 1.57 with a very wide confidence interval of (95% CI= 0.19,12.56) for infants with IgG levels ≥ 20 U/mL and the OR and CI remained consistent throughout all thresholds of IgG for vaccinated infants. (Table 4, Fig 3A, 3B) The wide confidence intervals may have been a result of small sample size in each of the thresholds we used in models in stratified analyses.

For unvaccinated infants, in IgA threshold model, the OR was 0.55 (95% CI= 0.38, 0.88) for infants with IgA levels ≥ 20 U/mL, and OR was 0.2 (95% CI=0.05, 0.88) for infants with IgA levels ≥ 640 U/mL. This means that the odds of vaccinated infants with IgA levels with the

threshold of ≥ 20 U/mL are 0.55 times less likely to get rotavirus gastroenteritis than the odds of those with threshold of < 20 U/mL IgA levels. In IgG threshold model for vaccinated infants, the OR was 0.56 (95% CI= 0.33, 0.94) for infants with IgG levels ≥ 20 U/mL and the OR slowly decreased as the threshold of IgG levels increased with OR of 0.42 (95% CI=0.24,0.73) for infants with IgG levels ≥ 640 U/mL. This means that the odds of unvaccinated infants with IgG levels with the threshold of ≥ 20 U/mL are 0.56 times less likely to get rotavirus gastroenteritis than the odds of those with threshold of < 20 U/mL IgG levels. Same as above, only small differences were observed in the ORs with rather wide confidence intervals for IgG threshold model in vaccinated infants, thefore, we cannot conclude there is any sort of pattern of increased protection with increased threshold of Ig levels. (Fig 4A, 4B)

Discussion:

The purpose of this study was to compare IgA and IgG as correlates of protection against rotavirus gastroenteritis in low and middle income countries. We aimed to assess the thresholds of IgA and IgG antibodies units that best predict reduced risk of rotavirus gastroenteritis. Although no definitive pattern was identified for both IgA and IgG threshold models, the lowest OR was associated with the highest threshold (≥640) in our overall (non-stratified) and stratified analyses. Our results highlight that higher antirotavirus IgA and IgG levels provided better protection against the occurrence of rotavirus gastroenteritis in unvaccinated children of the MAL-ED cohort. Although neither IgA nor IgG showed 100 % protection against rotavirus gastroenteritis, the findings serve as threshold indicators of substantially reduced risk of severe gastroenteritis, as opposed to “perfect” predictors.

We found that higher IgA and IgG antibody titers were associated with lower odds of rotavirus gastroenteritis episodes. These findings are consistent with O'Ryan, M.L. *et al* study conducted in Texas that reported that IgA and IgG titers both correlated with reductions in both infection and diarrhea.[8] In contrast, a Danish study reported neither IgA nor IgG titers in the serum influenced the frequency of diarrhoea associated with infection, although the IgA titres did reduce disease severity. [9]

Prior studies have also looked at the IgA and IgG as individual correlate of protection. Baker J. et al., that found in high child mortality settings, seroconversion (IgA ≥ 20 U/mL) reduced the risk of severe rotavirus gastroenteritis (HR, 0.46; 95% CI, .25–.86)[6] and Velázquez et al found infants with IgA > 800 U/mL had an 84% reduced risk of rotavirus gastroenteritis (risk ratio, 0.16; 95% CI, .04–.64) until 2 years of age in Mexico [7]. In contrast, the same study, Velázquez et al found infants with an IgG titer >1:6400 were protected against rotavirus infection (adjusted Risk Ratio, 0.51; p <.001) but not against rotavirus diarrhea.[7] These difference may result from their study design, sample size, different sample population or analytical methods etc.

There are several strengths to this study. This is the largest and first multi-country study to examine rotavirus infection and disease in children of low- and middle-income countries using a standardized approach. This is the first time the MAL-ED data set is described and analyzed to evaluate both IgA and IgG as correlates of protection against rotavirus gastroenteritis. Our study addressed the gap in the literature on evaluation of IgG and IgA both as correlates of protection against rotavirus.

This study has several limitations. The lack of distribution in all thresholds of IgA made it difficult to assess the relationship between IgA thresholds and protection offered against rotavirus

gastroenteritis. The breastfeeding variable was a critical confounder to control for the maternal/transplacental transfer of IgA antibodies to infants via breast milk, but due to the very skewed distribution of population in currently breastfeeding category in this variable we were not able to include it in the model.To address the inconsistent vaccine rollout in various countries included in this study, we performed stratified analysis based on vaccination status. This also enabled us to compare the natural immunity for unvaccinated infants versus vaccine induced immunity in vaccinated infants.

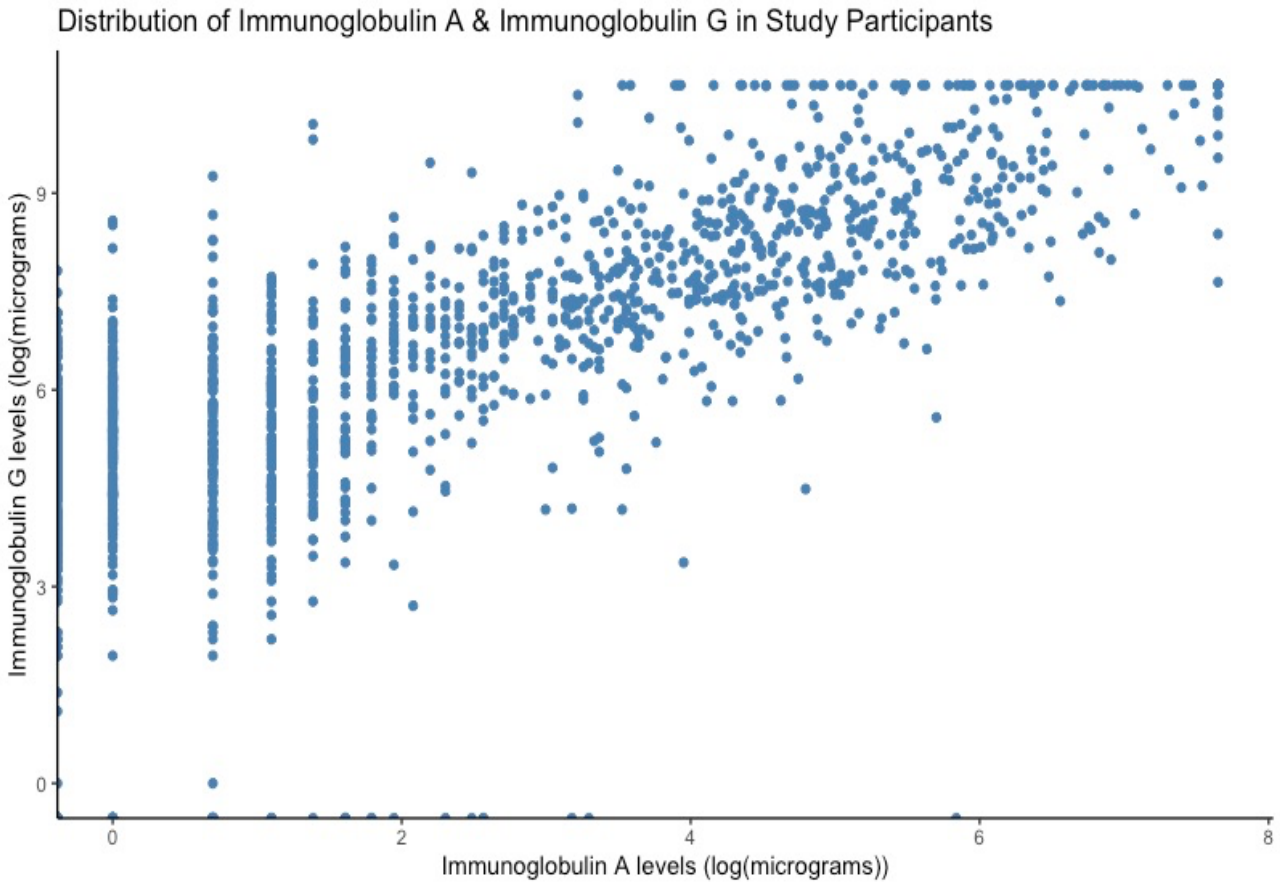
These findings have implications for future rotavirus vaccine evaluations, showing that improved vaccine strategy targeting to increase in both antirotavirus IgA and IgG in infants may correspond with stronger protection against rotavirus gastroenteritis. Future directions will be to examine both IgA and IgG as a "combined" correlate of protection against rotavirus gastroenteritis. Further, future studies should investigate IgG and IgA as correlates of protection separately, compared to combined and likely stronger correlate of protection. As a part of improving vaccine strategy, the clinical trials should examine the IgA/IgG levels, to predict whether booster shots of next generation rotavirus vaccines are needed and the timing of booster shots in order to gain higher vaccine efficacy in order to guide the policy decisions.

Tables and Figures:

TABLE 1			
	RVGE No **(N=1061)**	RVGE Yes **(N=243**)	Overall **(N=1304)**
IgA Thresholds			
<20	662 (62.4%)	173 (71.2%)	835 (64.0%)
(20-40)	98 (9.2%)	15 (6.2%)	113 (8.7%)
(40-80)	79 (7.4%)	11 (4.5%)	90 (6.9%)
(80-160)	77 (7.3%)	13 (5.3%)	90 (6.9%)
(160-320)	59 (5.6%)	11 (4.5%)	70 (5.4%)
(320-640)	44 (4.1%)	15 (6.2%)	59 (4.5%)
>640	42 (4.0%)	5 (2.1%)	47 (3.6%)
IgG Thresholds			
<20	70 (6.6%)	26 (10.7%)	96 (7.4%)
(20-40)	26 (2.5%)	9 (3.7%)	35 (2.7%)
(40-80)	54 (5.1%)	24 (9.9%)	78 (6.0%)
(80-160)	108 (10.2%)	30 (12.3%)	138 (10.6%)
(160-320)	125 (11.8%)	31 (12.8%)	156 (12.0%)
(320-640)	111 (10.5%)	26 (10.7%)	137 (10.5%)
>640	567 (53.4%)	97 (39.9%)	664 (50.9%)
Sex			
Male	527 (49.7%)	119 (49.0%)	646 (49.5%)
Female	534 (50.3%)	124 (51.0%)	658 (50.5%)
RV Vaccination Status			
Yes	604 (56.9%)	172 (70.8%)	776 (59.5%)
No	457 (43.1%)	71 (29.2%)	528 (40.5%)
Breastfeeding Status			
Current	1010 (95.2%)	238 (97.9%)	1248 (95.7%)
Ever	49 (4.6%)	5 (2.1%)	54 (4.1%)
Never	2 (0.2%)	0 (0%)	2 (0.2%)
Length- for- Age Z score			
Mean (SD)	-0.999 (1.11)	-1.10 (1.05)	-1.02 (1.10)
Median [Max and Min]	-1.00 [-4.43, 3.31]	-1.18 [-4.82, 1.63]	-1.01 [-4.82, 3.31]
WAMI Index			
Mean (SD)	0.583 (0.225)	0.557 (0.167)	0.578 (0.216)
Median [Max and Min]	0.590 [0, 1.00]	0.550 [0.170, 0.980]	0.580 [0, 1.00]

Table 2						
	Vaccinated			Unvaccinated		
	RVGE No (N=457)	RVGE Yes (N=71)	Overall (N=528)	RVGE No (N=604)	RVGE Yes (N=172)	Overall (N=776)
IgA Thresholds						
<20	315 (68.9%)	49 (69.0%)	364 (68.9%)	347 (57.5%)	124 (72.1%)	471 (60.7%)
(20-40)	46 (10.1%)	6 (8.5%)	52 (9.8%)	52 (8.6%)	9 (5.2%)	61 (7.9%)
(40-80)	33 (7.2%)	6 (8.5%)	39 (7.4%)	46 (7.6%)	5 (2.9%)	51 (6.6%)
(80-160)	25 (5.5%)	5 (7.0%)	30 (5.7%)	52 (8.6%)	8 (4.7%)	60 (7.7%)
(160-320)	18 (3.9%)	3 (4.2%)	21 (4.0%)	41 (6.8%)	8 (4.7%)	49 (6.3%)
(320-640)	8 (1.8%)	1 (1.4%)	9 (1.7%)	36 (6.0%)	14 (8.1%)	50 (6.4%)
>640	12 (2.6%)	1 (1.4%)	13 (2.5%)	30 (5.0%)	4 (2.3%)	34 (4.4%)
IgG Thresholds						
<20	14 (3.1%)	1 (1.4%)	15 (2.8%)	56 (9.3%)	25 (14.5%)	81 (10.4%)
(20-40)	7 (1.5%)	4 (5.6%)	11 (2.1%)	19 (3.1%)	5 (2.9%)	24 (3.1%)
(40-80)	15 (3.3%)	2 (2.8%)	17 (3.2%)	39 (6.5%)	22 (12.8%)	61 (7.9%)
(80-160)	41 (9.0%)	7 (9.9%)	48 (9.1%)	67 (11.1%)	23 (13.4%)	90 (11.6%)
(160-320)	64 (14.0%)	9 (12.7%)	73 (13.8%)	61 (10.1%)	22 (12.8%)	83 (10.7%)
(320-640)	64 (14.0%)	13 (18.3%)	77 (14.6%)	47 (7.8%)	13 (7.6%)	60 (7.7%)
>640	252 (55.1%)	35 (49.3%)	287 (54.4%)	315 (52.2%)	62 (36.0%)	377 (48.6%)
Sex						
Male	244 (53.4%)	38 (53.5%)	246 (46.6%)	290 (48.0%)	86 (50%)	400 (51.5%)
Female	213 (46.6%)	33 (46.5%)	282 (53.4%)	314 (52.0%)	86 (50%)	376 (48.5%)
Breastfeeding Status						
Current	426 (93.2%)	70 (98.6%)	496 (93.9%)	584 (96.7%)	168 (97.7%)	752 (96.9%)
Ever	29 (6.3%)	1 (1.4%)	30 (5.7%)	20 (3.3%)	4 (2.3%)	24 (3.1%)
Never	2 (0.4%)	0 (0%)	2 (0.4%)	0 (0%)	0 (0%)	0 (0%)
Length- for- Age Z score						
Mean (SD)	-0.838 (1.22)	-1.26 (1.01)	-0.895 (1.20)	-1.12 (0.993)	-1.04 (1.06)	-1.10 (1.01)
Median [Max and Min]	-0.950 [-4.24, 3.31]	-1.36 [-3.50, 1.63]	-1.01 [-4.24,3.31]	-1.03 [-4.43, 2.49]	-0.995 [-4.82, 1.37]	-1.02 [-4.82,2.49]
WAMI Index						
Mean (SD)	0.7 (0.173)	0.541 (0.156)	0.678 (0.179)	0.494 (0.220)	0.563 (0.171)	0.509 (0.212)
Median [Max and Min]	0.730 [0.230,0.980]	0.530 [0.230, 0.980]	0.700 [0.230, 0.980]	0.500 [0,1.00]	0.560 [0.10,0.50]	0.520 [0,1.00]

Figure 1


Distribution of Immunoglobulin A & Immunoglobulin G in Study Participants
Immunoglobulin G levels (log(micrograms)
0
3
6
9
0
2
4
6
8
Immunoglobulin A levels (log(micrograms))

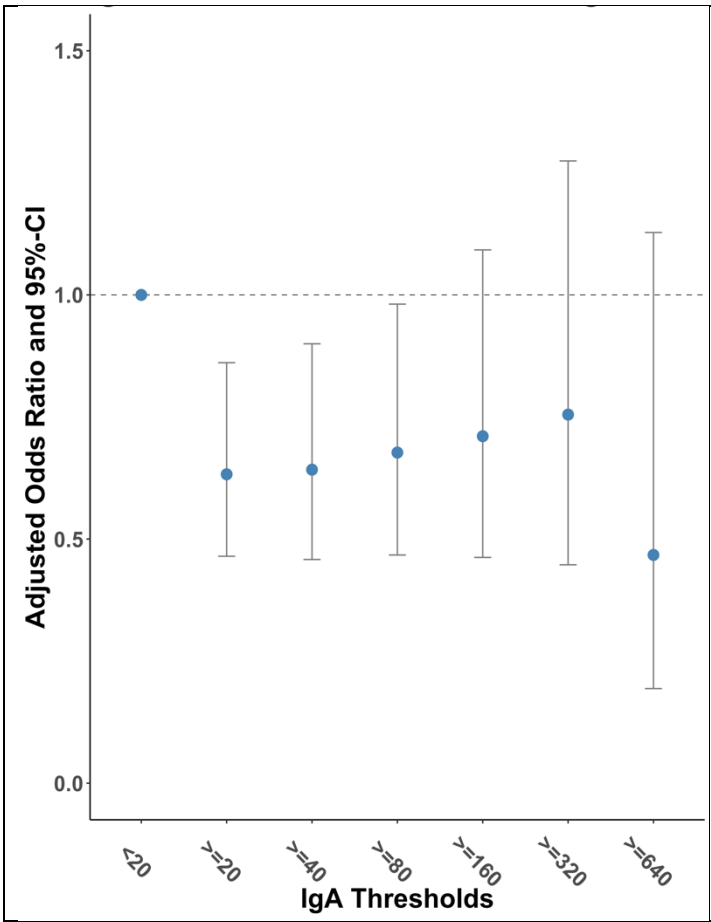


Fig 2A shows IgA thresholds and protection against rotavirus gastroenteritis among all infants

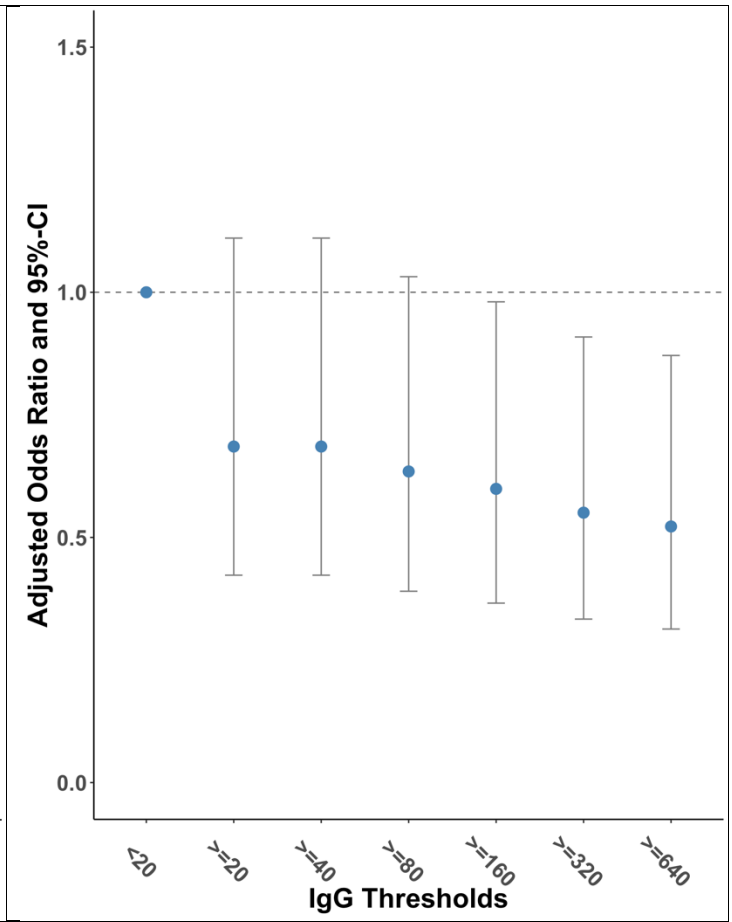


Fig 2B shows IgG thresholds and protection against rotavirus gastroenteritis among all infants

Note: For all thresholds, the comparison group was the <20 titer group.

Table 3

Thresholds (in U/mL)	**IgA** OR (95% CI)	**IgG** OR (95% CI)
≥ 20	0.63 (0.46, 0.85)	0.68 (0.42, 1.27)
≥ 40	0.64 (0.45, 0.89)	0.67 (0.42,1.37)
≥ 80	0.67 (0.46, 0.97)	0.63 (0.39, 1.03)
≥ 160	0.71 (0.45, 1.07)	0.59 (0.37, 0.99)
≥ 320	0.75 (0.43, 1.25)	0.55 (0.33,0.92)
≥ 640	0.46 (0.17, 1.04)	0.52 (0.31, 0.88)

*Note: For all thresholds, the comparison group was the <20 titer group.

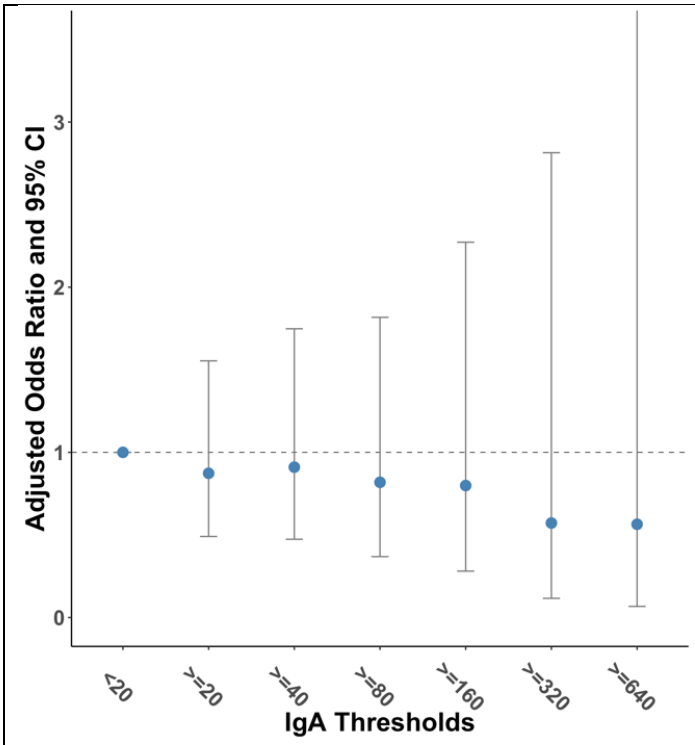


Fig 3A shows IgA thresholds and protection against gastroenteritis among vaccinated infants

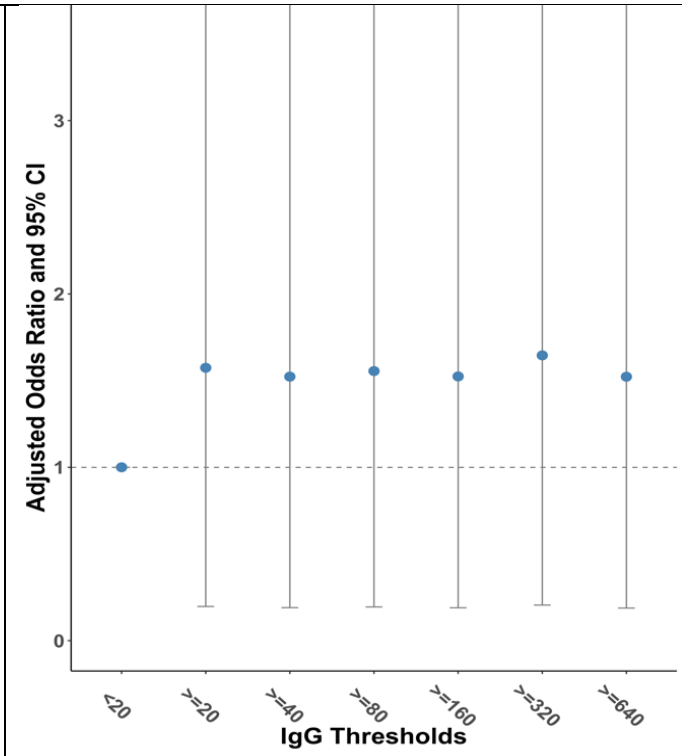


Fig 3B shows IgG thresholds and protection against gastroenteritis among vaccinated infants

Note: For all thresholds, the comparison group was the <20 titer group. Please note that due to small sample size in each of the threshold models shown above, the confidence intervals are very wide(See table 4),

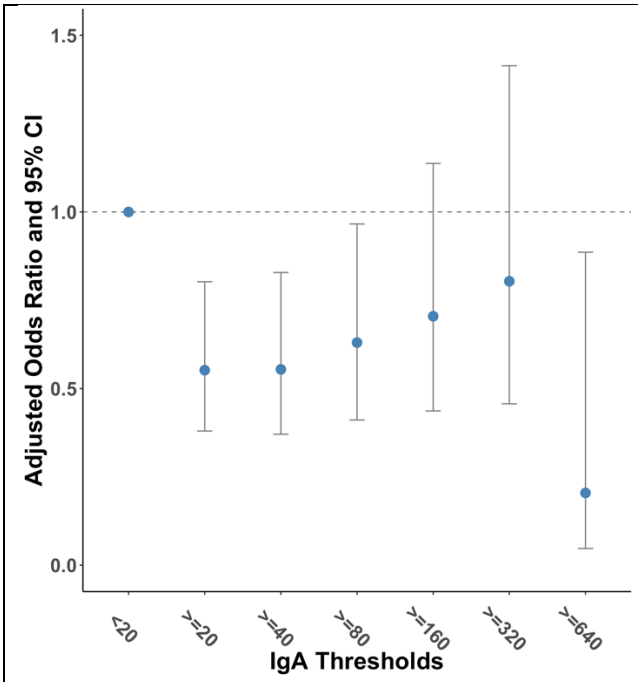


Fig 4A shows IgA thresholds and protection against gastroenteritis among unvaccinated infants

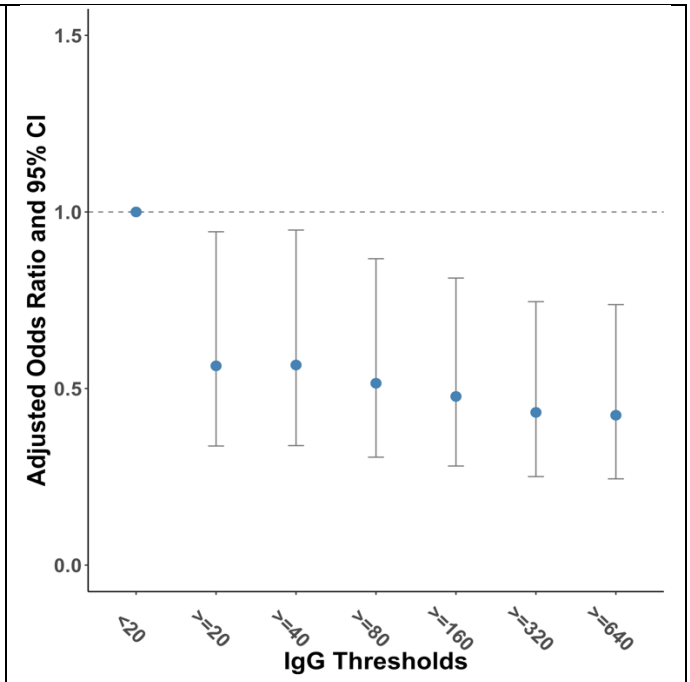


Fig 4B shows IgG thresholds and protection against gastroenteritis among unvaccinated infants

Note: For all thresholds, the comparison group was the <20 titer group.

Table 4		
Vaccinated Infants (N=528)		
Thresholds (in U/mL)	**IgA** OR (95% CI)	**IgG** OR (95% CI)
≥ 20	0.87 (0.49, 1.55)	1.57 (0.19, 12.56)
≥ 40	0.91 (0.47, 1.74)	1.52 (0.19, 12.18)
≥ 80	0.81 (0.37, 1.81)	1.55 (0.19,12.43)
≥ 160	0.79 (0.28, 2.27)	1.52 (0.18, 12.24)
≥ 320	0.57 (0.11, 2.81)	1.64 (0.20, 13.19)
≥ 640	0.56 (0.06, 4.73)	1.52 (0.18, 12.33)

*Note: For all thresholds, the comparison group was the <20 titer group

Table 5		
Unvaccinated Infants (N=776)		
Thresholds (in U/mL)	**IgA** OR (95% CI)	**IgG** OR (95% CI)
≥ 20	0.55 (0.38, 0.80)	0.56 (0.33, 0.94)
≥ 40	0.55 (0.37, 0.82)	0.56 (0.34, 0.94)
≥ 80	0.63 (0.41, 0.96)	0.51 (0.30, 0.86)
≥ 160	0.70 (0.43, 1.13)	0.47 (0.28, 0.81)
≥ 320	0.80 (0.45, 1.41)	0.43 (0.25, 0.74)
≥ 640	0.20 (0.05, 0.88)	0.42 (0.24, 0.73)

*Note: For all thresholds, the comparison group was the <20 titer group.

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