

Homotypic neutralizing antibody responses exceed heterotypic responses to Rotarix in Bangladeshi infants

Daniel Velasquez Portocarrero

iqm6@cdc.gov

Centers for Disease Control and Prevention

Jennifer Hull

Centers for Disease Control and Prevention

Yuhuan Wang

Centers for Disease Control and Prevention

Umesh Parashar

Centers for Disease Control and Prevention

Kalequ Zaman

International Centre for Diarrhoeal Disease Research

Baoming Jiang

Centers for Disease Control and Prevention

Article

Keywords:

Posted Date: October 18th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11144194/v1>

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Additional Declarations: No competing interests reported.

Abstract

Oral rotavirus vaccines are less immunogenic and less effective in many high-burden settings, and genotype-specific neutralizing responses may help explain this gap. Monovalent Rotarix has shown lower effectiveness against fully heterotypic strains in some analyses, while post-vaccine surveillance has documented continued circulation and emergence of DS-1-like reassortant strains; the contribution of vaccine-mediated immune selection remains uncertain. We analyzed paired sera from 99 baseline rotavirus IgA-seronegative Bangladeshi infants who received Rotarix at 6 and 10 weeks in a randomized phase 4 clinical trial for rotavirus IgA, IgG, and neutralizing antibodies (NAb) to homotypic Rotarix G1P[8] and heterotypic DS-1 G2P[4]. The primary endpoint was maintenance or increase in antibody titer from 6 to 22 weeks, defined as fold-change ≥ 1 . Rotavirus IgA seroconversion occurred in 46/99 infants. NAb maintenance/increase was more frequent for G1P[8] than G2P[4] overall (49/99 versus 28/99; paired risk difference 21.2 percentage points; $P < 0.001$) and among IgA responders (38/46 versus 20/46; $P < 0.001$). Rotarix induced stronger homotypic than heterotypic antibody response dynamics, especially among IgA responders.

Trial registration: ClinicalTrials.gov NCT02847026; registered 27/07/2016.

Introduction

Rotavirus remains a major cause of severe diarrhea in young children, with the largest burden in low-resource settings [1]. Recent Global Burden of Diseases (GBD) 2023 estimates indicate that rotavirus remains the leading cause of diarrhoeal-disease deaths globally and in children younger than five years, with mortality concentrated in South Asia and sub-Saharan Africa [2]. Although live oral rotavirus vaccines have reduced global morbidity and mortality, vaccine performance remains lower in many high-burden settings. A 24-country MNSSTER-V pooled analysis confirmed this performance gradient, reporting higher complete-series vaccine effectiveness in low/medium child-mortality settings than in high child-mortality strata [3]. Multiple host, environmental, and viral factors may contribute, including maternal antibody, breastfeeding, enteric coinfections, intestinal microbiota, histo-blood group antigen phenotype, malnutrition, and genetic diversity of circulating rotavirus strains [4].

Rotaviruses are classified by VP7 and VP4, which define G and P genotypes and are major targets of neutralizing antibodies [5]. Homotypic neutralizing antibodies are directed against antigenically similar strains, whereas heterotypic antibodies recognize antigenically distinct strains. Rotarix is a monovalent live-attenuated human G1P[8] vaccine, whereas G2P[4] strains are fully heterotypic to Rotarix and commonly have a DS-1-like genetic backbone. Pooled clinical-trial and post-licensure effectiveness data suggest that protection after monovalent vaccination may be lower against fully heterotypic strains, including G2P[4], than against homotypic or partially heterotypic strains [6, 13–16]. In parallel, post-vaccine-era surveillance has documented continued circulation or re-emergence of DS-1-like strains and DS-1-like reassortants, including G2P[4] and, in some settings, equine-like or other animal-derived G3 strains on DS-1-like genetic backgrounds. These genotype shifts should not be interpreted as vaccine escape by themselves, because rotavirus strain patterns also reflect natural temporal fluctuations,

reassortment, importation, host population immunity, and surveillance intensity. Nevertheless, they highlight the need for mechanistic studies that directly compare vaccine-induced neutralizing antibody dynamics against homotypic and heterotypic strains. Recent reverse-genetics data further support this rationale by showing that VP7 genotype can influence susceptibility to systemic neutralizing antibodies [17].

The parent randomized phase 4 clinical trial in Bangladesh compared immune responses to Rotarix and RotaTeq and identified factors associated with serum rotavirus IgA response [4]. In this post hoc immunogenicity substudy, we applied a microneutralization assay to paired sera from Rotarix recipients who were rotavirus IgA-seronegative before vaccination. We compared neutralizing antibody dynamics to homotypic Rotarix G1P[8] and heterotypic DS-1 G2P[4] strains.

Results

The NAb analytic cohort was broadly similar to eligible nonincluded infants

The neutralizing-antibody analytic cohort included 99 infants who were rotavirus IgA-seronegative at 6 weeks and had paired sera available for G1P[8] and G2P[4] NAb testing. Compared with eligible Rotarix IgA-negative infants who were not included in the substudy, the analytic cohort was similar for sex, anthropometric z scores, breastfeeding, baseline IgG titer, and baseline IgA titer. Age at enrollment showed a standardized difference of 0.20, but the absolute difference was small. Secretor phenotype showed a small imbalance, with secretors representing 75/97 known phenotypes in the NAb cohort versus 254/367 among eligible nonincluded infants (Table 1).

Table 1

Baseline characteristics of infants included in the NAb analytic cohort compared with eligible Rotarix IgA-negative infants not included in the substudy.

Characteristic	NAb analytic cohort N = 99	Eligible Rotarix IgA-negative not included N = 375	Standardized difference
Age at dose 1 or enrollment, days, mean (SD)	44.8 (1.3)	44.5 (1.4)	0.20
Sex, male, n/N (%)	47/99 (47.5%)	196/375 (52.3%)	0.10
Weight-for-length z score at dose 1, mean (SD)	-0.26 (1.02)	-0.25 (1.09)	0.00
Length-for-age z score at dose 1, mean (SD)	-0.66 (1.24)	-0.74 (1.10)	0.07
Exclusive breastfeeding at both Rotarix doses, n/N (%)	29/99 (29.3%)	99/375 (26.4%)	0.07
Secretor phenotype among known, n/N (%)	75/97 (77.3%)	254/367 (69.2%)	0.18
Secretor phenotype not determined, n/N (%)	2/99 (2.0%)	8/375 (2.1%)	NA

Baseline IgG correlated with homotypic and heterotypic NAb titers

At 6 weeks, all infants in the analytic cohort were seropositive for rotavirus IgG and for NAb to both G1P[8] and G2P[4] strains. Baseline rotavirus IgG titers correlated with NAb titers to both G1P[8] (Spearman $\rho = 0.49$, 95% CI 0.33 to 0.63; $P = 2.35e-07$) and G2P[4] ($\rho = 0.54$, 95% CI 0.39 to 0.67; $P = 6.98e-09$), consistent with maternally derived rotavirus antibody contributing to baseline neutralizing activity (Fig. 1; **Supplementary Table S1**).

Homotypic G1P[8] NAb maintenance/increase exceeded heterotypic G2P[4]

At 22 weeks, 46/99 infants (46.5%) had rotavirus IgA seroconversion. Maintenance/increase in antibody titer (fold-change ≥ 1) responses were observed in 40/99 infants (40.4%) for rotavirus IgG, 49/99 (49.5%) for G1P[8] NAb, and 28/99 (28.3%) for G2P[4] NAb (Table 2, Fig. 2a). In paired analysis, G1P[8] NAb maintenance/increase exceeded G2P[4] by 21.2 percentage points (95% CI 10.6 to 31.8; paired RR 1.75, 95% CI 1.31 to 2.45; exact McNemar $P = 3.24e-04$).

Table 2

Primary endpoint of maintenance/increase in antibody titer from 6 to 22 weeks defined as fold-change ≥ 1 .

Group	Rotavirus IgG	G1P[8] NAb	G2P[4] NAb	G1 minus G2 paired risk difference pp (95% CI)	G1/G2 paired RR (95% CI)	Exact McNemar P
Overall	40/99 (40.4%)	49/99 (49.5%)	28/99 (28.3%)	21.2 (10.6 to 31.8)	1.75 (1.31 to 2.45)	3.24e-04
IgA nonresponders	6/53 (11.3%)	11/53 (20.8%)	8/53 (15.1%)	5.7 (-7.7 to 19.0)	1.38 (0.64 to 3.40)	0.581
IgA responders	34/46 (73.9%)	38/46 (82.6%)	20/46 (43.5%)	39.1 (23.6 to 54.6)	1.90 (1.44 to 2.79)	4.01e-05

The homotypic-heterotypic difference was concentrated among IgA responders. Among IgA responders, G1P[8] NAb maintenance/increase occurred in 38/46 infants (82.6%) and G2P[4] NAb maintenance/increase in 20/46 (43.5%), corresponding to a paired risk difference of 39.1 percentage points (95% CI 23.6 to 54.6; $P = 4.01e-05$). Among IgA nonresponders, G1P[8] and G2P[4] maintenance/increase were lower and more similar [11/53 (20.8%) versus 8/53 (15.1%); $P = 0.581$]. The paired response-pattern pie charts showed that 19/46 (41.3%) IgA responders maintained or increased G1P[8] NAb without a corresponding G2P[4] response (Fig. 2b).

Formal paired interaction models supported IgA-specific homotypic advantage

In paired GEE (generalized estimating equations) models, the strain $\times$ IgA seroconversion interaction indicated that the G1P[8]–G2P[4] difference in maintenance/increase was larger among IgA responders than among IgA nonresponders. On the additive risk-difference scale, the interaction was 33.5 percentage points (95% CI 13.2 to 53.7; $P = 0.001$), indicating that the homotypic advantage was larger among IgA responders. On the multiplicative risk-ratio scale, the interaction ratio was 1.38 (95% CI 0.61 to 3.13; $P = 0.438$), showing that interaction inference depended on scale. In the continuous log₂ fold-change model, the strain $\times$ IgA-response interaction corresponded to a geometric mean ratio of 1.83 (95% CI 1.24 to 2.71; $P = 0.002$), supporting stronger G1P[8] than G2P[4] response dynamics among IgA responders (Table 4).

Table 4
Formal paired models evaluating strain, IgA response, and strain × IgA-response interaction.

Model	Term	Metric	Estimate (95% CI)	P value
Binary FC ≥ 1 endpoint, additive GEE	Strain: G1P[8] vs G2P[4] among IgA nonresponders	Risk difference or difference-in-differences	5.7 pp (-7.6 to 18.9)	0.402
Binary FC ≥ 1 endpoint, additive GEE	IgA responder vs nonresponder for G2P[4]	Risk difference or difference-in-differences	28.4 pp (11.1 to 45.6)	0.001
Binary FC ≥ 1 endpoint, additive GEE	Strain × IgA response interaction	Risk difference or difference-in-differences	33.5 pp (13.2 to 53.7)	0.001
Binary FC ≥ 1 endpoint, log-link GEE	Strain: G1P[8] vs G2P[4] among IgA nonresponders	Relative risk or ratio of RRs	1.37 (0.65 to 2.92)	0.407
Binary FC ≥ 1 endpoint, log-link GEE	IgA responder vs nonresponder for G2P[4]	Relative risk or ratio of RRs	2.88 (1.40 to 5.91)	0.004
Binary FC ≥ 1 endpoint, log-link GEE	Strain × IgA response interaction	Relative risk or ratio of RRs	1.38 (0.61 to 3.13)	0.438
Continuous log2 fold-change GEE	Strain: G1P[8] vs G2P[4] among IgA nonresponders	Geometric mean ratio or ratio of ratios	1.23 (0.97 to 1.57)	0.090
Continuous log2 fold-change GEE	IgA responder vs nonresponder for G2P[4]	Geometric mean ratio or ratio of ratios	2.24 (1.57 to 3.18)	7.55e-06
Continuous log2 fold-change GEE	Strain × IgA response interaction	Geometric mean ratio or ratio of ratios	1.83 (1.24 to 2.71)	0.002

Continuous fold-change analyses supported the binary endpoint

Continuous analyses of fold-change supported the primary binary endpoint (Table 3, Fig. 3). Overall, the geometric mean fold-change from 6 to 22 weeks was 0.75 (95% CI 0.60 to 0.94) for G1P[8] NAb and 0.46 (95% CI 0.38 to 0.56) for G2P[4] NAb. The within-infant G1P[8]/G2P[4] geometric mean fold-change ratio was 1.63 (95% CI 1.33 to 2.00; P = 7.44e-06). Among IgA responders, the ratio was 2.26 (95% CI 1.64 to 3.11; P = 6.06e-06); among IgA nonresponders, it was 1.23 (95% CI 0.96 to 1.58; P = 0.099).

Table 3

Antibody GMTs and fold-change dynamics overall and by rotavirus IgA seroconversion status.

Endpoint	Overall N = 99	IgA nonresponders N = 53	IgA responders N = 46
IgG GMT at 6 weeks	2,455 (1,940 to 3,106)	5,187 (4,075 to 6,603)	1,037 (805 to 1,334)
IgG GMT at 22 weeks	1,245 (1,028 to 1,507)	1,123 (862 to 1,464)	1,401 (1,055 to 1,860)
IgG geometric mean fold-change	0.51 (0.38 to 0.67)	0.22 (0.17 to 0.28)	1.35 (0.92 to 1.98)
G1P[8] NAb GMT at 6 weeks	140 (121 to 162)	182 (150 to 222)	103 (85 to 125)
G1P[8] NAb GMT at 22 weeks	105 (90 to 123)	71 (61 to 83)	165 (132 to 206)
G1P[8] NAb geometric mean fold-change	0.75 (0.60 to 0.94)	0.39 (0.32 to 0.48)	1.60 (1.18 to 2.16)
G2P[4] NAb GMT at 6 weeks	305 (256 to 362)	438 (354 to 542)	201 (159 to 253)
G2P[4] NAb GMT at 22 weeks	140 (121 to 162)	139 (113 to 170)	142 (114 to 176)
G2P[4] NAb geometric mean fold-change	0.46 (0.38 to 0.56)	0.32 (0.26 to 0.39)	0.71 (0.52 to 0.95)
Within-infant G1/G2 fold-change ratio	1.63 (1.33 to 2.00); P = 7.44e-06	1.23 (0.96 to 1.58); P = 0.099	2.26 (1.64 to 3.11); P = 6.06e-06

Antibody correlations and sensitivity analyses were consistent with primary findings

Figure 4 extends the primary binary endpoint by showing the continuous relationships among post-vaccination antibody responses. G1P[8] NAb fold-change correlated strongly with 22-week IgA titer ($\rho = 0.67$, 95% CI 0.54 to 0.77; $P = 5.03\text{e-}14$), whereas the corresponding G2P[4] correlation was positive but more modest ($\rho = 0.40$, 95% CI 0.22 to 0.55; $P = 3.81\text{e-}05$). Thus, infants with stronger systemic IgA responses tended to have greater NAb fold-change for both strains, but the association was more pronounced for the homotypic G1P[8] response. Figure 4 also shows the relationship of strain-specific NAb fold-change with IgG fold-change, supporting the interpretation that the $\text{FC} \geq 1$ primary endpoint reflects a broader pattern of post-vaccination antibody response rather than an isolated dichotomous cutoff.

In sensitivity analyses using no more than a one-dilution decline (fold-change ≥ 0.5), G1P[8] responses remained more frequent than G2P[4] responses overall and among IgA responders (**Supplementary**

Table S2 and Supplementary Figure S1). In the more stringent four-fold-rise sensitivity endpoint (fold-change ≥ 4), responses were uncommon but again more frequent for G1P[8] than G2P[4] among IgA responders [12/46 (26.1%) versus 4/46 (8.7%); **Supplementary Table S3**]. Higher baseline IgG and NAb titers were inversely correlated with 22-week IgA titers, including baseline IgG ($\rho=-0.66$, 95% CI -0.76 to -0.53), baseline G1P[8] NAb ($\rho=-0.39$, 95% CI -0.55 to -0.21), and baseline G2P[4] NAb ($\rho=-0.50$, 95% CI -0.63 to -0.34) (**Supplementary Figure S2**). Categorized baseline-antibody plots showed the same pattern descriptively: infants in higher baseline rotavirus IgG categories tended to have higher baseline G1P[8] and G2P[4] NAb titers, supporting the interpretation that pre-vaccination NAb titers reflected pre-existing, likely maternally derived, serum antibody rather than vaccine-induced infant responses (**Supplementary Figure S3**).

Discussion

In this post hoc immunogenicity substudy of Bangladeshi infants who received Rotarix, homotypic G1P[8] neutralizing antibody response dynamics exceeded heterotypic G2P[4] dynamics. The difference was evident using the primary binary endpoint of maintenance/increase in antibody titer and was supported by continuous fold-change analyses. The distinction was most pronounced among infants who had rotavirus IgA seroconversion, suggesting that systemic IgA response identifies infants with broader vaccine-induced antibody dynamics while still revealing a homotypic advantage.

The data do not indicate that absolute G2P[4] NAb titers were lower than G1P[8] titers at 22 weeks. In fact, G2P[4] GMTs were higher than G1P[8] GMTs at baseline and remained numerically higher at 22 weeks. However, the interpretation of these absolute titers differs from that of vaccine-associated response dynamics. Because all infants were IgA-seronegative at 6 weeks yet seropositive for IgG and NAb to both strains, baseline G2P[4] NAb likely reflected maternally derived or broadly cross-reactive antibody rather than infant-generated immunity. After vaccination, G1P[8] showed stronger maintenance/increase and fold-change than G2P[4], particularly among IgA responders, suggesting that G1P[8] NAb at 22 weeks included a larger vaccine-induced component, whereas G2P[4] NAb may have remained more influenced by waning maternal antibody. This distinction may help reconcile higher absolute G2P[4] titers with the emergence or persistence of heterotypic DS-1-like and animal-derived rotavirus strains in the post-vaccine era: passive serum antibody at early infancy does not necessarily imply durable population-level protection against antigenically divergent strains later.

These findings should not be interpreted as evidence that G2P[4] strains are intrinsically poorly neutralized in all settings. Rather, they suggest that in this infant cohort, Rotarix-induced response dynamics were more strongly directed toward the vaccine-matched G1P[8] strain. The emergence of G2P[4], DS-1-like, or equine-like reassortant strains globally after vaccine introduction is likely multifactorial, involving antigenic differences in VP7/VP4, full-genome constellation, reassortment, host population immunity, and natural temporal genotype fluctuations. Because our assay included only one DS-1 G2P[4] comparator strain, it remains to be determined whether post-vaccine genotype shifts are driven by VP7 genotype, VP4 genotype, DS-1-like backbone, or strain-specific antigenic features.

Additionally, recent reverse-genetics data provide mechanistic support for genotype-specific differences in neutralization susceptibility. A VP4/VP7 monoreassortant panel showed that VP7 genotype was a major determinant of susceptibility to systemic neutralizing antibodies and identified genotypes with reduced susceptibility after vaccination or natural infection [17].

Baseline IgG correlated with baseline neutralizing activity to both strains, supporting the interpretation that maternally derived antibody contributed to pre-vaccination neutralizing titers. Higher baseline antibody titers were inversely associated with post-vaccination fold-change and IgA response, a pattern compatible with maternal antibody interference. A recent neonatal oral rotavirus vaccination model showed that maternal antibodies can block vaccine replication and blunt subsequent immune activation, suggesting maternal antibody-mediated vaccine clearance as a mechanism of interference [18]. Our data are consistent with this mechanism, although maternal and infant-derived antibodies could not be distinguished in serum, and fold-change is mathematically influenced by baseline titer.

This study has limitations. It measured immunologic outcomes rather than clinical rotavirus disease, so it cannot establish a protective threshold for NAb. The sample size was modest for multivariable modeling, and IgA seroconversion should be interpreted as a post-vaccination proxy rather than a baseline determinant. The paired interaction model formally supported the IgA-specific homotypic advantage on the additive and continuous fold-change scales, but multiplicative risk-ratio interaction was imprecise. The assay compared one homotypic and one heterotypic strain, so findings may not generalize to all Wa-like or DS-1-like genotypes.

These findings support genotype-specific neutralization as a mechanistic readout for evaluating oral rotavirus vaccine performance. Future studies should link strain-specific neutralizing antibody dynamics, viral whole-genome background, mucosal immunity, and clinical endpoints to define immune markers of durable cross-genotype protection in high-burden settings.

Methods

Study design and participants

This was a post hoc immunogenicity substudy nested within a randomized, controlled, open-label, parallel, phase 4 trial conducted in urban slums in Dhaka, Bangladesh, between September and December 2016. The parent trial was prospectively registered at ClinicalTrials.gov before participant enrollment began (NCT02847026; registered 27/07/2016) [4]. The present analysis was restricted to infants in the Rotarix arm/source population who were rotavirus IgA-seronegative at 6 weeks and had paired serum samples available at 6 and 22 weeks for neutralizing-antibody testing. A CONSORT-style substudy flow diagram is provided as Supplementary Figure S4.

No separate formal sample-size calculation was performed for the neutralizing-antibody substudy. The analytic sample was determined by substudy eligibility, availability of paired sera at 6 and 22 weeks, and availability of valid strain-specific neutralization measurements.

Patient and public involvement

Patients and the public were not involved in the design, conduct, analysis, or reporting of this post hoc immunogenicity substudy beyond participation of infants and their parents or legal guardians in the parent trial.

Procedures and assays

Infants received two oral doses of Rotarix at 6 and 10 weeks of age. Serum samples were collected at 6 weeks before dose 1 and at 22 weeks, approximately 12 weeks after dose 2. Rotavirus-specific serum IgA and IgG were measured by EIA [4]. Rotavirus NABs were measured by microneutralization assay using heat-inactivated sera, two-fold serial dilution, trypsin-activated Rotarix G1P[8] or DS-1 G2P[4], and MA104 cells [19]. The NAb titer was defined as the reciprocal of the highest dilution producing > 70% reduction in absorbance compared with virus-only controls.

Outcomes

The primary binary endpoint was maintenance/increase in antibody titer from 6 to 22 weeks, defined as fold-change ≥ 1 . This endpoint was evaluated for rotavirus IgG, G1P[8] NAb, and G2P[4] NAb. The main sensitivity endpoint was no more than one-dilution decline, defined as fold-change ≥ 0.5 , and an additional stringent sensitivity endpoint was four-fold rise, defined as fold-change ≥ 4 . The main continuous endpoint was log2 fold-change from 6 to 22 weeks.

Statistical analysis

Antibody titers were summarized as GMTs with 95% confidence intervals after log transformation. Because G1P[8] and G2P[4] NAb responses were measured in the same infants, paired binary responses were compared using exact McNemar tests, paired risk differences, and paired relative risks. Paired G1P[8]/G2P[4] fold-change ratios were estimated from within-infant log2 fold-change differences and back-transformed. To formally assess whether the strain-specific difference in NAb response varied by IgA seroconversion status, paired GEE models were fitted with infant as the clustering unit. Models included strain, IgA seroconversion, and a strain $\times$ IgA seroconversion interaction term. Additive GEE models estimated risk differences and difference-in-differences; log-link GEE models estimated relative risks; and Gaussian GEE models of log2 fold-change estimated geometric mean ratios. Spearman correlation coefficients were reported with approximate 95% CIs using Fisher z transformation. Exploratory modified Poisson models with robust standard errors estimated adjusted relative risks for maintenance/increase. Analyses of the primary paired outcomes used all 99 infants with complete paired G1P[8] and G2P[4] measurements. Two infants with indeterminate secretor phenotype were excluded only from models that included secretor status; no imputation was performed. The paired G1P[8] versus G2P[4] comparison for maintenance/increase was designated as the primary analysis for the current post hoc reanalysis; regression models, correlation analyses, subgroup analyses, interaction models, and sensitivity analyses were considered exploratory and were not adjusted for multiplicity. Statistical analyses were performed using Python 3.13.5 with NumPy 2.3.5, pandas 2.2.3, SciPy 1.17.0,

statsmodels 0.14.6, matplotlib 3.10.8, and openpyxl 3.1.5. Reproducible Python code, package-version information, derived statistical outputs, and figure source-data files are available at [https://doi.org/\[ZENODO DOI\]](https://doi.org/[ZENODO DOI]).

Protocol and analysis-plan availability

The parent trial protocol (PR-15105) is provided as a separate submission file. The present neutralizing-antibody substudy and updated analyses were post hoc; no prospective substudy-specific statistical analysis plan was prepared. A dated post hoc analysis plan, reproducible Python code, and figure source data files are available at DOI 10.5281/zenodo.22904251.

Reporting checklist

Reporting of the parent randomized trial and the present post hoc immunogenicity substudy followed applicable CONSORT 2025 recommendations. A completed CONSORT 2025 checklist with manuscript page references and a CONSORT-style substudy flow diagram are provided with the submission.

Harms

Harms and adverse events were assessed during the parent trial and have been reported previously [4]. No new safety or harms outcomes were evaluated in the present post hoc immunogenicity substudy.

Ethics

The parent study protocol PR-15105 was approved by the Institutional Review Board and Centre Director of the International Centre for Diarrhoeal Disease Research, Bangladesh. The protocol was shared with the US Centers for Disease Control and Prevention, which deferred to the icddr,b Institutional Review Board. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and applicable local regulatory requirements. Written informed consent was obtained from the parents or legal guardians of all participating infants before enrollment.

Declarations

Competing interests

The authors declare no competing interests.

CDC disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Funding

The parent clinical trial and the present study were funded by the US Centers for Disease Control and Prevention. CDC investigators contributed to study design, laboratory testing, data analysis, interpretation, and manuscript preparation. No commercial funding was received for this work. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Author Contribution

D.V.P. and B.J. conceived the neutralizing-antibody substudy. K.Z. implemented the parent clinical trial. J.H., Y.W., and D.V.P. contributed to laboratory assays. D.V.P. performed the statistical analyses and drafted the manuscript. D.V.P., J.H., Y.W., U.P., K.Z., and B.J. interpreted the data, revised the manuscript, and approved the final version.

Acknowledgements

The authors thank the participating families, field teams, and laboratory staff involved in the parent trial and neutralizing antibody assays.

Data Availability

Figure source data, derived analysis tables, and supporting metadata are publicly available in Zenodo at the DOI 10.5281/zenodo.22904251. De-identified participant-level analytic data are not publicly posted because they derive from a human infant clinical trial and are subject to ethical, institutional, and data-use restrictions imposed by the International Centre for Diarrhoeal Disease Research, Bangladesh, and the US Centers for Disease Control and Prevention. De-identified participant-level data may be made available to qualified researchers upon reasonable request to the corresponding author, subject to review and approval by icddr,b and CDC, completion of any required data-use agreement, and agreement not to attempt participant re-identification or onward transfer of the data.

Code availability

The Python code used to reproduce the statistical analyses, tables, figures, paired generalized estimating equation models, sensitivity analyses, and figure source-data files is publicly available in Zenodo at the DOI 10.5281/zenodo.22904251. The archived record includes executable analysis scripts, a README, Python and package-version information, derived statistical outputs, and source-data files.

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Figures

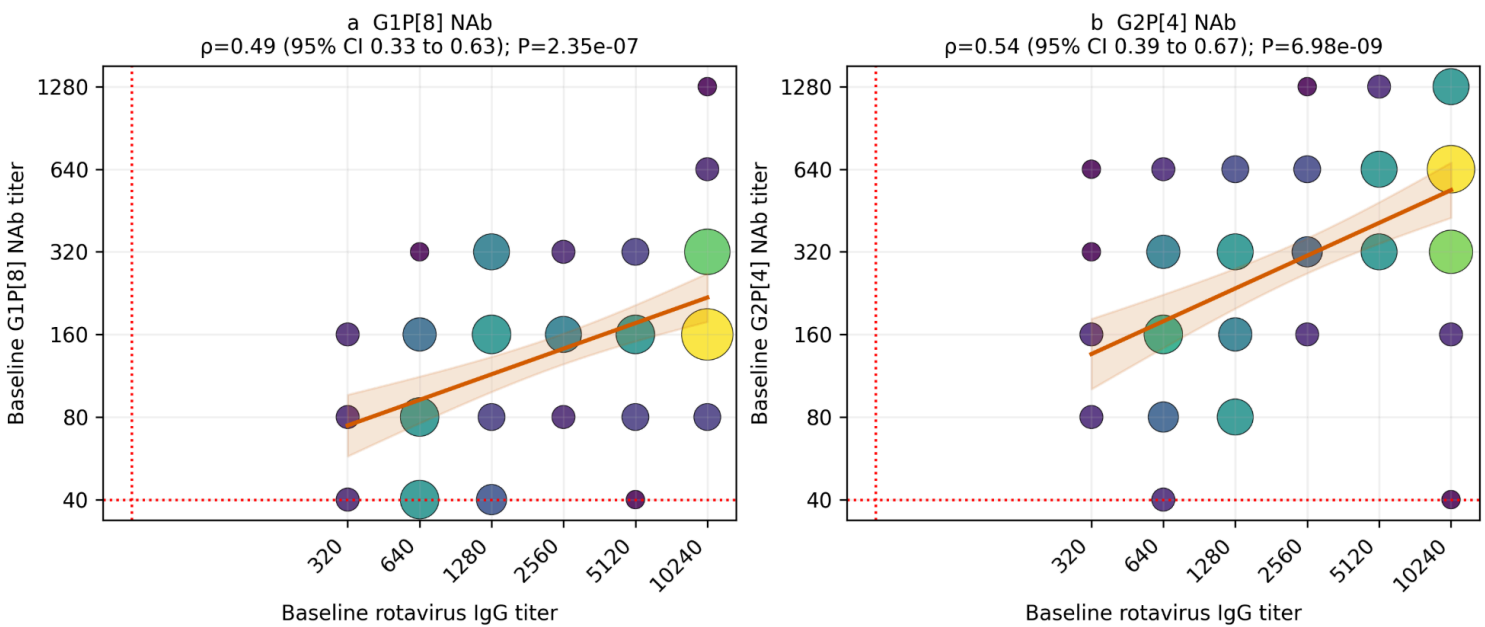


Figure 1

Baseline rotavirus IgG and strain-specific neutralizing antibody titers. a, Weighted correlation plot showing rotavirus IgG titers and homotypic G1P[8] NAb titers at 6 weeks. b, Weighted correlation plot showing rotavirus IgG titers and heterotypic G2P[4] NAb titers at 6 weeks. Each marker represents a unique x-y titer pair; marker area and shading are proportional to the number of infants with identical values. Solid trendlines and shaded bands show linear regression and 95% CI for the mean fitted in log2-transformed titer space. Red dotted lines indicate seropositivity cutoffs.



Figure 2

Primary maintenance/increase endpoint for homotypic and heterotypic NAb responses. a, Proportion of infants with maintenance/increase in titer from 6 to 22 weeks, defined as fold-change ≥ 1 , overall and by IgA seroconversion status. b, Colorblind-safe pie charts of paired G1P[8] and G2P[4] response patterns for the primary endpoint among IgA nonresponders (b1, left) and IgA responders (b2, right). Pie slices show counts, denominators, percentages, and the four paired categories: G1+/G2+, G1+/G2-, G1-/G2+, and G1-/G2-.

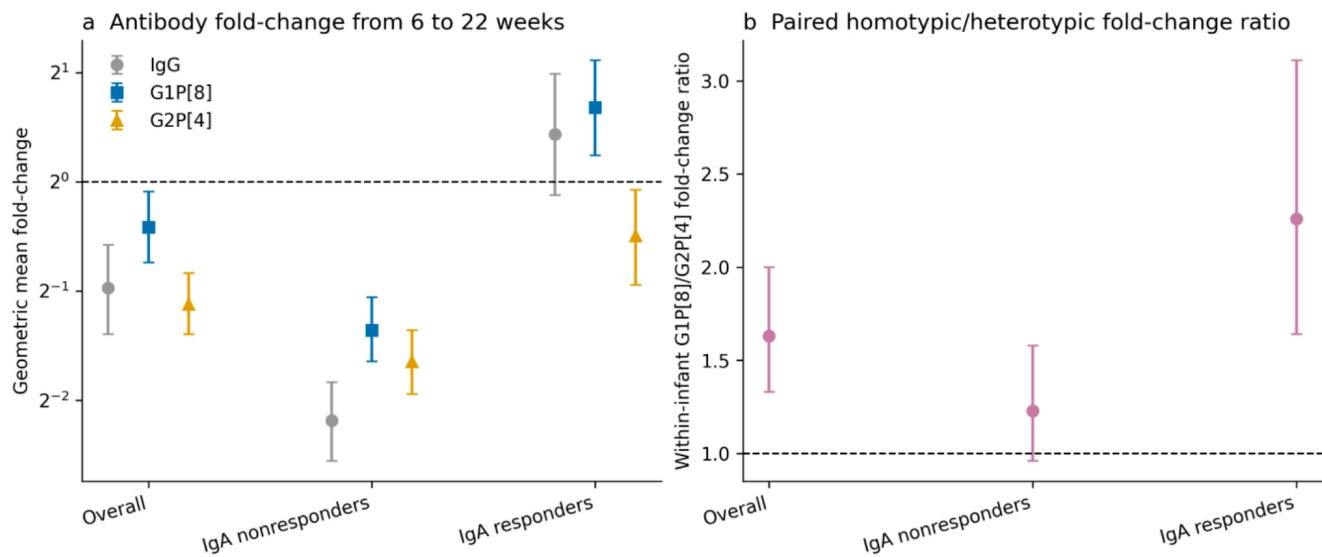


Figure 3

Continuous antibody fold-change dynamics by IgA seroconversion status. a, Geometric mean fold-change from 6 to 22 weeks with 95% CI for rotavirus IgG, G1P[8] NAb, and G2P[4] NAb. The dashed line indicates fold-change=1. b, Within-infant G1P[8]/G2P[4] geometric mean fold-change ratio with 95% CI. Values above 1 indicate higher G1P[8] than G2P[4] fold-change.

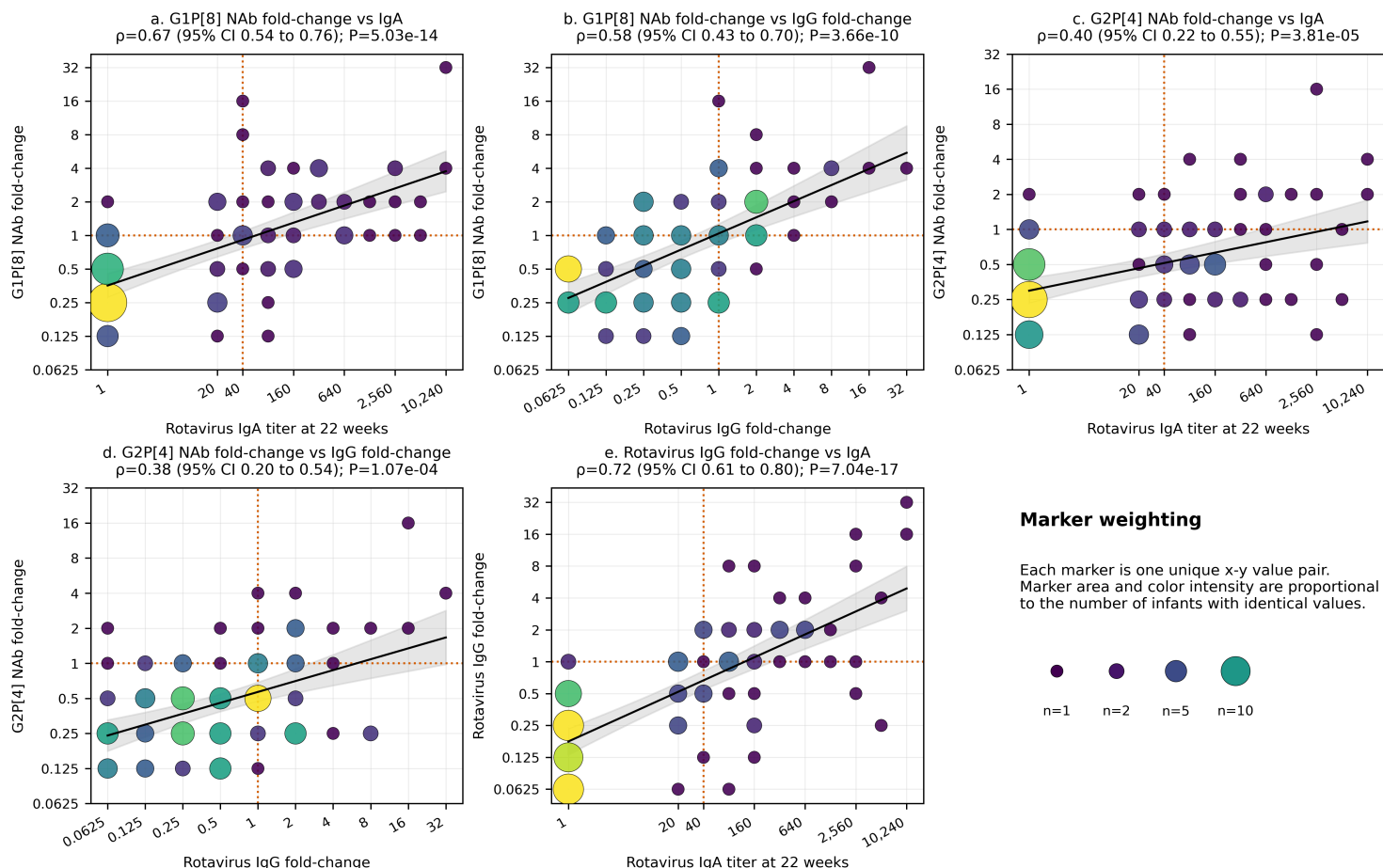


Figure 4

Continuous correlations among post-vaccination antibody response measures. Panels show Spearman correlations between G1P[8] NAb fold-change, G2P[4] NAb fold-change, rotavirus IgG fold-change, and 22-week rotavirus IgA titer. The figure complements the primary $FC \geq 1$ maintenance/increase endpoint by displaying the underlying continuous antibody-response dynamics. Each marker represents a unique x-y pair; marker area and shading are proportional to the number of infants with identical values. Solid trendlines and shaded bands show linear regression and 95% CI for the mean fitted in the displayed log2-transformed scale. Red dotted lines indicate IgA seropositivity or fold-change reference thresholds. NAb, neutralizing antibody.

Supplementary Files

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