IDSA — Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Plasma Rotavirus-specific IgA and Risk of Rotavirus Vaccine Failure in Infants in Malawi

Louisa Pollock,[1,2,a,] Aisleen Bennett,[1,2] Khuzwayo C. Jere,[1,2,3] Jonathan Mandolo,[2] Queen Dube,[4] Naor Bar-Zeev,[2,5] Robert S. Heyderman,[2,6] Nigel A. Cunliffe,[1,7,b] and Miren Iturriza-Gomara[1,7,c]

[1]Centre for Global Vaccine Research, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom; [2]Malawi Liverpool Wellcome Trust Clinical Research Programme, Kamuzu University of Health Sciences, Blantyre, Malawi; [3]Department of Biomedical Sciences, Kamuzu University of Health Sciences, Blantyre, Malawi; [4]Department of Paediatrics, Kamuzu University of Health Sciences, Blantyre, Malawi; [5]International Vaccine Access Center, Dept. International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA; [6]Research Department of Infection, Division of Infection and Immunity, University College London, London, United Kingdom; and [7]National Institute for Health Research Health Protection Research Unit in Gastrointestinal Infections at University of Liverpool, Liverpool, United Kingdom[a]Present affiliation: University of Glasgow, Glasgow, UK.[b]Present affiliation: National Institute for Health Research (NIHR) Health Protection Research Unit, Gastrointestinal Infections, University of Liverpool, in partnership with Public Health England, in collaboration with University of Warwick.[c]Present affiliation: Centre for Vaccine Innovation and Access, PATH, Geneva, Switzerland.

Background. Rotavirus vaccine efficacy is reduced in low-income populations, but efforts to improve vaccine performance are limited by lack of clear correlates of protection. Although plasma rotavirus (RV)-specific immunoglobulin A (IgA) appears strongly associated with protection against rotavirus gastroenteritis in high-income countries, weaker association has been observed in low-income countries. We tested the hypothesis that lower RV-specific IgA is associated with rotavirus vaccine failure in Malawian infants.

Methods. In a case-control study, we recruited infants presenting with severe rotavirus gastroenteritis following monovalent oral rotavirus vaccination (RV1 vaccine failures). Conditional logistic regression was used to determine the odds of rotavirus seronegativity (RV-specific IgA < 20 U/mL) in these cases compared 1:1 with age-matched, vaccinated, asymptomatic community controls. Plasma RV-specific IgA was determined by enzyme-linked immunosorbent assay for all participants at recruitment, and for cases at 10 days after symptom onset. Rotavirus infection and genotype were determined by antigen testing and reverse transcription-polymerase chain reaction, respectively.

Results. In 116 age-matched pairs, infants with RV1 vaccine failure were more likely to be RV-specific IgA seronegative than controls: odds ratio, 3.1 (95% confidence interval [CI], 1.6–5.9), *P* = .001. In 60 infants with convalescent serology, 42/45 (93%; 95% CI. 81–98) infants seronegative at baseline became seropositive. Median rise in RV-specific IgA concentration following acute infection was 112.8 (interquartile range, 19.1–380.6)-fold.

Conclusions. In this vaccinated population with high residual burden of rotavirus disease, RV1 vaccine failure was associated with lower RV-specific IgA, providing further evidence of RV-specific IgA as a marker of protection. Robust convalescent RV-specific IgA response in vaccine failures suggests differences in wild-type and vaccine-induced immunity, which informs future vaccine development.

Keywords. rotavirus; IgA; vaccine; Malawi; gastroenteritis.

Introduction of rotavirus vaccines into childhood immunization programs has reduced global child deaths from diarrheal disease [1], but current vaccines are less effective in low-income, high-mortality countries than in higher income settings [2]. Multiple explanations for this disparity have been proposed, including factors that may inhibit the initial vaccine response [3–6] and factors that may increase rotavirus exposure or increase susceptibility to rotavirus disease in later infancy [7–9]. Efforts to assess and improve rotavirus vaccines have been hampered by the lack of a proven correlate or surrogate marker of protection against rotavirus disease [10]. Biomarkers associated with protection are required to reduce the need for very large-scale clinical trials focussed on increasingly rare clinical outcomes [10].

In studies of immune response to natural rotavirus infection, plasma rotavirus (RV)-specific immunoglobulin A (IgA) levels were shown to increase with repeated exposure to natural infection, and were associated with reduced risk of future RV disease [11]. Higher concentrations of RV-specific IgA, and in some studies IgG, were associated with reduced likelihood of severe rotavirus disease in early case-control studies [12]. In studies of response to oral RV vaccines, postimmunization RV-specific IgA has been correlated with vaccine efficacy at population level [13, 14]. At an individual level, in a recent pooled analysis of monovalent oral rotavirus vaccine (RV1) trial data, Baker et al [15] demonstrated that RV-specific IgA seroconversion (defined

https://doi.org/10.1093/cid/ciab895

as postimmunization RV-specific >20 U/mL) was strongly associated with protection against rotavirus gastroenteritis. However, although seroconversion and RV-specific IgA titers postimmunization were very strongly associated with protection in low child-mortality countries, a much weaker association was observed in high child-mortality countries. Similarly, Lee et al [16] found that postimmunization RV-specific IgA was a suboptimal correlate of protection against rotavirus gastroenteritis in infants in Bangladesh. The extent to which plasma RV-specific IgA may be a mechanistic correlate of protection, causally responsible for observed protection, or a nonmechanistic proxy marker of an alternative, likely mucosal, protective immune response is debated [17, 18]. Individual data on RV-specific IgA measured at time of presentation with rotavirus gastroenteritis would add new information to further inform this debate.

Malawi is a low-income country with high rotavirus burden and relatively low (~ 50%) rotavirus vaccine efficacy [19], which introduced RV1 nationally in 2012, with vaccine coverage reaching 95% by 2015 [20]. We previously demonstrated that low serum RV-specific IgA is associated with increased rotavirus load in vaccinated Malawian children with acute gastroenteritis [21]. We now test the hypothesis that low RV-specific IgA is associated with increased risk of clinical rotavirus vaccine failure.

METHODS

This was a prospective case-control study. Ethical approval was granted by the University of Malawi College of Medicine (P.09/14/1624) and University of Liverpool (00758) Research Ethics Committees. All participants were recruited following informed consent from a parent/guardian.

Infants presenting with symptoms of gastroenteritis to 3 primary healthcare centers and a referral hospital in Blantyre, Malawi, between January 2015 and January 2017 were screened for recruitment within the local diarrheal surveillance platform [20]. Cases (vaccine failures) met the following eligibility criteria: aged between 10 weeks and 1 year; received 2 doses of oral RV1 documented in their hand-held health record; severe gastroenteritis, defined as Vesikari score ≥11 [22]; and rotavirus positive by rapid stool immunochromatography test (RotaStrip, Coris Bioconcept, Belgium).

Age-matched community controls were recruited in a 1:1 ratio. Global positioning system locations were randomly generated (using R 3.1.1, The R Foundation for Statistical Computing) within recruitment site healthcare catchment areas to search for controls. Controls met the following eligibility criteria: born within ±30 days of matched case; received 2 doses of RV1; and reported no diarrhea within 1 week before recruitment.

Data Collection and Anthropometry

Demographic and socioeconomic data were collected by structured interview. Nutritional status was determined by measurement of weight, length, and mid-upper arm circumference, compared with World Health Organization (WHO) age-determined z scores [23]. Infants with weight for height z score <-3 standard deviations and infants older than 6 months of age with a mid-upper arm circumference <11.5 cm were considered to have severe acute malnutrition [24]. Weight of cases with signs of some or severe dehydration noted on admission was adjusted by 5% or 10%, respectively [25]. The z-score restrictions were applied in accordance with WHO guidelines so that biologically implausible outliers were removed [26].

Sample Collection

All participants had stool, saliva, and plasma samples collected at time of presentation with gastroenteritis (cases) or recruitment (controls). Rotavirus gastroenteritis cases had a convalescent plasma sample taken 10 days following onset of diarrhea, corresponding to the peak rise in RV-specific IgA following acute infection in infants [27].

Laboratory Methods

For detailed laboratory methods see Supplementary Methods. Nucleic acid was extracted from stool from gastroenteritis cases and community controls using the Qiagen Viral RNA Mini-Kit (Qiagen, Germany). Reverse transcription (RT) using random primers was used to generate complementary DNA [28]. VP6 quantitative (q)RT-polymerase chain reaction (PCR) was used detect rotavirus. Community controls with VP6 ≥100 copies/mL by qRT-PCR were considered to have asymptomatic rotavirus infection, those with VP6 <100 copies/mL (analytical sensitivity) were considered rotavirus negative. Genotyping was undertaken by heminested PCR and gel electrophoresis was undertaken for all rotavirus positive samples possessing a qRT-PCR cycle threshold below 35 [29].

Plasma RV-specific IgA was determined by antibody-sandwich enzyme-linked immunosorbent assay (ELISA) [30]. Quantification was made by comparison to a standard plasma [31], reported as geometric mean concentration (GMC) in units per milliliter (U/mL). RV-specific IgA seropositivity was defined as a GMC >20 U/mL [32].

Histo-blood group antigen (HBGA) phenotyping was determined as described in Pollock et al [5]. In brief, antigens A, B, H, and Lewis a and b were detected in saliva by ELISA, using specific monoclonal antibodies, detected by peroxidase conjugated anti-mouse IgM. Infants with detectable salivary A, B, or H antigen were classified as secretors. Where detection of A, B, and H antigens was negative or borderline, secretor status was confirmed by ELISA to detect lectin antigen [33].

Statistical Analysis

All statistical analysis was performed in StataIC version 13.1 (StataCorp). Summary statistics for demographics, nutritional status, and socioeconomic factors were reported for cases and controls. Continuous variables were compared by *t* test for parametric and Wilcoxon rank-sum test for nonparametric data. Categorical variables were compared by χ^2 test, or Fisher exact test where cell values were less than 10.

The primary outcome measure was the odds of RV-specific IgA <20 U/mL in cases compared with controls calculated by conditional logistic regression. With 1:1 controls, a sample size of 137 cases was estimated to achieve 80% power to detect an odds ratio of 2.0 (alpha 0.05), assuming 50% RV-specific IgA seropositivity in controls (assumption based on vaccine trial data [19]). In addition, comparison of RV-specific IgA as a continuous variable between cases and controls was made by Wilcoxon rank-sum test. Receiver operating characteristic (ROC) analysis was used to evaluate the utility of RV-specific IgA concentration in discriminating between vaccine failures and community controls. A cutoff point of RV-specific IgA concentration that best predicted case-control status was determined by maximizing Youden's index ((sensitivity + specificity) – 1) [34, 35].

RESULTS

A total of 196 rotavirus-positive infants meeting eligibility criteria were identified. Seven-six infants did not participate, mainly because of the requirement for blood sampling. A total of 120 severe rotavirus gastroenteritis cases were therefore recruited.

The median age of presentation of cases was 9.0 months (interquartile range [IQR] 7.6–10.6). The median age at recruitment for community controls was 9.8 months (IQR 8.3–11.1). The median Vesikari score was 14 (IQR 13–16).

Genotype was determined for 116/120 (97%) rotavirus gastroenteritis cases. Four genotypes accounted for >75% of total genotypes: G1P[8] (32%), G2P[4] (26%), G12P[6] (10%), and G2P[6] (9%).

There were no significant differences between cases and controls in weight or height for age *z* scores, sanitation, or socioeconomic factors, human immunodeficiency virus exposure, male sex, or low birth weight (Table 1). As previously reported [5], cases were significantly less likely than controls to have nonsecretor HBGA phenotype.

Comparison of RV-specific IgA Between RV1 Vaccine Failures and Community Controls

RV-specific IgA was determined for 117 rotavirus gastroenteritis cases (vaccine failures) and 119 community controls. RV-specific IgA was generally low in this population: 55% of community controls were seronegative (RV-specific IgA <20 U/mL) (Table 2). There was no difference in baseline RV-specific IgA by nonsecretor HBGA phenotype (Supplementary Table 1). In those with detectable RV-specific IgA, RV-specific IgA concentration was significantly lower in RV1 vaccine failures compared with controls (Table 2). In 116 age-matched pairs, the odds of being seronegative were more than 3 times higher in vaccine failures compared with controls: odds ratio, 3.1 (95% confidence interval [CI], 1.6–5.9), *P* = .001.

In ROC analysis, the inverse of the RV-specific IgA concentration showed some utility in discriminating between vaccine failures and controls, with an area under the curve of 0.61 (95% CI, 0.54–0.68) (Supplementary Figure 1).

RV-specific IgA Response to Natural Infection in RV1 Vaccine Failures

Paired presentation and convalescent plasma samples were available for 60/120 (50%) rotavirus gastroenteritis cases. The median number of days after illness onset at convalescent sampling was 10 (IQR 9–12). The distribution of baseline RV-specific IgA was similar in those with convalescent serology data available to those without (Supplementary Table 2). Of 45 infants seronegative at presentation, 42 (93%; 95% CI, 81–98) were seropositive at follow-up. The follow-up GMC was significantly higher than baseline (Wilcoxon matched-pairs signed-rank test, *P* < .0001) (Table 3). The median convalescent rise in RV-specific IgA concentration was 112.8 (IQR 19.1–380.6)-fold.

DISCUSSION

In this high mortality, high disease burden population in Malawi, lower RV-specific IgA measured at time of disease presentation was strongly associated with increased odds of RV1 clinical vaccine failure. This provides further evidence that RV-specific IgA is associated with clinical protection at individual, as well as population, level. Our findings are consistent with a post hoc analysis of data from a phase III efficacy trial of RV1 conducted in Malawi and South Africa, where postimmunization RV-specific IgA seropositivity was associated with reduced risk of subsequent severe rotavirus gastroenteritis (odds ratio, 0.39 [95% CI, 0.29–0.52], *P* < .001) [14]. Our data are also consistent with a large pooled analysis of RV1 trial data across 16 countries, which showed that higher postimmunization RV-specific IgA levels were associated with reduced cumulative incidence of severe rotavirus gastroenteritis [15].

Using ROC analysis, RV-specific IgA concentration was shown to have relatively limited utility in discriminating between RV1 vaccine failures and controls in this population. This could reflect the limitations of the case-control design, in which some community controls could also be at risk of vaccine failure if exposed to infection, and in which some cases could already have had rising IgA in response to acute infection. However, it could also support the hypothesis that RV-specific IgA is a surrogate marker, rather than an absolute correlate, of

Table 1. Host and Socioeconomic Factors: RV1 Vaccine Failures and Community Controls

Characteristic[a]	RV1 Vaccine Failures	Community Controls	P
Infant characteristics			
Male	73, 61% (52–69%)	61, 51% (42–60%)	.12[b]
Exposed to HIV[c]	17, 14% (9–22%)	19, 16% (10–24%)	.72[b]
Low birth weight (<2.5 kg)	14/112, 13% (8–20%)	12/118, 9% (5–16%)	.44[b]
Nonsecretor	14/119, 12% (7–19%)	34/120, 28% (21–37%)	.001[b]
Nutritional status			
Median weight for age *z*-score (IQR)[d]	-0.37 (-1.39 to 0.45)	-0.42 (-1.0 to 0.36)	.88[e]
Median length for age *z*-score (IQR)	-0.68 (-1.75 to 0.95)	-0.76 (-1.98 to -0.06)	.19[e]
Median weight for length *z*-score (IQR)[d]	-0.62 (-1.58 to 0.37)	-0.05 (-1.32 to 0.88)	.11[e]
Sanitation and socioeconomic factors			
Median household size (IQR)	5 (4–6)	4 (3–6)	.13[e]
Nonpiped water source	22, 19% (12–27%)	17, 14% (9–22%)	.37[b]
Time to access water			.52[b]
<5 min	27, 23% (16–31%)	20, 17% (11–25%)	
5–30 min	52, 44% (35–53%)	52, 44% (36–54%)	
>30 min	40, 34% (26–43%)	45, 38% (30–48%)	
Pit-latrine type toilet	116, 97% (91–99%)	115, 96% (90–98%)	.73[b]
Electricity at home	61, 50% (42–59%)	55, 46% (37–55%)	.44[b]
One or more household members with salary	82, 68% (59–76%)	76, 63% (54–72%)	.41[b]
Household food insecurity	40, 33% (25–42%)	36, 30% (22–39%)	.58[b]
Median age of head of household in years (IQR)	30 (26–37)	30 (27–34)	.40[e]
Median years of maternal education (IQR)	8 (5–11)	9 (7–11)	.32[e]

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; RV, rotavirus; RV1, monovalent oral rotavirus vaccine.

[a]All proportions reported as number (proportion, 95% confidence interval of proportion). Denominator for all proportions n = 120 for both cases and controls unless stated otherwise.

[b]χ^2 test.

[c]11/17 RV gastroenteritis cases exposed to HIV and 15/19 community controls exposed to HIV had a negative HIV DNA polymerase chain reaction at 6 weeks old. One community control was known to be infected with HIV and on antiretrovirals. Status of remaining infants exposed to HIV was unknown.

[d]Weight adjusted for dehydration status by adding 5% for some dehydration and 10% for severe dehydration.

[e]Wilcoxon rank-sum test.

Table 2. Comparison of RV-specific IgA Among RV1 Vaccine Failures and Community Controls

	RV1 Vaccine Failures	Community Controls	P
Undetectable RV-specific IgA	55/117	41/119	.05[a]
n, % (95% CI)	47 (38–56)	34 (26–44)	
RV-specific IgA <20 U/mL	89/117	66/119	.001[a]
n, % (95% CI)	76 (67–83)	55 (46–64)	
RV-specific IgA concentration[b] Median (IQR) U/mL	18.1 (7.1–53.1) U/mL	48 (14.5–146.7) U/mL	.01[c]

Abbreviations: IgA, immunoglobulin A; CI, confidence interval; RV, rotavirus; RV1, monovalent oral rotavirus vaccine.

[a]χ^2 test.

[b]In infants with detectable RV-specific IgA.

[c]Wilcoxon rank-sum test.

protection [10, 18]. The extent to which RV-specific IgA is associated with protection across populations varies: Baker et al [15] found that for any given threshold for IgA, greater protection was conferred in low child-mortality countries compared with high child-mortality countries, but were unable to identify specific confounding factors that explained this difference. Nevertheless, they concluded that a seroconversion threshold of 20 U/mL remained a practical and informative measure of protection.

Lee et al [16] found that uniformly poor RV-specific IgA responses postimmunization in infants in Bangladesh, together with rapidly waning immunity in some infants, reduced the utility of RV-specific IgA as a correlate of protection. Postimmunization RV-specific IgA responses in Malawian infants are similarly poor compared with higher income countries. In a recent Malawian birth cohort, we found only 24% of infants seroconverted [5]. Despite this, we demonstrate that most infants with clinical rotavirus vaccine failure show a robust RV-specific IgA response to natural infection. Almost all infants demonstrated a significant rise in RV-specific IgA titers within 10 days of symptom onset, similar to the immune response seen in unvaccinated infants [27, 36]. In this population, 93% of infants seronegative at baseline seroconverted following acute infection. Of note, infants with low RV-specific IgA at

Table 3. RV-specific IgA Response to Natural Infection in RV1 Vaccine Failures

	RV1 Vaccine Failures[a] at Presentation	RV1 Vaccine Failures Convalescent Sample
Undetectable RV-specific IgA	29/60	1/60
n, % (95% CI)	48 (36–61)	2 (0.2–11)
RV-specific IgA <20 U/mL	45/60	3/60
n, % (95% CI)	75 (62–85)	5 (2–15)
RV-specific IgA concentration[b] Median (IQR,) U/mL	18.8 (6.4–54.7)	433.5 (130.3–896.4)

Abbreviations: IgA, immunoglobulin A; CI, confidence interval; RV, rotavirus; RV1, monovalent oral rotavirus vaccine.

[a]RV gastroenteritis cases with convalescent sample available.

[b]Infants with detectable RV-specific IgA.

baseline, and of similar age to our study population, show a less consistent response to challenge with "booster" live, oral rotavirus vaccination. Thus, in Mali, a booster (fourth) dose of oral pentavalent rotavirus vaccine (RV5) at age 9 months resulted in seroconversion in 56.9% of infants who were seronegative at baseline [37], whereas in Bangladesh only 43.6% of baseline seronegative infants seroconverted following receipt of a third booster dose of RV1 [38]. Lower immune responses to booster doses of vaccine compared with natural infection could reflect continuing vaccine-specific inhibitory factors persistent in later infancy or may reflect a higher virus inoculum in natural infection. There is evidence from early studies of RV1 and of the live rotavirus vaccine ORV-116E that higher vaccine doses generate a more robust immune response [39–41].

A key strength of our study is that we measured RV-specific IgA at time of presentation with severe gastroenteritis. The main limitation is that the cross-sectional case-control design means that we are unable to determine whether low RV-specific IgA at time of presentation relates to poor initial response to vaccination, or to waning immunity. Rotavirus vaccine effectiveness appears to be lower in the second year of life in some low-income countries, but the extent to which this may reflect waning immunity is unclear [42]. This is an important distinction because it could determine whether efforts should continue to focus on improving magnitude and duration of initial vaccine response, or designing optimal vaccine booster strategies. A cohort study determining the dynamics of RV-specific IgA response from vaccination throughout the first year of life could address this issue.

This study provides data in a rotavirus-vaccinated population of RV-specific IgA measured at time of rotavirus clinical vaccine failure. Further data from diverse populations are required to determine whether a particular level of RV-specific IgA can be considered broadly protective, or at least highly predictive of protection against clinical vaccine failure. These data take us closer to the identification of a reliable protective threshold would greatly assist efforts to improve the performance of current vaccines, and the evaluation of new vaccines.

References

1. Burnett E, Jonesteller CL, Tate JE, Yen C, Parashar UD. Global impact of rotavirus vaccination on childhood hospitalizations and mortality from diarrhea. J Infect Dis **2017**; 215:1666–72.
2. Jonesteller CL, Burnett E, Yen C, Tate JE, Parashar UD. Effectiveness of rotavirus vaccination: a systematic review of the first decade of global postlicensure data, 2006-2016. Clin Infect Dis **2017**; 65:840–50.
3. Church JA, Rogawski McQuade ET, Mutasa K, et al. Enteropathogens and rotavirus vaccine immunogenicity in a cluster randomized trial of improved water, sanitation and hygiene in rural Zimbabwe. Pediatr Infect Dis J **2019**; 38:1242–8.
4. Church JA, Chasekwa B, Rukobo S, et al. Predictors of oral rotavirus vaccine immunogenicity in rural Zimbabwean infants. Vaccine **2020**; 38:2870–8.
5. Pollock L, Bennett A, Jere KC, et al. Nonsecretor histo-blood group antigen phenotype is associated with reduced risk of clinical rotavirus vaccine failure in Malawian infants. Clin Infect Dis **2019**; 69:1313–9.
6. Baker JM, Tate JE, Leon J, Haber MJ, Lopman BA. Antirotavirus IgA seroconversion rates in children who receive concomitant oral poliovirus vaccine: a secondary, pooled analysis of phase II and III trial data from 33 countries. PLoS Med **2019**; 16:e1003005.
7. Lopman BA, Pitzer VE, Sarkar R, et al. Understanding reduced rotavirus vaccine efficacy in low socio-economic settings. PLoS One **2012**; 7:e41720.
8. Mohan VR, Karthikeyan R, Babji S, et al; Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) Network Investigators. Rotavirus infection and disease in a multisite birth cohort: results from the MAL-ED study. J Infect Dis **2017**; 216:305–16.
9. Naylor C, Lu M, Haque R, et al; PROVIDE study teams. Environmental enteropathy, oral vaccine failure and growth faltering in infants in Bangladesh. EBioMedicine **2015**; 2:1759–66.

10. Angel J, Steele AD, Franco MA. Correlates of protection for rotavirus vaccines: Possible alternative trial endpoints, opportunities, and challenges. Hum Vaccin Immunother **2014**; 10:3659–71.

11. Lewnard JA, Lopman BA, Parashar UD, et al. Naturally acquired immunity against rotavirus infection and gastroenteritis in children: paired reanalyses of birth cohort studies. J Infect Dis **2017**; 216:317–26.

12. Jiang B, Gentsch JR, Glass RI. The role of serum antibodies in the protection against rotavirus disease: an overview. Clin Infect Dis **2002**; 34:1351–61.

13. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. J Infect Dis **2013**; 208:284–94.

14. Cheuvart B, Neuzil KM, Steele AD, et al. Association of serum anti-rotavirus immunoglobulin A antibody seropositivity and protection against severe rotavirus gastroenteritis: analysis of clinical trials of human rotavirus vaccine. Hum Vaccin Immunother **2014**; 10:505–11.

15. Baker JM, Tate JE, Leon J, Haber MJ, Pitzer VE, Lopman BA. Postvaccination serum antirotavirus immunoglobulin A as a correlate of protection against rotavirus gastroenteritis across settings. J Infect Dis **2020**; 222:309–18.

16. Lee B, Carmolli M, Dickson DM, et al. Rotavirus-specific immunoglobulin A responses are impaired and serve as a suboptimal correlate of protection among infants in Bangladesh. Clin Infect Dis **2018**; 67:186–92.

17. Grimwood K, Lund JC, Coulson BS, Hudson IL, Bishop RF, Barnes GL. Comparison of serum and mucosal antibody responses following severe acute rotavirus gastroenteritis in young children. J Clin Microbiol **1988**; 26:732–8.

18. Clarke E, Desselberger U. Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings. Mucosal Immunol **2015**; 8:1–17.

19. 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.

20. Bar-Zeev N, Jere KC, Bennett A, et al; Vaccine Effectiveness and Disease Surveillance Programme, Malawi (VACSURV) Consortium. Population impact and effectiveness of monovalent rotavirus vaccination in urban Malawian children 3 years after vaccine introduction: ecological and case-control analyses. Clin Infect Dis **2016**; 62(Suppl 2):S213–9.

21. Bennett A, Pollock L, Jere KC, et al. Duration and density of fecal rotavirus shedding in vaccinated Malawian children with rotavirus gastroenteritis. J Infect Dis **2020**; 222:2035–40.

22. Givon-Lavi N, Greenberg D, Dagan R. Comparison between two severity scoring scales commonly used in the evaluation of rotavirus gastroenteritis in children. Vaccine **2008**; 26:5798–801.

23. World Health Organization. WHO child growth standards. Geneva, Switzerland: WHO Press, **2006**.

24. World Health Organization and UNICEF. WHO child growth standards and the identification of severe acute malnutrition in infants and children. Geneva, Switzerland: WHO Press, **2009**.

25. World Health Organization. The treatment of diarrhoea: a manual for physicians and other senior health workers. 4th rev ed. Geneva: WHO Press, **2005**.

26. World Health Organisation. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser **1995**; 854:1–452.

27. Hjelt K, Grauballe PC, Schiøtz PO, Andersen L, Krasilnikoff PA. Intestinal and serum immune response to a naturally acquired rotavirus gastroenteritis in children. J Pediatr Gastroenterol Nutr **1985**; 4:60–6.

28. Iturriza-Gomara M, Green J, Brown DW, Desselberger U, Gray JJ. Comparison of specific and random priming in the reverse transcriptase polymerase chain reaction for genotyping group A rotaviruses. J Virol Methods **1999**; 78:93–103.

29. European Rotavirus Surveillance Network (EuroRotaNet). Available at: www.eurorotanet.com. Accessed 15 July 2020.

30. Paul A, Gladstone BP, Mukhopadhya I, Kang G. Rotavirus infections in a community based cohort in Vellore, India. Vaccine **2014**; 32(Suppl 1):A49–54.

31. Paul A, Babji S, Sowmyanarayanan TV, et al. Human and bovine rotavirus strain antigens for evaluation of immunogenicity in a randomized, double-blind, placebo-controlled trial of a single dose live attenuated tetravalent, bovine-human-reassortant, oral rotavirus vaccine in Indian adults. Vaccine **2014**; 32:3094–100.

32. Bernstein DI, Sack DA, Rothstein E, et al. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. Lancet **1999**; 354:287–90.

33. Nordgren J, Nitiema LW, Ouermi D, Simpore J, Svensson L. Host genetic factors affect susceptibility to norovirus infections in Burkina Faso. PLoS One **2013**; 8:e69557.

34. Youden WJ. Index for rating diagnostic tests. Cancer **1950**; 3:32–5.

35. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biom J **2005**; 47:458–72.

36. Cunliffe NA, Gondwe JS, Kirkwood CD, et al. Effect of concomitant HIV infection on presentation and outcome of rotavirus gastroenteritis in Malawian children. Lancet **2001**; 358:550–5.

37. Haidara FC, Tapia MD, Sow SO, et al. Evaluation of a booster dose of pentavalent rotavirus vaccine coadministered with measles, yellow fever, and meningitis a vaccines in 9-month-old Malian infants. J Infect Dis **2018**; 218:606–13.

38. Burnett E, Lopman BA, Parashar UD. Potential for a booster dose of rotavirus vaccine to further reduce diarrhea mortality. Vaccine **2017**; 35:7198–203.

39. Vesikari T, Karvonen A, Korhonen T, et al. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine in adults, toddlers and previously uninfected infants. Vaccine **2004**; 22:2836–42.

40. Dennehy PH, Brady RC, Halperin SA, et al; North American Human Rotavirus Vaccine Study Group. Comparative evaluation of safety and immunogenicity of two dosages of an oral live attenuated human rotavirus vaccine. Pediatr Infect Dis J **2005**; 24:481–8.

41. Bhandari N, Sharma P, Taneja S, et al; Rotavirus Vaccine Development Group. A dose-escalation safety and immunogenicity study of live attenuated oral rotavirus vaccine 116E in infants: a randomized, double-blind, placebo-controlled trial. J Infect Dis **2009**; 200:421–9.

42. Pitzer VE, Bennett A, Bar-Zeev N, et al. Evaluating strategies to improve rotavirus vaccine impact during the second year of life in Malawi. Sci Transl Med **2019**; 11:eaav6419.