Association of serum antibodies with protection against rotavirus infection and disease in South Indian children

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Abstract

Serum antibodies play an important role in natural protection from rotavirus infection and disease, but conflicting estimates of association have emerged from epidemiological studies in different geographical settings. In this study, we aim to assess the relationship between pre-existing serum immunoglobulin (Ig)G and IgA titers with protection against rotavirus infection and disease in a birth cohort of Indian children. Children were recruited at birth and followed up for 36 months. Stool samples were collected every 2 weeks and during episodes of diarrhea and serum samples were obtained at least every 6 months. The incidence rate of rotavirus infection and diarrhea was 0.9 (95% CI: 0.88, 0.99) and 0.2 (95% CI: 0.19, 0.25) episodes per child year, respectively. The risk of rotavirus infection and diarrhea decreased with age, while antibody titers (IgG and IgA) increased with age. After adjusting for age and number of previous infections, higher levels of IgG and IgA were independently associated with reduced risk of rotavirus infection. However, we did not find a clear association of IgG or IgA with rotavirus diarrhea risk or a threshold level of protection. The study supports a correlation of serum antibodies in reducing the risk of rotavirus infections, however the potential of serum antibody titer as a correlate of protection is not clear for children in lower income settings.

Keywords

Antibody; Immunity; Protection; Rotavirus; Diarrhea

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Conflict of interest statement
None declared.
1. Introduction

Infection with both natural rotavirus and oral rotavirus vaccines stimulates production of IgM, IgA and IgG antibodies. While it is thought that secretory IgA production in the small intestine is directly involved with protection against infection and disease, infection also stimulates humoral responses that may correlate with protection. Both animal models and studies in humans have indicated that serum antibodies have a relationship with protection against rotavirus infections and disease [1]. An apparent relation of serum IgA response with protection against disease has served as a tool in the clinical development of live oral vaccines for rotavirus [2]. Currently, two live oral rotavirus vaccines (Rotarix [RV1] GSK biologicals and Rotateq [RV5] Merck & Co.) have been successfully evaluated in large-scale clinical trials, are licensed in over 100 countries and are in routine use in over 40 countries [3,4]. However, both vaccine protection and immunogenicity is considerably reduced in middle and lower income countries compared to high income settings [5–7].

Past studies have attempted to identify how serum antibodies protect against natural rotavirus infections. However, findings from these studies have been inconsistent and mechanisms or effectors of protection from natural infection remain unclear. Studies have found reduced risk was associated with serum IgA and not IgG [8], with IgG and not IgA [9] while others did not find association with serum antibodies at all [10]. Further, it is unclear whether serum antibodies are directly involved in protection or merely reflect a recent infection. This incomplete understanding of the correlates of protection from rotavirus infection and disease remains a critical limiting factor in understanding differences in rotavirus vaccine effectiveness. Moreover, identification of a suitable correlate of protection would facilitate evaluation of new vaccines or new vaccine strategies (e.g. alternative schedules) by reducing the need for large scale trials with disease endpoints. This is particularly important as many candidate rotavirus vaccines are currently in development and testing of efficacy of these vaccines in a placebo controlled trial may raise ethical and logistical concerns given the availability of licensed vaccines in many countries.

In this study, we aim to assess the association between pre-existing serum antibody levels and subsequent rotavirus infection and disease risk in a birth cohort from an impoverished community in south India, where natural rotavirus infection has been shown to confer less protection than in higher income settings [11,12].

2. Materials and methods

2.1. Study design

The study was conducted in the urban slums of Vellore, as described elsewhere [13]. Briefly, a cohort of 452 newborns was recruited at birth from March 2002 to August 2003. The children were followed until 36 months of age. Written, informed consent was obtained from a parent of each child before enrollment. Stool samples were collected during all diarrheal episodes. Surveillance stool samples were collected once in two weeks. A blood sample was collected from the mother at time of child birth. For all children in the cohort, a blood sample was collected at the time of birth (cord blood) or within the first week and at least every six months for three years.
2.2. Definitions

An ‘episode of diarrhea’ was defined as three or more watery stools in 24 h or, in breastfed children, an increased number of daily stools considered to be diarrhea by the mother. The day following the return of the child’s bowel movements to normal marked the end of a diarrheal episode. Two episodes of diarrhea were separated by an interval of at least 48 h of normal bowel movements. A ‘rotavirus infection’ was defined as the detection of virus in stool or a fourfold increase in anti-rotavirus IgG or a three-fold change in IgA levels in sequential sera. An infection was considered asymptomatic if a child did not have diarrhea for the week prior to or following detection of rotavirus in stool, or if a child seroconverted with no diarrhea between the two serum sample collections. An infection was defined as symptomatic when rotavirus was identified in stool during the week preceding or following the diarrheal episode.

2.3. Laboratory methods

2.3.1. Detection and characterization of rotavirus in stool—All surveillance and diarrheal stool samples were screened for rotavirus antigen by enzyme linked immunosorbent assay (ELISA, Rotavirus IDEIA, UK). All rotavirus positive surveillance stool samples were retested with the use of same ELISA to improve specificity. Surveillance samples that were positive by both ELISA were genotyped by means of reverse transcription polymerase chain reaction (RT-PCR). All diarrheal stool samples were screened by means of ELISA and tested by RT-PCR assay even if the screening ELISA was negative. A stool sample was considered positive when positive either by two ELISA tests or by RT-PCR (Fig. 1).

2.3.2. Testing for anti-rotavirus IgA and IgG in serum—For each child, the serum specimens obtained at birth and at 6-month intervals thereafter were analyzed for antirotavirus IgA and IgG antibodies by means of an antibody-sandwich enzyme immunoassay. The IgA or IgG titer was determined by comparing the optical density values from sample wells with a standard curve based on pooled human serum samples.

2.4. Data analysis

The two primary outcomes of interest were rotavirus infection and rotavirus diarrhea. We first conducted exploratory data analysis to determine the nature of the relationship between rotavirus infection/diarrhea rates with age, number of previous infections and antibody titers. Antibody titers were log transformed and geometric mean titers (GMTs) were calculated by adding one to each antibody value in order to derive logs for zero values.

As a measure of protection, we estimated the effect that the preexisting antibody levels had on the subsequent infection rates. We restricted our analysis only to the first episode, whenever there were multiple rotavirus infections between two sero-surveys. These episodes and time between infection to the next survey were excluded, since we only had a measure of serum antibodies at the time of each serosurvey and could not account for responses resulting from infections between sero-surveys.
We then used multivariable Poisson regressions to model the risk of rotavirus infection or disease as a function of pre-existing antibody levels. Serum antibody titers for IgG and IgA were categorized into deciles to examine whether a dose–response relationship exists between antibody titers and protection against rotavirus infection or disease. The antibody level was included as a categorical variable in the model and lowest decile was chosen as the reference category. We adjusted for age (in 6-month age intervals) and the number of previous infections, as both were strong predictors of rotavirus infection and disease. To account for the correlation among multiple responses from each child, a generalized estimating equations approach with an exchangeable correlation structure was used. Similar models were fitted to explore the association of serum antibody titers according to severity of rotavirus diarrhea. However, the models utilizing mild to moderate and moderate to severe diarrhea as outcome variables did not converge, so are not presented.

3. Results

The study sample included 373 children who completed the longitudinal follow up for 36 months. During the study period, we observed a total of 1103 rotavirus infections and 282 rotavirus diarrheal episodes based on serum and stool examination. Here, we restricted the present analysis to 1016 infections that were the first episodes in each sero-survey period. Of which, 370 (36%) were primary and 646 (64%) were subsequent infections. The total number of symptomatic infections and asymptomatic infections were 237 (23%) and 779 (77%) respectively. The overall incidence of rotavirus infection was 0.9 episodes/child-year (95% CI: 0.88, 0.99); the incidence of rotavirus associated diarrhea was 0.2 episodes/child-year (95% CI: 0.19, 0.25).

When stratified by age groups, the risk of rotavirus infection decreased from 9.3 episodes per 100 child-months in 0–5-month olds to 6.5 episodes per 100 child-months among 24–29-month olds. Children 0–5 months had the highest incidence for rotavirus associated diarrhea: 3.4 episode/100 child-months. Diarrheal incidence decreased thereafter (Fig. 2).

Serum anti-rotavirus IgG and IgA antibody titers increased with age (Fig. 3). The geometric mean titers for IgG and IgA at birth were 1231 and five, respectively. The high initial mean IgG levels were assumed to reflect maternal antibodies; after these waned (by 6 months) mean levels of IgG increased steadily with the age of the child. Mean IgA levels increased progressively with age. Fig. 4 shows the antibody responses according to the total number of previous infections. Geometric mean antibody levels for IgG and IgA tend to increase with an increasing number of previous infections.

There was a strong association between pre-existing levels of IgG and the subsequent rotavirus infection (Table 1). After controlling for age and number of previous infections, the rate of rotavirus infection decreased progressively with increasing levels of IgG (as categorized by deciles). The rate of rotavirus infection was reduced by 72% (95% CI: 58–81%) among children with IgG values > 20,818 compared with those with values ≤100. For IgA, we also found a progressive decrease in the rate of rotavirus infections, with increasing antibody titers up to the 10th decile (IgA > 619). Perhaps counter intuitively, despite the
rates of rotavirus infection decreasing with age, the IRR increased with age after controlling for previous infections and IgA/IgG.

However, there were no significant relationships between IgA and IgG antibody titers with rotavirus disease. The rate of rotavirus disease decreased steeply up to an IgG titer of 1164–1667 (5th decile), then increased to 1667–2838, after which little additional benefit was observed (Table 2). Likewise, a similar non-significant relationship was observed between IgA and rotavirus disease. In both the IgG and IgA the effect of age was not significant after controlling for previous infections and antibody titers.

The number of previous infections remained a significant inverse predictor of both infection and disease risk even after accounting for IgG and IgA levels in the multivariable models.

4. Discussion

We found that pre-existing serum levels of IgG and IgA antibodies were significantly associated with reduced risk for rotavirus infection, even after controlling for age and number of previous rotavirus infections. In particular, our results indicate a clear, significant dose–response relationship between serum antibody titers of both IgG and IgA with rotavirus infection. However, pre-existing antibody titers were not associated with a graded reduction in risk of rotavirus diarrhea. Further, we did not find a threshold level of antibody titers that clearly represented protection against rotavirus infection or disease.

Several studies have evaluated the degree of protection from pre-existing serum antibody titers against rotavirus infection and disease [1,2,9,10,12,14–16]. A study among Danish children with rotavirus gastroenteritis or asymptomatic infection found that pre-existing levels of IgA, but not IgG correlate with protection [8]. In contrast, IgG titers correlated with protection against symptomatic rotavirus diarrhea in children from Bangladesh [9]. A seminal study from Mexico [12] indicated that serum IgA could be a marker of protection against rotavirus infection and moderate to severe diarrhea. This study used a similar longitudinal approach to ours, to account for effects of previous exposure to rotavirus infections and to provide information on age of acquisition of antibodies. They reported serum IgG titers of >6400 and IgA titers of >800 correlated with lower risk of rotavirus infection and complete protection against moderate to severe diarrhea, though these titers were substantially higher than those found in a US study (IgG titers of >800 and IgA titers of >200) [14]. In our present study, we did not find evidence for a threshold level for IgG and IgA titers that associated with protection; there were still children infected with elevated levels (up to the 10th decile: IgG > 20,818, IgA > 619) of antibody titers. Though we observed gradual reduction in risk with increasing antibody titers, the magnitude of protection is lower in our study. One possible explanation for this finding could be that early infections and frequent reinfections result in a less robust immune response, that confers some degree of protection against infection, but no clear relationship with disease [11]. We previously reported protection from prior infections was not complete in this cohort and was lower than was shown in Mexico [17] and Guinea Bissau [18].
An interesting finding in our study is that previous infections still induced some level of protection, after taking antibody levels into account in multivariable models. Serum antibodies are presumed to reflect responses to previous infections and therefore, we expected that the variable indicating number of previous infections would ‘work through’ antibody titers, which we expected to be more proximally related to infection and disease. However, our results indicate the previous infections might be important in their own right and that serum antibody titers in themselves may not be an ideal indicator of protection. A number of previous studies have assessed the influence of pre-existing antibody levels on rotavirus infection/diarrhea, however these studies have not assessed or reported how age and previous infections affect protection, once antibody titers are taken into account in this analysis. Secretory IgA in the intestine may be required to prevent infection or attenuate severity [19]. Secretory IgA generally correlates with serum IgA [20], but in tropical settings where infection with various pathogens is frequent, IgA levels in the gut may be more transient. In vaccine trial settings where IgA titers were low (GMT < 90 U/ml) vaccine efficacy tends to drop considerably in the second year of life [2].

A limitation of our study is that we are unable to directly measure the effects of antibody titers according to the severity of diarrhea (moderate to severe and mild to moderate). Because of the relatively small number of severe disease episodes, parameter estimates from our regression models did not converge. A second limitation of the study was relatively long intervals between serological sampling (approximately 6 months) which might have caused us to miss some infections. Further, we assumed that the first episode between two serosurveys would better reflect on pre-existing antibody levels. We think this assumption is realistic as antibody levels may vary after the initial rotavirus infection or diarrhea and associating with a level that does not account for infections during the intermittent period might lead to inaccurate estimates.

In conclusion, we found that serum antibody levels are associated with reduced risk for subsequent rotavirus infection in infants in south India. The results suggest no clear threshold for antibody levels that correlated with protection of either infection or disease. The lack of a clear correlate in this population is at odds with a similar study in Mexico (which found such a threshold) but may be consistent with other observation of reduced protection from both natural rotavirus exposure and oral vaccination in low socio-economic settings compared to middle and high income settings.

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Fig. 1.
Flow diagram of algorithm for testing surveillance and diarrhea stool samples for rotavirus (shaded boxes represent specimens considered rotavirus positive).
Fig. 2.
Incidence (per 100-child months) of rotavirus infection and diarrhea, by age.
Fig. 3.
Serum antibody titers (GMTs-antilog of mean of the log-titer transformations, 95% CI) according to age 1 (horizontal line in the middle indicates the GMTs and the hollow box represents the 95% CI).

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Fig. 4.
Serum antibody titers (GMTs-antilog of mean of the log-titer transformations, 95% CI) according to the number of previous infections (*horizontal line in the middle indicates the GMTs and the hollow box represents the 95% CI*).
Table 1
Multivariable Poisson regression results of antibody levels (IgG and IgA) to rotavirus (RV) infections.

| Variables         | RV infection rate [95% CI]$^a$ | IgG  | P values$^c$ | RV infection rate [95% CI]$^a$ | IgA  | P values$^c$ |
|-------------------|-------------------------------|------|--------------|-------------------------------|------|--------------|
|                   | IRR$^b$ | 95% CI       |      |                  | IRR$^b$ | 95% CI       |      |
| Age (months)      |                                |      |              |                                |      |              |
| 0–5               | 9.3 [8.1–10.6]                 | 1    | [Ref]        | 1.26 [1.03, 1.56]              | 0.025|
| 6–11              | 8.7 [7.6–10.1]                 | 1.04 | [0.82, 1.30] | 0.77                          |      |
| 12–17             | 8.0 [6.9–9.3]                  | 1.35 | [1.06, 1.74] | 0.02                          |      |
| 18–23             | 6.7 [5.7–7.8]                  | 1.43 | [1.09, 1.87] | 0.01                          |      |
| 24–29             | 6.5 [5.5–7.6]                  | 1.70 | [1.28, 2.25] | <0.001                        |      |
| 30+               | 7.3 [6.3–8.6]                  | 2.24 | [1.68, 3.01] | <0.001                        |      |
| Previous infections|                                |      |              |                                |      |              |
| 0                 | 13.2 [11.9–14.6]               | 1    | [Ref]        | 1.26 [1.03, 1.56]              | 0.025|
| 1                 | 7.9 [7.1–8.7]                  | 0.48 | [0.40, 0.57] | <0.001                        |      |
| 2                 | 5.6 [4.9–6.4]                  | 0.32 | [0.25, 0.41] | <0.001                        |      |
| 3+                | 4.3 [3.6–5.4]                  | 0.22 | [0.16, 0.31] | <0.001                        |      |
| Deciles: IgG      |                                |      |              |                                |      |              |
| 1 (<100)          | 13.2 [11.3–15.7]               | 1    | [Ref]        | 13.4 [12.2–14.8]              | 1    | [Ref] |
| 2 (100–367)       | 11.2 [9.4–13.3]                | 0.99 | [0.77, 1.27] | 0.92                          |      |
| 3 (368–767)       | 10.5 [8.8–12.5]                | 0.97 | [0.75, 1.24] | 0.80                          |      |
| 4 (768–1164)      | 8.8 [7.3–10.7]                 | 0.88 | [0.67, 1.16] | 0.36                          | 1–18 | 9.3 [7.7–11.3] | 0.82 | [0.65, 1.06] | 0.127     |
| 5 (1165–1667)     | 7.9 [6.5–9.6]                  | 0.74 | [0.56, 0.99] | 0.04                          | 19–36| 7.4 [6.1–9.1] | 0.66 | [0.50, 0.85] | 0.001     |
| 6 (1668–2838)     | 6.7 [5.4–8.4]                  | 0.63 | [0.47, 0.84] | 0.002                         | 37–66| 6.4 [5.2–7.9] | 0.62 | [0.47, 0.80] | <0.001    |
| 7 (2839–5271)     | 8.3 [6.8–10.1]                 | 0.81 | [0.61, 1.07] | 0.13                          | 67–110| 6.1 [4.9–7.6]| 0.59 | [0.45, 0.78] | <0.001    |
| 8 (5272–9324)     | 5.9 [4.7–7.4]                  | 0.57 | [0.42, 0.78] | <0.001                        | 111–219| 5.0 [3.9–6.3]| 0.48 | [0.35, 0.64] | <0.001    |
| 9 (9325–20,817)   | 5.1 [4.0–6.5]                  | 0.52 | [0.37, 0.71] | <0.001                        | 220–618| 5.0 [4.0–6.3]| 0.46 | [0.35, 0.64] | <0.001    |
| 10 (>20,818)      | 2.6 [1.9–3.5]                  | 0.28 | [0.19, 0.42] | <0.001                        | >619 | 3.3 [2.5–4.3] | 0.32 | [0.23, 0.46] | <0.001    |

$^a$ Incidence rates (per 100-child months) in each category.

$^b$ Adjusted incidence rate ratios.

$^c$ P values were calculated using Wald test.
Table 2

Multivariable Poisson regression results of antibody levels (IgG and IgA) to rotavirus (RV) diarrhea.

| Variables       | RV diarrhea rate [95% CI] | IgG    | 95% CI | P values<sup>c</sup> | RV diarrhea Rate [95% CI] | IgA    | 95% CI | P-values<sup>c</sup> |
|-----------------|---------------------------|--------|--------|----------------------|---------------------------|--------|--------|--------------------|
| **Age (months)**|                           |        |        |                      |                           |        |        |                    |
| 0–5             | 3.40 [2.8–4.3]             | 1      | Ref    |                      | 1.39 [0.97, 1.98]         | 0.067  |        |                    |
| 6–11            | 3.20 [2.5–4.0]             | 1.41   | [0.93, 2.17] | 0.105 | 1.13 [0.72, 1.79]         | 0.580  |        |                    |
| 12–17           | 1.60 [1.2–2.2]             | 1.12   | [0.66, 1.87] | 0.664 | 0.97 [0.56, 1.68]         | 0.916  |        |                    |
| 18–23           | 1.00 [0.7–1.5]             | 0.87   | [0.47, 1.60] | 0.648 | 1.28 [0.69, 2.31]         | 0.440  |        |                    |
| 24–29           | 0.90 [0.6–1.5]             | 1.18   | [0.62, 2.23] | 0.623 | 0.77 [0.34, 1.74]         | 0.531  |        |                    |
| 30+             | 0.50 [0.2–0.9]             | 0.71   | [0.30, 1.69] | 0.442 |                      |        |        |                    |
| **Previous infections** |                   |        |        |                      |                           |        |        |                    |
| 0               | 4.3 [3.6–5.1]              | 1      | Ref    |                      | 1.39 [0.97, 1.98]         | 0.067  |        |                    |
| 1               | 1.9 [1.5–2.3]              | 0.43   | [0.29, 0.61] | <0.001 | 0.41 [0.27, 0.63]         | <0.013 |        |                    |
| 2               | 0.6 [0.4–1.0]              | 0.15   | [0.08, 0.27] | <0.001 | 0.13 [0.06, 0.25]         | <0.001 |        |                    |
| 3+              | 0.6 [0.3–1.0]              | 0.10   | [0.04, 0.27] | <0.001 | 0.09 [0.04, 0.25]         | <0.001 |        |                    |
| **Deciles: IgG** |                   |        |        |                      |                           |        |        |                    |
| 1 (<100)        | 3.3 [2.3–4.5]              | 1      | Ref    |                      |                      |        |        |                    |
| 2 (100–367)     | 2.6 [1.9–3.8]              | 0.88   | [0.52, 1.46] | 0.614 |                      |        |        |                    |
| 3 (368–767)     | 1.8 [1.2–2.7]              | 0.65   | [0.36, 1.17] | 0.156 |                      |        |        |                    |
| 4 (768–1164)    | 2.0 [1.4–2.9]              | 0.84   | [0.46, 1.51] | 0.556 | 1–18 | 2.3 [1.6–3.4] | 1.25 [0.75, 2.07] | 0.376  |
| 5 (1165–1667)   | 1.4 [1.0–2.3]              | 0.54   | [0.27, 1.04] | 0.067 | 19–36 | 1.4 [0.9–2.2] | 0.72 [0.40, 1.29] | 0.281  |
| 6 (1668–2838)   | 1.6 [1.1–2.5]              | 0.73   | [0.40, 1.32] | 0.303 | 37–66 | 1.0 [0.6–1.7] | 0.73 [0.38, 1.39] | 0.350  |
| 7 (2839–5271)   | 2.1 [1.4–3.1]              | 0.94   | [0.52, 1.67] | 0.823 | 67–110 | 1.1 [0.6–1.8] | 0.86 [0.44, 1.63] | 0.648  |
| 8 (5272–9324)   | 1.5 [0.9–2.3]              | 0.73   | [0.39, 1.39] | 0.345 | 111-219 | 0.8 [0.4–1.4] | 0.62 [0.30, 1.27] | 0.196  |
| 9 (9325–20,817) | 1.6 [1.1–2.5]              | 0.97   | [0.52, 1.78] | 0.927 | 220–618 | 0.8 [0.4–1.4] | 0.62 [0.29, 1.26] | 0.193  |
| 10 (>20,818)    | 0.5 [0.3–1.0]              | 0.40   | [0.17, 0.97] | 0.045 | >619 | 1.1 [0.7–1.8] | 0.60 [0.30, 2.13] | 0.539  |

<sup>a</sup> Incidence rates (per 100-child months) in each category.

<sup>b</sup> Adjusted incidence rate ratios.

<sup>c</sup> P values were calculated using Wald test.