Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Gladstone BP, Ramani S, Mukhopadhya I, et al. Protective effect of natural rotavirus infection in an Indian birth cohort. N Engl J Med 2011;365:337-46.

Additional tables for supplementary information

Supplementary Table 1: Comparison of three birth cohorts evaluating rotavirus infection

	Mexico[7]	Guinea-Bissau[8]	Vellore, India
No. of children recruited and completing follow up	200 (77% follow up)	200 (49% - 102 lost to follow up before completion of 2 years)	452 (373 with 99.5% follow up)
Frequency of visits and stool	1/week, 1/week + diarrhea	1/week, 1/week	2/week, 1 in 2 weeks + diarrhea
Infections identified	316, 57% stool and 77% serology	116, all stool	1103, 48% stool and 76% serology
Order of infection	52% first infections, 48% subsequent	81% first infections, 19% subsequent	30% first infections, 70% subsequent
Time to infection	34% infected by 6 mo	26% infected by 6 mo	53% infected by 6 mo
Symptoms in 1st infection	47%	44%	30%
Severity	No moderate to severe diarrhea after two infections	No severity data	22.4% of 3rd or later rotavirus diarrhea were moderate or severe
Protection from primary infection against subsequent	38%, 77%	52%, 70%	39%, 43%

infection, diarrhea			
Protection against severe diarrhea	100% after two infections	75% against all diarrhea in the same season after one infection	79% after three infections

Supplementary Table 2: Comparison of children who had less than five and five or more rotavirus infections from the cohort of 373 children who completed 3 years of follow up

Characteristics of children	Less than 5 rotavirus infections (n=331)	Five or more rotavirus infections (n=42)	P-value
Religion			
Hindu	151	25	
Muslim	164	16	
Christian	16	1	
Type of family			
Joint	66	7	0.359
Extended	91	16	
Nuclear	174	19	
Number of household members			
≤5	219	28	0.321
>5	112	14	
Socio-economic status			
Class I	201	19	0.456
Class II	130	23	
Bidi-working household			
Yes	154	19	0.875
No	177	23	
Birth weight			
Missing	7	1	
<2.5 kg	39	4	0.802

≥2.5 kg	285	37	
Number of siblings			
0	106	12	0.65
≥1	225	30	
Mean (SD) number of illnesses experienced in three years			
All morbidity	32.6 (14.3)	43.4 (14.2)	<0.001
GI illness	5.8 (4.1)	9.5 (5.5)	<0.001
Respiratory illness	19.6 (8.7)	24.6 (7.5)	<0.001
Nutritional status at 12, 24 and 36 months			
Malnourished at all time points	154	20	0.894
Ever malnourished	269	34	0.961
Wasted at all time points	7	0	
Ever wasted	67	10	0.59
Stunted at all time points	154	20	0.894
Ever stunted	263	32	0.624
Underweight at all time points	80	9	0.695
Underweight at any time point	167	255	0.268

Supplementary Table 3: Protection from natural rotavirus infection against subsequent rotavirus infection and diarrhea in a birth cohort of 417 children who completed at least three months of a planned three year follow up

Outcome and no. of previous infections	No. of episodes	Incidence per 100 child months †	Unadjusted relative risk (95% CI*)	Adjusted relative risk‡ (95% CI*)	Adjusted efficacy§ (95% CI) %
Any infection¶					
0	407	14.26			
1	350	8.35	0.59 (0.51 – 0.68)	0.59 (0.51 - 0.68)	31 (32 – 49)
2	242	6.72	0.47 (0.40 – 0.55)	0.47 (0.40 - 0.55)	53 (45 – 60)
3	102	4.67	0.33 (0.26 – 0.41)	0.32 (0.26 - 0.40)	68 (60 – 74)
Any diarrhea					
0	120	4.21			
1	99	2.36	0.56 (0.43 – 0.73)	0.56 (0.43 - 0.73)	44 (27 -57)
2	44	1.22	0.29 (0.21 – 0.41)	0.28 (0.20 - 0.40)	72 (60 – 80)
3	19	0.87	0.21 (0.13 – 0.34)	0.19 (0.12 - 0.31)	81 (69 -88)
Moderate to severe diarrhea					
0	19	0.67			
1	21	0.5	0.75 (0.41 – 1.40)	0.75 (0.40 - 1.39)	25 (-39 – 60)
2	10	0.28	0.42 (0.19 – 0.90)	0.40 (0.19 - 0.86)	60 (14 - 81)
3	3	0.14	0.21 (0.06 – 0.70)	0.19 (0.06 - 0.65)	81 (35 -94)
Mild diarrhea					

0	89	3.12			
1	74	1.77	0.57 (0.42 – 0.77)	0.56 (0.41 – 0.77)	44 (23 – 59)
2	33	0.92	0.29 (0.20 – 0.44)	0.28 (0.19 - 0.42)	72 (58 – 81)
3	16	0.73	0.24 (0.14 – 0.40)	0.22 (0.13 - 0.37)	78 (63 – 87)
Unknown status					
0	114	4			
1	69	1.65	0.41 (0.31 – 0.56)	0.41 (0.31 – 0.56)	59 (44 – 69)
2	53	1.47	0.37 (0.27 – 0.51)	0.37 (0.26 – 0.51)	63 (49 – 74)
3	14	0.64	0.16 (0.09 – 0.28)	0.16 (0.09 – 0.27)	84 (73 – 91)
Asymptomatic infections					
0	173	6.06			
1	182	4.34	0.72 (0.58 – 0.88)	0.72 (0.58 – 0.88)	28 (12 - 42)
2	145	4.03	0.66 (0.53 – 0.83)	0.66 (0.53 – 0.82)	34 (18 – 47)
3	69	3.16	0.52 (0.39 – 0.69)	0.51 (0.39 – 0.68)	49 (32 – 61)

*CI denotes confidence interval.

†The group with no previous infections was monitored for 2853 child-months; the group with one previous infection for 4191 child-months; the group with two previous infections for 3601 child-months; and the group with three previous infections for 2186 child-months.

‡The risk was adjusted for sex, hygiene status and involvement in bidi-work.

§Efficacy was calculated as the percent reduction in the risk of an outcome as compared with the risk for children who were not yet infected.

¶This category includes symptomatic infections, asymptomatic infections, and infections for which the symptom status was ‘unknown’.

Supplementary Table 4: Protection from natural rotavirus infection against subsequent rotavirus infection and diarrhea in a cohort of 373 children who completed three years of follow up, with rotavirus infections identified in stool by PCR alone

Outcome and no. of previous infections	No. of episodes	Incidence per 100 child months †	Unadjusted relative risk (95% CI**)	Adjusted relative risk‡ (95% CI**)	Adjusted efficacy§ (95% CI) %
Any infection¶					
0	365	13.45			
1	336	8.31	0.62 (0.53-0.72)	0.62 (0.53-0.72)	38 (28-47)
2	223	6.24	0.46 (0.39-0.55)	0.46 (0.39-0.54)	54 (46-61)
3	92	4.49	0.33 (0.27-0.42)	0.33 (0.26-0.41)	67 (59-74)
Any diarrhea					
0	112	4.09			
1	95	2.35	0.49 (0.37-0.66)	0.49 (0.37-0.66)	51 (34-63)
2	40	1.12	0.19 (0.13-0.27)	0.18 (0.13-0.27)	82 (73-87)
3	18	0.87	0.11 (0.06-0.18)	0.10 (0.06-0.18)	90 (82-94)
Moderate to severe diarrhea					
0	18	0.66			
1	21	0.52	0.77 (0.39-1.49)	0.75 (0.39-1.45)	25 (-45-61)
2	8	0.22	0.27 (0.11-0.65)	0.26 (0.11-0.63)	74 (37-89)
3	5	0.24	0.23 (0.08-0.69)	0.22 (0.08-0.66)	78 (34-92)
Mild diarrhea					
0	84	3.07			

1	70	1.73	0.49 (0.35-0.69)	0.49 (0.35-0.69)	51 (31-65)
2	31	0.87	0.21 (0.13-0.32)	0.20 (0.13-0.32)	80 (68-87)
3	13	0.63	0.12 (0.06-0.22)	0.12 (0.06-0.22)	88 (78-94)
Unknown status					
0	103	3.76			
1	70	1.73	0.46 (0.34-0.62)	0.46 (0.34-0.62)	54 (38-66)
2	52	1.45	0.38 (0.27-0.54)	0.38 (0.27-0.54)	62 (46-73)
3	13	0.63	0.17 (0.09-0.30)	0.16 (0.09-0.30)	84 (70-91)
Asymptomatic infections					
0	153	5.59			
1	171	4.23	0.76 (0.61-0.94)	0.76 (0.61-0.94)	24 (6-39)
2	131	3.67	0.66 (0.52-0.83)	0.65 (0.52-0.82)	35 (18-48)
3	61	2.98	0.53 (0.40-0.72)	0.53 (0.39-0.71)	47 (29-61)

**CI denotes confidence interval.

†The group with no previous infections was monitored for 2737 child-months; the group with one previous infection for 4043 child-months; the group with two previous infections for 3576 child-months; and the group with three previous infections for 2049 child-months.

‡The risk was adjusted for sex, hygiene status and involvement in bidi-work.

§Efficacy was calculated as the percent reduction in the risk of an outcome as compared with the risk for children who were not yet infected.

¶This category includes symptomatic infections, asymptomatic infections, and infections for which the symptom status was ‘unknown’.

Statistical analysis

The protective effect of rotavirus infection and of strain specific infection of the major genotypes against subsequent infection were studied, with protective efficacy calculated as 1 minus the adjusted relative risks for a specific outcome (infection, diarrhea, severe diarrhea) of 1, 2 or 3 infections versus no infection. The number of previous infections was included in these models as a dummy variable, with the reference group being the group with no previous infections. Shared gamma frailty survival models were used to obtain relative risks and confidence intervals adjusted for the repeat infections within a child. For each outcome, a parametric regression survival model[26] with exponential distribution was fitted, using the length of time from birth to the occurrence of the first infection and the interval between infections, including time-independent covariates. The child-months at risk for a first infection were counted from the first day of follow up until the child became infected. The number of child-months at risk for subsequent infections was defined by the interval between infections. The outcomes assessed for the first and subsequent infections were any rotavirus infection, asymptomatic infection, any rotavirus-associated diarrhea and mild, moderate or severe rotavirus-associated diarrhea. The incidence of each outcome was calculated as the number of episodes per 100 child-months at risk.

Adjustment for potential confounders

Our previously reported analysis for all morbidity had shown that male gender, low personal and household hygiene and bidi-work (the making of indigenous cigarettes) in the household were associated with increased risk of respiratory and gastrointestinal disease. In addition, higher order of birth ($p=0.07$) and lower maternal education ($p=0.04$) were associated with increased risk of diarrheal disease, but not duration of exclusive or any breastfeeding or size of household. The latter may be because of the high levels and very similar breastfeeding patterns and housing conditions in the slum areas where the study was conducted. Therefore,

these variables were evaluated as potential confounders in the adjusted model and gender, hygiene and bidi-work in the household were retained in the final model to calculate the adjusted relative risks and protective efficacy of prior rotavirus infections.

Parametric regression survival modeling

Survival models were fitted using the exponential distribution and the proportional hazards metric, yielding a model parameterization of:

$$\lambda_j = \exp(x_j\beta)$$

for x_j a vector of covariates and β a vector of regression coefficients.

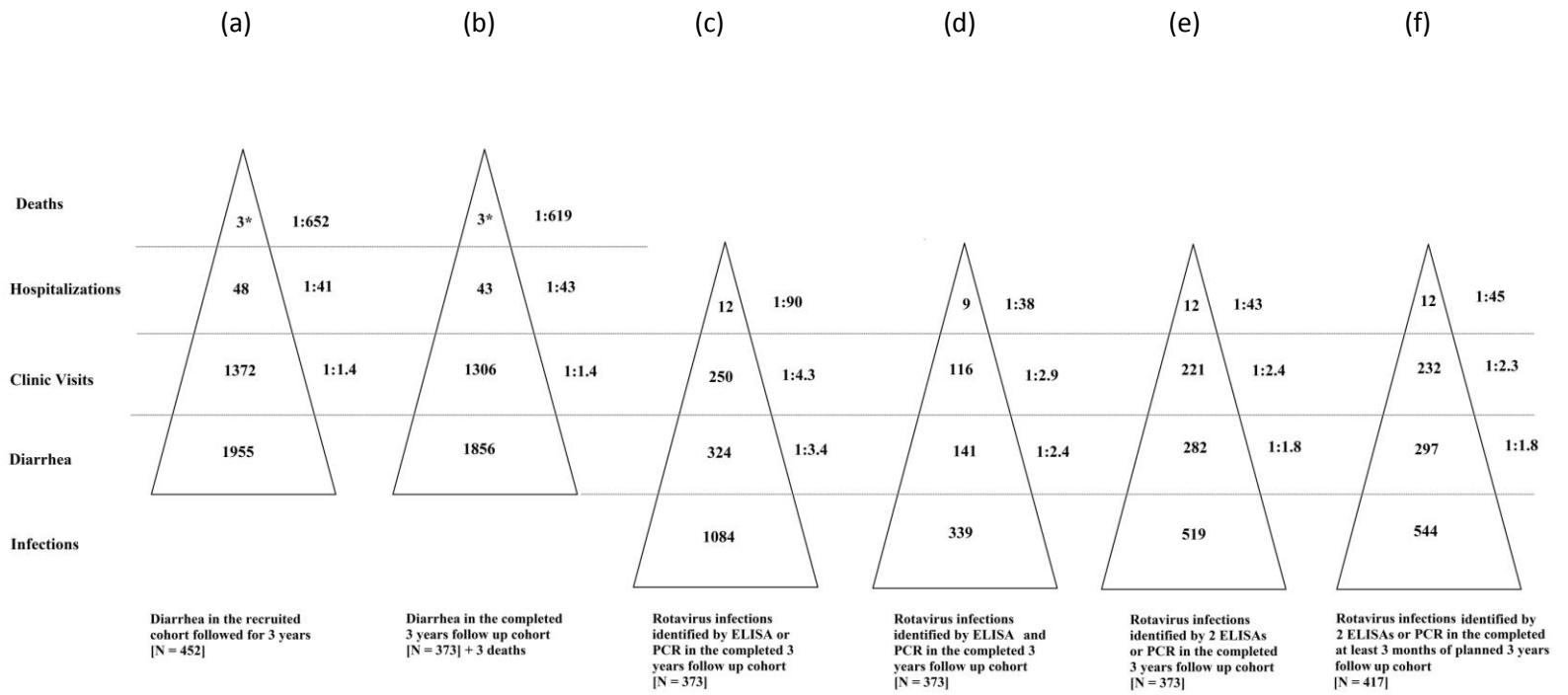
Gamma shared frailty was used to account for the repeat infections within a child. This model assumes a constant hazard over time.

Two contrasting models were used to investigate robustness to our assumption of a constant hazard. First, a Cox model with robust standard errors was fit; this model does not specify a functional form for the baseline hazard but the robust standard errors adjust standard errors to account for repeat infections within a child. Second, a proportional hazards Weibull model with gamma shared frailty was fit; this model allows the hazard to monotonically increase or decrease over time but includes shared frailty to account for repeat infections. While Weibull provided us with better log-likelihoods for a few outcomes, we chose to present the data using a single model (the Poisson model with gamma frailty) which was shown to be robust and consistent with our understanding of rotavirus infection and biology.

Figure legend for Supplementary figure

Figure 1: Incidence of diarrhea and rotavirus infections detected by stool testing alone in the birth cohort. Pyramids (a) and (b) represent the number of diarrheal episodes, clinic visits, hospitalizations and deaths due to diarrhea in the recruited cohort of 452 children and in the completely followed up cohort of 373 children, respectively. Pyramids (c), (d) and (e) provide a comparison of rotavirus infections and diarrhea when evaluated using three different definitions of detection of rotavirus in stool – (c) by ELISA or PCR positivity as the most sensitive definition, (d) ELISA and PCR as the most specific definition or (e) two ELISAs or PCR, which was used in this study. Figure 1 (f) shows the results of rotavirus infection and diarrhea when an additional 44 children who had at least three months of follow up and at least one serum sample collected are included.

These data do not include infections detected by serology. The ratios indicate the distributions of each category relative to the base of the pyramid. Two deaths which were not associated with diarrheal disease are not shown.


(a)
(b)
(c)
(d)
(e)
(f)
Deaths
Hospitalizations
Clinic Visits
Diarrhea
Infections
3*
1:652
48
1:41
1372
1:1.4
1955
3*
1:619
43
1:43
1306
1:1.4
1856
12
1:90
250
1:4.3
324
1:3.4
1084
9
1:38
116
1:2.9
141
1:2.4
339
12
1:43
221
1:2.4
282
1:1.8
519
12
1:45
232
1:2.3
297
1:1.8
544
Diarrhea in the recruited cohort followed for 3 years [N = 452]
Diarrhea in the completed 3 years follow up cohort [N = 373] + 3 deaths
Rotavirus infections identified by ELISA or PCR in the completed 3 years follow up cohort [N = 373]
Rotavirus infections identified by ELISA and PCR in the completed 3 years follow up cohort [N = 373]
Rotavirus infections identified by 2 ELISAs or PCR in the completed 3 years follow up cohort [N = 373]
Rotavirus infections identified by 2 ELISAs or PCR in the completed at least 3 months of planned 3 years follow up cohort [N = 417]