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Exposure to Human and Bovine Noroviruses in a Birth Cohort in Southern India from 2002 to 2006

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Human and bovine norovirus virus-like particles were used to evaluate antibodies in Indian children at ages 6 and 36 months and their mothers. Antibodies to genogroup II viruses were acquired early and were more prevalent than antibodies to genogroup I. Low levels of IgG antibodies against bovine noroviruses indicate possible zoonotic transmission.

Noroviruses (NoVs) are nonenveloped, single-stranded positive-sense RNA viruses, accounting for ~50% of gastroenteritis outbreaks worldwide (1). Seroepidemiological surveys using NoV virus-like particles (VLPs) as antigens show exposure to NoVs across the globe (2–7), with high seroprevalence in children <5 years of age; seroprevalence can reach 100% in adults (2, 8, 9). Analyses of bovine strains suggest close relation to human NoV genogroup I (GI) and GII.3 strains (10–14). Studies from the Netherlands found that 22% of the population had antibodies to bovine NoV, with veterinarians having high frequencies of antibodies (15), raising the possibility of zoonotic transmission (16). In this study, sera from children in a birth cohort and their mothers were used to assess exposure to human and bovine norovirus genogroups in early life and adulthood.

The study population was a birth cohort from semiurban slums in Vellore, South India, recruited and monitored from 2002 to 2006, with sample collection as previously described (17–19). Maternal sera at delivery and sera from children at 6 and 36 months were tested for IgG antibodies against human and bovine viruses. Diarrheal samples from calves were collected from a veterinary clinic and a commercial dairy farm in 2007 and 2008 (20). Written informed consent was obtained from parents of all children; the study was approved by the Institutional Review Board of the Christian Medical College, Vellore, India.

The NoV GIII and NB VLPs were obtained from Linda Saif, Ohio State University (21). Validation assays were carried out prior to use of bovine VLPs using 20 bovine sera from a veterinary clinic. Goat anti-bovine IgG-horseradish peroxidase (IgG-HRP; (Jackson ImmunoResearch Inc., United States) was added, followed by addition of 3,3′,5,5′-tetramethylbenzidine substrate solution. The reaction was stopped with 2 M sulfuric acid after 15 min, and optical density (OD) was measured at 450 nm.

Serum IgG was detected using plates coated overnight with 2 μg/ml of human and bovine VLPs in phosphate-buffered saline (PBS) at 4°C, and the plates were blocked using 10% skim milk in PBS. Diluted serum samples were added to uncoated and VLP-coated wells and incubated. Anti-human IgG (Southern Biotech, United States) was added, followed by goat anti-mouse IgG-HRP (human adsorbed; Southern Biotech) and the substrate 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate) solution; the reaction was stopped using 1% SDS solution, and OD was measured at 405 nm.

The assays for human and bovine VLPs differed in the standards and controls included on each plate. The GI and GII standard curves were 2-fold dilutions of positive human sera starting at a 1:100 dilution, and the GIII and Nebraska bovine (NB) viruses used 2-fold dilutions of purified human IgG (Sigma-Aldrich, United States) starting at a 2-μg/ml concentration. The mean ODs for standards, controls, and samples were calculated if the difference between replicates was an OD of <0.1. If the margin of error for the internal reference included on every plate was more than 15% from the expected value, the plates were rejected.

Viral RNA was extracted from stool samples available from children positive for serum antibodies against bovine NoVs, by the guanidium isothiocyanate-silica method (22). Bovine diarrheal samples were additionally subjected to CF11 purification (23). cDNA was generated by reverse transcription in the presence of hexamers (Pharmacia Biotech, United Kingdom). Primers specific to NoV GIII and NB were used (24), and amplification was detected on a 2% agarose gel.

Data were imported into GraphPad Prism, version 4.03. The serum used for the standard curve was assigned an arbitrary value of 0.25. The lowest serum IgG concentration that could be calculated from the linear portion of the standard curve was used as a cutoff. Net absorbance was calculated by subtracting the negative-well OD from the test well OD. The net absorbance for each of the VLPs was plotted by Spearman's rank correlation (r_s) with a 95% significance level to assess the possibility of IgG cross-reactivity by STATA 10.0 (STATA Corp., United States) (15).

Enzyme-linked immunosorbent assay (ELISA) validation of the bovine sera showed 90% (18/20) and 85% (17/20) positivity for NoV GIII and NB antibodies, respectively. Among 6- and 36-month-old children, seroconversion patterns suggest a lower level of exposure to GI than GII (Fig. 1; Table 1), and the geometric mean concentration (GMC) for NB was higher than for NoV GIII (Table 1).

Cross-reactivity between VLPs was checked using Spearman's rank correlations. NoV GIII was positively correlated with NoV

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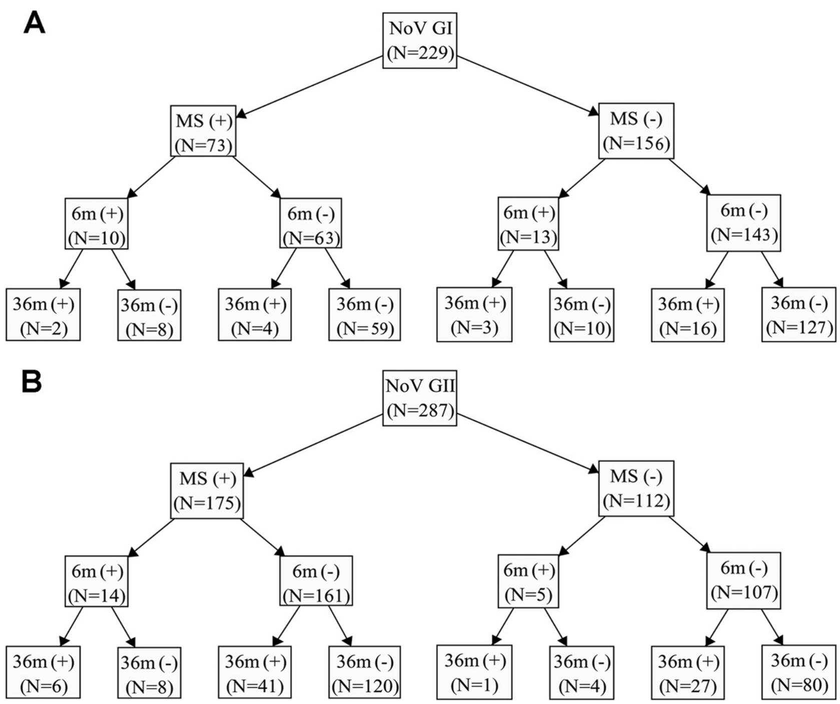

FIG 1 Seroconversion data of mothers (MS) and children at the ages of 6 and 36 months for NoV GI (A) and GII (B).

GI and NoV GII, indicating partial cross-reactivity. NB negatively correlated with NoV GI and NoV GII, indicating a greater specificity. Positive correlation between NoV GI and GII indicates partial cross-reactivity (Fig. 2). The GI and GII VLPs previously evaluated for specificity using the antisera prepared against the expressed VLPs showed that they were antigenically distinct (25). Studies have also shown the specificity of GIII and NB VLPs, with no cross-reactivity observed with GI and GII with the antiserum reagents produced (21, 26). Sequence comparison has shown a low level of amino acid sequence identity between human and bovine norovirus strains, suggesting that antigenically and genetically, the strains are distinct (27). But cross-reactivity cannot be completely ruled out, as limited antigenic cross-reactivity between NoVs of different genogroups may occur, as evidenced by the use of VLPs and antisera generated from panels of NoV genotypes of genogroups I and II (28).

TABLE 1 Prevalence and geometric mean concentration of IgG antibodies to VLPs of noroviruses GI, GII, and GIII and NB virus in serum samples obtained from children at 6 and 36 months of age and their mothers in a southern Indian urban community birth cohort study

Serum source	VLP	No. with IgG (% positive)	GMC	95% confidence interval
Mothers	NoV GI	73 (31.9)	25.43	21.79–29.68
	NoV GII	175 (61)	52.46	47.20–58.32
	NoV GIII	33 (10.7)	0.56	0.50–0.62
	NB	1 (0.4)	1.1	
6-mo-old children	NoV GI	22 (9.6)	9.55	8.20–11.20
	NoV GII	20 (7)	9.72	7.94–11.90
	NoV GIII	1 (0.3)	0.29	0.26–0.31
	NB	7 (2.6)	7.25	2.93–17.96
36-mo-old children	NoV GI	25 (10.9)	12.73	10.41–15.57
	NoV GII	77 (26.8)	32.29	27.06–37.53
	NoV GIII	24 (7.8)	0.41	0.38–0.45
	NB	12 (4.4)	2.95	1.20–4.37

Of the 249 animal samples screened, one was positive for NoV GIII. Sequence analysis by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed 81% identity to a GIII.1 bovine norovirus (Aba Z5/2002/HUN, EU360814.1). No samples were positive for NB virus. Of 99 stool samples from children screened for NoV GIII, 1 sample was positive by PCR but failed sequencing. No samples were positive for NB norovirus.

The analysis of sera from infants at 6 months, when maternal antibodies are expected to have waned, provides a baseline from which an increase can be used to demonstrate exposure to noroviruses in early life. The inclusion of mothers' sera allowed comparison with adults living in a similar environment. Antibodies to genogroup II are acquired rapidly in early life, with at least 20% of children seroconverting between 6 and 36 months, while rates of acquisition of antibodies are lower for genogroup I. The antibody acquisition rate is best documented for pediatric populations but varies among adults and countries (5, 7, 9, 29–32) (Table 2).

Among adults, the prevalence of antibody to bovine norovirus was lower (NoV GIII, 10.7%) than 20% and 26.7%, rates reported in Europe (15, 33). The low value of Spearman's rank correlation indicated a moderate cross-reactivity of antibodies against NoV GIII to NoV GI and GII, but this alone could not explain the observed seroreactivity to bovine VLPs. This is similar to other studies which show limited cross-reactivity between human and bovine NoV strains (14, 15, 21, 34). The differences in the prevalence rates of antibody to the VLPs tested likely indicate different levels of exposure to these viruses, with significant differences in

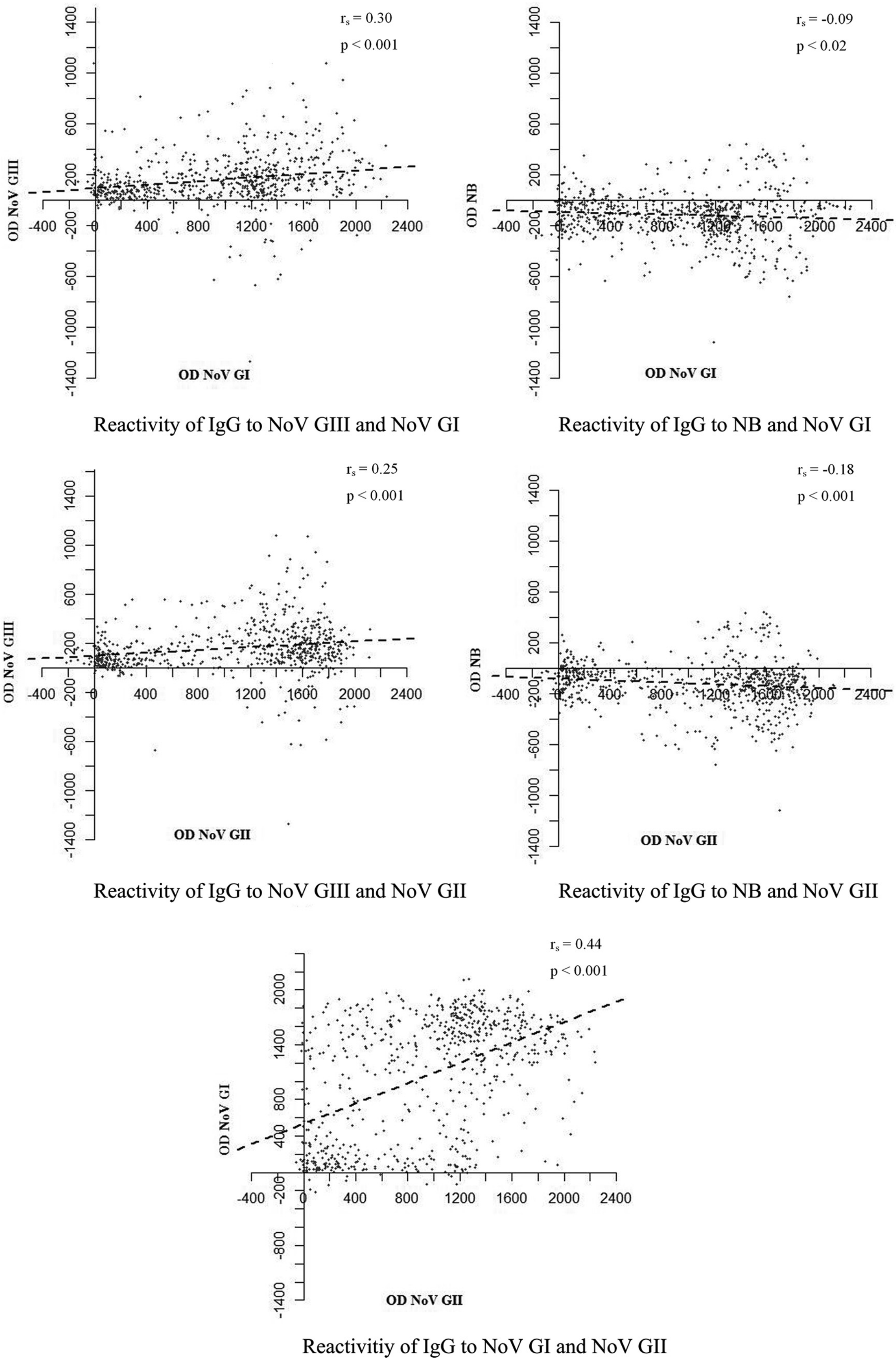

FIG 2 Scatter plot of the absorbance values at 405 nm for serum IgG reactivity against NoV GI, GII, GIII, and NB in the tested sera. Spearman correlation coefficient is calculated to check for cross-reactivity between the different norovirus genogroups.

TABLE 2 Seroprevalence to noroviruses GI and GII from studies in different geographic regions

Continent	Country or region	*n*	Age (yr)	M/F ratio[a]	% positive		Reference
					GI	GII	
North America	United States	308	0–>60		34		35
	United States	295	18–≥50	149:146	66.1		36
Europe	Belgium	133	20–≥50	80:53	76.6		36
	France	1,078	0–>70	475:603		74.1	37
	Finland	492	0–14			63.4	38
	Italy	1,729	0–95	799:930	28.7	91.2	31
	Switzerland	91	20–≥50	91:0	69.2		36
	United Kingdom	3,250	0–≥90	1,638:1,612	73.3		2
	Yugoslavia	76	18–≥50	38:38	53.9		36
Asia	Bangladesh	104	18–≥50		69.2		36
	China	588	17–24	298:290	88.9	90	7
	China	1,109	0–>60	577:532	89	91	32
	Ecuador	123	18–≥50	63:60	78		36
	Finland	492	0–14			63.4	38
	Indonesia	50	20–39	25:25	90		39
	Indonesia	50	20–37	25:25		88	30
	Japan	324	20–60	122:202		87	30
	Japan	400	1–>60		23.8	64.3	5
	Japan	380	20–>50	129:251	81		39
	Kuwait	414	0–>100		93.6	98	4
	Nepal	55	18–≥50	25:30	75		36
	Singapore	50	20–39	50:0	64		39
	Singapore	50	20–37	50:0		82	30
Australia	Papua New Guinea	50	20–49	18:32	100		39
	Papua New Guinea	50	20–49	18:32		86	30
Africa	South Africa	488	3–87	215:273	98	99	40
	South Africa	686	15–49	0:686	96	96	40
	South Africa and Southern Africa	2,358	0–>70		94.4	96.5	29

[a] M, male; F, female.

exposure between human genogroups and bovine NoVs. The prevalence of antibodies against the bovine noroviruses indicates either cross-reactivity or possible zoonotic transmission, but no direct evidence was found by screening of human and bovine stool samples. These studies broaden our understanding of NoV epidemiology and highlight the importance of sequential samples to determine exposure at the individual and population levels.

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