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The Etiological Roles of Astrovirus, Sapovirus, and Norovirus as a Cause of Diarrheal Episodes in a Birth Cohort of Indian Children: 2002 – 2006

By

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Epidemiology

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# The Etiological Roles of Astrovirus, Sapovirus, and Norovirus as a Cause of Diarrheal Episodes in a Birth Cohort of Indian Children: 2002 – 2006

By

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2020

## Abstract

The Etiological Roles of Astrovirus, Sapovirus, and Norovirus as a Cause of Diarrheal Episodes in a Birth Cohort of Indian Children: 2002 – 2006

By Megan Irving

**BACKGROUND**
Diarrheal diseases are a leading cause of global under-five mortality with nearly 500,000 deaths each year, nearly one-quarter of which occur in India. Following introduction of routine rotavirus vaccination in India in 2016, diarrheal etiology is likely to change. We studied the roles of astrovirus, sapovirus, and norovirus genotypes 1 and 2 as etiologic drivers of diarrhea in a birth cohort in South India prior to rotavirus vaccine introduction.

**METHODS**
We reanalyzed diarrheal and routine surveillance stool samples from a birth cohort study of children in Vellore, India between 2002 and 2003. Samples were tested for presence of astrovirus, sapovirus, and norovirus genotypes 1 and 2 using real time reverse transcriptase-polymerase chain reaction. We calculated unadjusted and age-adjusted pathogen-specific odds ratios (ORs) for diarrhea using logistic regression modeling.

**RESULTS**
We analyzed 1,122 diarrheal and 1,085 routine surveillance stool samples from 337 children. Overall, pathogen presence decreased with age in both diarrheal and routine samples. Norovirus genotype 2 was the most prevalent pathogen found among both diarrheal (14%) and routine samples (14%). Presence of sapovirus was higher among diarrheal (13%) versus routine samples (8%). Astrovirus was present in 7% of diarrheal and 3% of routine samples. After adjusting for age, only sapovirus was associated with increased odds of diarrhea (OR = 1.57, 95% confidence interval (CI): 1.19, 2.08). Though the null was within the 95% CI, astrovirus was associated with increased odds of diarrhea, when adjusted for age and sex (OR = 1.50, 95% CI: 0.88, 2.57). Increased odds of diarrhea were not seen in the adjusted models for norovirus genotype 1 (OR = 0.81, 95% CI: 0.54, 1.22) or norovirus genotype 2 (OR = 0.95, 95% CI: 0.74, 1.22), which may be accounted for by common norovirus reinfection and partial conferred immunity.

**DISCUSSION**
Sapovirus is important in diarrheal etiology among children under 12 months of age in South India. The presence of astrovirus and norovirus were not significant drivers of diarrheal episodes. Our findings suggest that interventions targeted at sapovirus infections in 0 to 12-month-old children have the potential to greatly reduce childhood diarrheal disease morbidity in South India.

# The Etiological Roles of Astrovirus, Sapovirus, and Norovirus as a Cause of Diarrheal Episodes in a Birth Cohort of Indian Children: 2002 – 2006

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**Table of Contents**

**Introduction:**

Diarrheal diseases are estimated to be the fifth leading cause of death in children under five years of age worldwide, resulting in nearly half a million deaths annually (1). Considerable improvements have been made in diarrheal disease burden – globally, the rate of childhood mortality due to diarrheal illness has decreased by 16.6% since 2007, currently estimated at 74.3 deaths per 100,000 (1,2). The highest rates of mortality from diarrheal illness occur in Sub-Saharan Africa and South Asia; India accounts for 22% of these deaths (3,4). With a population of 1.34 billion, India is comprised of 29 states, 7 union territories, and varying levels of social and economic development (5). Etiology of diarrheal disease burden is not well understood in India, in part due to the extreme development heterogeneity between states and union territories (5).

Rotavirus is a leading cause of diarrheal disease globally, with the highest rotavirus-associated mortality rates in sub-Saharan Africa, Southeast Asia, and South Asia (1,6). Following guidance from the World Health Organization (WHO), rotavirus vaccine was recommended to be included in the Universal Immunisation Programme in India in 2014 (7,8). Routine rotavirus vaccination began in four Indian states in 2016 and continues to be introduced to additional states (9). There is a wealth of global literature regarding the etiological role of rotavirus as a leading cause of diarrheal diseases, which continues to expand as countries follow the WHO's recommendation and add rotavirus vaccine into their routine immunization programs (6,7,10-13). The etiologic drivers of diarrheal disease are likely to shift as rotavirus vaccine coverage increases.

Bacterial and parasitic infections such as Cryptosporidium and *Shigella* contribute to the global burden of diarrheal mortality in children under five years, however, previous

research has found viral diarrhea to be more common than diarrhea caused by bacterial or parasitic pathogens (12,14). In addition to rotavirus, astrovirus, sapovirus, and norovirus are three viral pathogens that contribute significantly to the burden of childhood diarrheal episodes (10,11,14,15). In a cohort of 1,715 children from eight low-resource settings (including Vellore, India), astrovirus, sapovirus, and norovirus were among the top ten pathogens contributing to diarrheal disease in the first two years of life (14). As routine rotavirus vaccination becomes increasingly common, it is possible a combination of astro-, sapo-, and noroviruses could become even more prevalent. These single-stranded RNA viruses spread via the fecal-oral route and are often self-limiting (15-20). Astroviruses can infect humans and other species (16). There are three divergent groups of human astroviruses, as well as high potential for zoonotic spillover (16). Sapoviruses are highly diverse and clinically difficult to distinguish from noroviruses (18). Noroviruses are even more diverse and three of the five known norovirus genogroups can infect humans (20). While noroviruses can be mild and self-limiting, they can also lead to death in vulnerable populations, such as young children and the elderly (19).

Previous literature on the etiological roles of astrovirus, sapovirus, and norovirus as a cause of diarrheal episodes has primarily captured cases seeking health care (5,8,12,21). In recent years, two major studies have emerged that examine community level diarrheal disease (22,23). The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study used multisite birth cohort data to estimate the contributions of different pathogens to diarrheal episodes (14,22). The MAL-ED study found approximately 96% of diarrheal episodes could be attributed to ten pathogens (including

rotavirus, astrovirus, sapovirus, and norovirus) (14). Additionally, Bray et al. (2019) utilized a matched case-control design to study diarrhea in young children in Bangladesh (data from one of seven sites included in the Global Enteric Multicenter Study (GEMS)), matching children experiencing diarrheal episodes to controls from the community and analyzing stool samples to determine diarrheal etiologies (10,23). Bray et al. (2019) found rotavirus and norovirus to be the most prevalent pathogens, and that disease attributable to rotavirus and adenovirus was more common among infants. Gladstone et al. (2011) also conducted a community-based birth cohort study with routine stool testing as well as stool testing during diarrheal episodes providing an opportunity to better understand the etiology of diarrheal illness and underlying disease presence in children in India.

Diarrheal diseases are still a leading cause of under-five mortality rate, even with marked decreases in the past 30 years (1). In addition to the large mortality burden, diarrheal diseases cause significant morbidity in children (24-26). Repeated or persistent intestinal infections in formative childhood developmental years can chronically inhibit nutrient absorption, affecting physical and cognitive development (24). Malnourished children are more susceptible to longer duration, more severe, and more frequent future diarrheal diseases (24,25). More frequent diarrheal episodes are associated with delayed entry into first grade and diminished school performance, the latter often used as a measure of economic productivity (26). Gaining a better understanding of the pathogens contributing to diarrheal episodes drives prioritization of public health activities and is imperative in reduction of the under-five mortality rate and improvement of both life expectancy and quality of life, particularly among low- and middle-income countries.

Here, we analyze data that was collected and previously analyzed by Gladstone et al. (2011), who found that natural rotavirus infection protected against subsequent severe infection. The data collected included demographic characteristics as well as regular stool samples (routine and diarrheal) from a birth cohort of children from urban slums in South India (13). This previous study provided a possible explanation for the observed rotavirus vaccine effectiveness in India, which was lower than the effectiveness seen in other countries, however there is limited data available from India regarding acquired immunity to astrovirus, sapovirus, and norovirus (13). Our study aims to analyze the etiological roles of astrovirus, sapovirus, and norovirus as a cause of diarrheal episodes experienced by the same birth cohort of Indian children. We hypothesize that the presence of astrovirus, sapovirus, and norovirus is associated with diarrhea – stool samples collected during diarrheal episodes will test positive for these pathogens more commonly than stool samples collected via routine surveillance.

**Methods:**

*Study Design, Participant Enrollment, and Follow-Up*

The institutional review boards of Christian Medical College, Vellore; London School of Hygiene and Tropical Medicine, London; and Baylor College of Medicine approved the study that our analysis follows; Emory University received only deidentified data so was determined to be non-human subject research (13). Each enrolled child's parent or guardian provided written informed consent. The enrollment criteria and methods of data collection have been published previously (13,27,28). Briefly, 452 infants born between March 2002 and August 2003 from three slums in Vellore, India (Chinnallapuram, Ramanaickanpalayam, and Kasba) were enrolled at

birth. Gladstone et al.'s (2011) analysis was restricted to 373 children who completed all three years of follow-up; the 79 children who did not remain in the study for the entire follow-up period were not significantly different from those who remained in the study in baseline characteristics or pattern of infection prior to leaving the study. As previously described, 31 of the 79 children who did not complete all three years of follow-up left the study within three months of recruitment because the mothers had delivered in their natal homes and returned to their own homes outside of the study area after recruitment (13). Our analysis was further restricted, as 36 children were eliminated due to lack of stool sample testing data. Baseline characteristics of these 36 children did not differ significantly from the children whose samples were available (Table 1).

*Diarrheal Disease Surveillance*

Visits to the children's homes were made twice each week and routine stool samples were collected from enrolled children every two weeks. If diarrhea was reported, families were instructed to collect a stool sample from each diarrheal episode and a field worker visited the home each day until the end of the diarrheal episode. As described by Gladstone et al. (2011), "An episode of diarrhea was defined as three or more watery stools in a 24-hour period or, in breast-fed children, an increased number of daily stools considered to be diarrhea by the mother. The episode ended on the day after the day the child's bowel movements returned to normal." Diarrheal episodes had to be separated by at least 48 hours of normal bowel movements to be considered different episodes. The field worker collected a stool sample on the first reported day of diarrhea and every other day until the diarrheal episode ended. All stool samples were both tested on the date of collection and frozen for storage. Real time reverse transcriptase-polymerase chain

reaction (RT-qPCR) was used to detect the presence and viral load of astrovirus, sapovirus, and norovirus in a subset of stool samples frozen in Gladstone et al.'s (2011) biorepository.

*Statistical Analysis*

Gladstone et al. (2011) collected 26,902 routine surveillance and 4,759 diarrheal stool samples (from 1,829 total diarrheal episodes) in the original cohort study. Routine samples were selected for inclusion if there were 14 days between the sample and a preceding or succeeding diarrheal episode. Previous analysis showed that male sex, low maternal education, and low level of hygiene (measured via regularity of boiling water before drinking) were significantly associated with increased risk of gastrointestinal disease (13,27,28). Additionally, age has a well-studied association with gastrointestinal disease and diarrheal episodes (10,14). Our analysis was conducted in SAS Version 9.4. We performed a backwards selection, $\alpha = 0.05$, to determine which variables to include in our final models. Logistic regression models were fit for each individual pathogen (astrovirus, sapovirus, and norovirus genotypes 1 and 2) to determine odds ratios, confidence intervals, and chi-square p-values among diarrheal versus routine stool samples.

**Results:**

*Baseline Characteristics*

Demographic characteristics at baseline did not differ between children with RT-qPCR tested samples and those whose samples were unavailable for testing (Table 1). Seventy-eight tested samples were excluded due to missing values for age at the time of sample collection. This analysis examined 2,207 stool samples from Gladstone's

biorepository – 1,085 routine surveillance and 1,122 diarrheal. Half (50%) of the children whose samples were included in this analysis were female. The majority (98%) of these children were exclusively breastfed for some period less than six months. When asked how often water is boiled before drinking in the household, 94% of our study population reported “never” or “sometimes” boiling water. Very few mothers of the children in the study had a college education (3%) - the majority of mothers were educated between the primary and high school level (68%) and a quarter had no formal education (29%).

*Viral Pathogen Detection*

Two thirds (n=1,460, 66%) of all tested stool samples did not test positive for any of the pathogens included in this study. There was a significant difference between the number of diarrheal and routine stool samples that tested negative for at least one of the four tested pathogens (diarrheal: n=701, 62%; routine: n=759, 70%; $p<0.01$). Norovirus genotype 2 was present in the largest number of diarrheal samples (n=152, 14%), followed by sapovirus (n=141, 13%), however presence of both norovirus genotypes were not significantly different among diarrheal versus routine stool samples (norovirus g1: $p=0.32$, g2: $p=0.71$) (Table 2). Presence of sapovirus was higher among diarrheal versus routine stool samples (diarrheal: n=141, 13%; routine: n=91, 8%; $p<0.01$) (Table 2). Astrovirus was present in 84 (7%) diarrheal stool samples versus 30 (3%) routine stool samples ($p<0.01$) (Table 2).

Pathogen presence in stool samples tended to decrease with increasing age (Figure 1). Children in the oldest age group (30-35 months) had the lowest percentage of infection by any pathogen in both diarrheal and routine stool samples. Children aged 0-5 months and 6-11 months (infants, collectively) had nearly the same percentage of

astrovirus infection in diarrheal stool samples (30% and 29%) (Table 3). Astrovirus was significantly more likely to be present in diarrheal versus routine stool samples in children aged 0-5 months, 6-11 months, and 12-17 months ($p$=0.04, $p$<0.01, $p$=0.04) (Table 3). Of samples that tested positive for sapovirus, 51% of diarrheal and 42% of routine surveillance stool samples were from children under 12 months of age ($p$<0.01) (Table 2) (Table 3). The lack of significant difference in norovirus (genotypes 1 and 2) among diarrheal versus routine stool samples was consistent when samples were stratified by age group (Table 3).

*Regression Results*

Table 4 shows the odds of a having a diarrheal stool sample when the sample tested positive for a pathogen (astrovirus, sapovirus, norovirus genotype 1, or norovirus genotype 2) compared to the odds of having a routine stool sample when the sample tested positive for the same pathogen.

*Astrovirus*

Unadjusted models showed that the odds of a diarrheal stool sample were 2.85 times the odds of a routine stool sample among children infected with astrovirus (95% CI: 1.86, 4.36). Preliminary analysis showed significant interaction between astrovirus infection and sex, therefore sex, age in six-month categories, and the interaction between sex and astrovirus infection were retained in the final adjusted model. Other covariates were found to be non-significant and therefore were excluded. After adjusting for confounding, the odds of a diarrheal stool sample were 1.50 times the odds of a routine stool sample among children infected with astrovirus (95% CI: 0.88, 2.57).

*Sapovirus*

In our unadjusted model, the odds of diarrhea were 1.57 times the odds of normal stool among children infected with sapovirus (95% CI: 1.19, 2.07). Only age was retained in the final adjusted model for sapovirus and the odds ratio remained unchanged (OR= 1.57, 95% CI: 1.19, 2.08).

*Norovirus*

Unadjusted models of norovirus resulted in no significant findings (Table 4). Again, sex did not have significant interaction with infection and was therefore removed from the adjusted norovirus genotype 1 and norovirus genotype 2 models. Infection with norovirus genotype 1 or genotype 2 was not associated with diarrheal stool samples; this association did not change for either genotype when the model was adjusted for age (Table 4).

**Discussion:**

Diarrheal disease is a leading, yet preventable, cause of death in children under five years of age worldwide. We inferred the etiological roles of astrovirus, sapovirus, and norovirus (genotypes 1 and 2) as causes of diarrhea among a cohort of Indian children by examining the relationship between pathogen presence and type of stool sample (diarrheal or routine surveillance). Our research led us to a number of important findings. First, presence of all four pathogens generally decreased as age increased. Second, diarrheal stool samples tested positive for at least one pathogen more commonly than routine stool samples (38% and 16%, respectively). Third, only sapovirus was significantly associated with diarrheal versus routine stool samples when adjusted for confounding.

Our finding of decreasing pathogen presence with increasing age is consistent with literature that shows decreasing incidence of viral gastrointestinal diseases as children age - most dramatically as they reach 12 months of age (10,14). More than half of the diarrheal stool samples in our study that tested positive for astrovirus, sapovirus, or norovirus were from children under 12 months of age. Infants may be particularly vulnerable to infections with these pathogens due to immature active immunity and relatively limited maternal passive immunity against astrovirus and sapovirus infection (16,29,30). Stool samples that tested positive for sapovirus or norovirus followed a slightly different pattern than astrovirus, with fewer infections occurring between birth and five months of age and a spike in infections between six and twelve months of age. This could be a result of declining maternal antibodies between birth and three months of age and subsequent susceptibility and infection with sapovirus in the next few months of life (29). Previous studies describe potential differences in transfer of maternal sapovirus antibodies by country; our findings could be suggestive of low levels of maternal antibodies at birth with decline until six months of age (29,31).

Neither norovirus genotype was found more frequently among diarrheal stool samples than among routine samples, regardless of inclusion of covariates in the norovirus regression models. This contrasts with other research which found norovirus to be highly prevalent and associated with diarrheal outcomes (10,32). These contrary findings could be a result of different study populations or participant enrollment methods. Bray et al. (2019) and Okada et al. (2020) matched community controls to hospitalized case participants. Our study includes routine surveillance control samples and case samples from children experiencing a diarrheal episode at home and a small

number of children who were hospitalized for their diarrheal episodes. Hospitalization serves as a proxy for severe illness; both Bray et al. (2019) and Okada et al. (2020) are capturing only the most severe cases. Our enrollment methods allow for analysis of both mild and severe cases, which is imperative because norovirus infections tend to be mild and self-limiting (19). Though norovirus was not the main etiologic agent of diarrheal episodes seen in children in this Indian birth cohort, it was highly prevalent among diarrheal and routine stool samples from these children.

This study has several limitations. First, Gladstone et al.'s (2011) original study was designed to examine the protective effect of rotavirus infection on subsequent infection and disease, and thus was not powered for diarrheal etiology in each age group. This led to occasional small sample sizes when stratified and subsequent use of appropriate analytical methods (i.e. Fisher's Exact Test). Though thousands of routine and diarrheal samples were collected, we were limited to analysis of those samples that were properly stored in the biorepository and had associated age at time of sample collection data. Our comparison of included and excluded samples found no significant differences in baseline characteristics. Although rotavirus infection was originally studied in this cohort, rotavirus infection and severity data were not available for this analysis. Inclusion of these data in future studies (i.e. examining rate of co-infections with the different pathogens) would give a more robust picture of diarrheal disease etiology in this cohort. It is possible that socio-economic status (SES) plays a role in the presence of these pathogens; we attempted to account for SES in our logistic regression models through consideration of level of hygiene as a potential covariate, but we found it to be insignificant. Level of hygiene was measured as self-reported frequency of boiling

drinking water. This definition accounts for a possible route of exposure to pathogens included in the study but may not be a good measure of overall hygiene. Diarrheal etiology and enteric pathogen presence are often specific to the region in which a child lives, therefore these results are not necessarily generalizable to areas outside of slums in South India. Finally, our case-control study design is limited in understanding norovirus due to common reinfection and the importance of individual immune state in development of norovirus disease, particularly in this high transmission setting (33). Consideration of viral load, for example using RT-qPCR cycle threshold value with appropriate sensitivity and specificity (specifically, the ability to differentiate between disease and viral shedding), could strengthen norovirus-related case-control studies (33).

Our study describes the prevalence of astrovirus, sapovirus, and norovirus among a cohort of children in rural southern India in the pre-rotavirus vaccination era. Our findings are essential to understanding potential changes in diarrheal etiology following rotavirus vaccine introduction, can inform prevention efforts, and can help to guide future vaccine development and vaccine-use strategies. Our first major finding (decreasing pathogen presence with increasing age) shows us early diarrheal disease interventions should be targeted towards children under 12 months of age to reduce diarrheal morbidity. Sapovirus is an important target for future vaccines, as diarrheal samples have a 57% greater chance of testing positive for the virus than routine samples. Though norovirus and astrovirus were not found to be significantly associated with diarrheal outcomes, both were prevalent among routine and diarrheal stool samples. Regardless of diarrheal outcomes, reduction of enteric pathogen presence will reduce gastrointestinal illness and associated sequalae in children (34).

Our study examined the etiologic role of astrovirus, sapovirus, and norovirus among Indian children in the pre-rotavirus vaccine era. We identified sapovirus as an important cause of diarrheal episodes, specifically among infants. These findings provide a baseline understanding of diarrheal etiology prior to rotavirus vaccine introduction and suggest that interventions targeted at sapovirus infections among infants have the potential to greatly reduce both under five diarrheal disease morbidity and mortality in South India.

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## Tables

*Table 1. Characteristics of a Birth Cohort of Indian Children who Completed and did not Complete Three Years of Follow-up*

| | 3 Years of Follow-up (n=373) | Samples Unavailable for RT-PCR Testing (n=36) | Samples Available for RT-PCR Testing (n=337) | Chi-square p-value |
|---|---|---|---|---|
| | No. (%) | No. (%) | No. (%) | |
| Sex of child | | | | 0.71 |
| Female | 186 (49.9) | 19 (53.8) | 167 (49.6) | |
| Siblings (living in home) | | | | 0.10 |
| Yes | 255 (68.4) | 29 (80.6) | 226 (67.1) | |
| Type of family | | | | 0.36 |
| Joint | 73 (19.6) | 6 (16.7) | 67 (19.9) | |
| Extended | 107 (28.7) | 14 (38.9) | 93 (27.6) | |
| Nuclear | 193 (51.7) | 16 (44.4) | 177 (52.5) | |
| Number of household members | | | | 0.75 |
| ≤5 | 250 (67.0) | 25 (69.4) | 225 (66.8) | |
| >5 | 123 (33.0) | 11 (30.6) | 112 (33.2) | |
| Boil water before drinking? | | | | 0.24* |
| Daily | 19 (5.1) | 0 (0.0) | 19 (5.6) | |
| Sometimes | 101 (27.1) | 13 (36.1) | 88 (26.1) | |
| No | 253 (67.8) | 23 (63.9) | 230 (68.3) | |
| Maternal age at delivery | | | | 0.15 |
| ≤23 years | 188 (50.4) | 14 (38.9) | 174 (51.6) | |
| >23 years | 185 (49.6) | 22 (61.1) | 163 (48.4) | |
| Exclusive breastfeeding | | | | 1.00* |
| <6 months | 367 (98.4) | 36 (100.0) | 331 (98.2) | |
| ≥6 months | 6 (1.6) | 0 (0.0) | 6 (1.8) | |
| Maternal education | | | | 0.24 |
| None | 106 (28.4) | 7 (19.4) | 99 (29.4) | |
| Primary, middle, high school | 258 (69.2) | 29 (80.6) | 229 (68.0) | |
| College | 9 (2.4) | 0 (0.0) | 9 (2.7) | |
| Place of birth | | | | 0.04* |
| Hospital or health center | 362 (97.1) | 34 (94.4) | 328 (97.3) | |
| Home | 8 (2.1) | 0 (0.0) | 8 (2.4) | |
| Other | 3 (0.8) | 2 (5.6) | 1 (0.3) | |

| | | | | |
|---|---|---|---|---|
| Mode of delivery | | | | 0.78* |
| Normal vaginal | 339 (90.9) | 33 (91.7) | 306 (90.8) | |
| Instrument-aided | 9 (2.4) | 0 (0.0) | 9 (2.7) | |
| Cesarean | 25 (6.7) | 3 (8.3) | 22 (6.5) | |
| Religion | | | | 0.09* |
| Hindu | 176 (47.2) | 18 (50) | 158 (46.9) | |
| Muslim | 180 (48.3) | 14 (38.9) | 166 (49.3) | |
| Christian | 17 (4.6) | 4 (11.1) | 13 (3.9) | |
| Other | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Birth weight (kg) | | | | |
| Mean (sd) | 2.9 (0.5) | 3.0 (0.4) | 2.9 (0.5) | 0.47** |

*p-value obtained using Fishers Exact Test (cell values < 5)
**p-value obtained using Wilcoxon Rank Sum Test

*Table 2. Viral Pathogen Presence in Diarrheal and Routine Stool Samples from Children in an Indian Birth Cohort (2002 – 2006)*

| | Sample Type | | |
|---|---|---|---|
| | Diarrheal (n=1,122) | Routine (n=1,085) | Chi-square p-value |
| Pathogen | No. Positive (%) | No. Positive (%) | |
| Astrovirus | 84 (7.5) | 30 (2.8) | <0.01 |
| Sapovirus | 141 (12.6) | 91 (8.4) | <0.01 |
| Norovirus, g1 | 44 (3.9) | 52 (4.8) | 0.32 |
| Norovirus, g2 | 152 (13.6) | 153 (14.1) | 0.71 |

*Table 3. Prevalence of Astrovirus, Sapovirus, and Norovirus Infection Among Stool Samples from Children in an Indian Birth Cohort (2002 – 2006) by Age Group*

| | | Diarrheal | Routine | |
|---|---|---|---|---|
| | Age (months) | No. Positive (%) | No. Positive (%) | Chi-square p-value |
| Astrovirus | | n=84 | n=30 | |
| | 0-5 | 25 (29.8) | 12 (40) | 0.04 |
| | 6-11 | 24 (28.6) | 5 (16.7) | <0.01 |
| | 12-17 | 13 (15.5) | 4 (13.3) | 0.04* |
| | 18-23 | 12 (14.3) | 6 (20.0) | 0.16 |
| | 24-29 | 5 (6.0) | 3 (10.0) | 0.72* |
| | 30-35 | 5 (6.0) | 0 (0.0) | 0.06* |
| Sapovirus | | n=141 | n=91 | |
| | 0-5 | 28 (19.9) | 15 (16.5) | 0.06 |
| | 6-11 | 44 (31.2) | 23 (25.3) | 0.01 |
| | 12-17 | 16 (11.4) | 22 (24.2) | 0.28 |
| | 18-23 | 25 (17.7) | 15 (16.5) | 0.11 |
| | 24-29 | 21 (14.9) | 14 (15.4) | 0.21 |
| | 30-35 | 7 (5) | 2 (2.2) | 0.16* |
| Norovirus, g1 | | n=44 | n=52 | |
| | 0-5 | 14 (31.8) | 9 (17.3) | 0.36 |
| | 6-11 | 11 (22.7) | 18 (34.6) | 0.10 |
| | 12-17 | 8 (18.2) | 7 (13.5) | 0.82 |
| | 18-23 | 4 (9.1) | 8 (15.4) | 0.25* |
| | 24-29 | 6 (13.6) | 7 (13.5) | 0.77 |
| | 30-35 | 2 (4.6) | 3 (5.8) | 0.67* |
| Norovirus, g2 | | n=152 | n=153 | |
| | 0-5 | 51 (33.6) | 42 (27.5) | 0.48 |
| | 6-11 | 47 (30.9) | 57 (37.3) | 0.20 |
| | 12-17 | 28 (18.4) | 25 (16.3) | 0.70 |
| | 18-23 | 11 (7.2) | 14 (9.2) | 0.49 |
| | 24-29 | 8 (5.3) | 12 (7.8) | 0.34 |
| | 30-35 | 7 (4.6) | 3 (2) | 0.32 |

*p-value obtained using Fishers Exact Test (cell values < 5)

*Table 4. Adjusted and Unadjusted Odds Ratios for Diarrheal versus Routine Stool Samples for Children in an Indian Birth Cohort (2002 – 2006) by Pathogen*

| | Total Samples (n=2,207) | Diarrheal Samples (n=1,122) | Routine Samples (n=1,085) | Unadjusted OR (95% CI) | Chi-square p-value | Adjusted* OR (95% CI) | Chi-square p-value |
|---|---|---|---|---|---|---|---|
| Astrovirus Positive | 114 | 84 | 30 | 2.85 (1.86, 4.36) | <0.0001 | 1.50 (0.88, 2.57)** | 0.14 |
| Sapovirus Positive | 232 | 141 | 91 | 1.57 (1.19, 2.07) | 0.0015 | 1.57 (1.19, 2.08) | <0.01 |
| Norovirus g1 Positive | 96 | 44 | 52 | 0.81 (0.54, 1.22) | 0.32 | 0.81 (0.54, 1.22) | 0.32 |
| Norovirus g2 Positive | 305 | 152 | 153 | 0.95 (0.74, 1.22) | 0.72 | 0.95 (0.75, 1.21) | 0.68 |

*Adjusted for age (6-month categories)

**Adjusted for age (6-month categories) and sex

## Figures

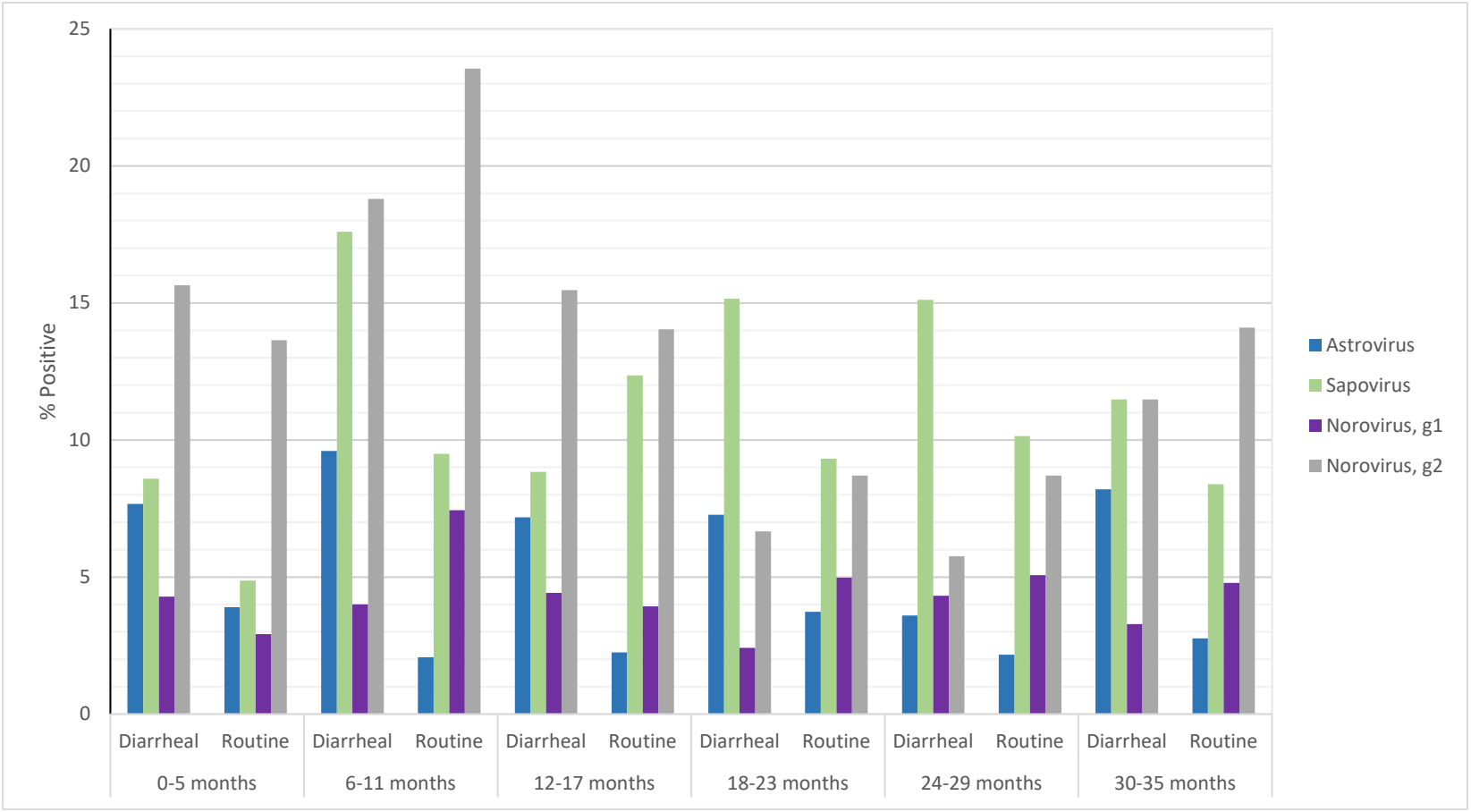


*Figure 1. Distribution of Pathogens Among Diarrheal and Routine Surveillance Stool Samples with a Positive RT-qPCR Test in an Indian Birth Cohort (2002 – 2006)*