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Kelly Wahl Date

Norovirus and Rotavirus Prevalence in Pediatric Patients with Underlying Gastrointestinal Dysfunction in Atlanta, GA, 2012-2013

By

Kelly N. Wahl

Master of Public Health

Epidemiology

_____

Christine L. Moe, PhD, MS
Committee Chair

_____

Amy E. Kirby, PhD, MPH
Committee Member

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By

Kelly N. Wahl

B.A.
Kenyon College
2012

Thesis Committee Chair: Christine L. Moe, PhD, MS

An abstract of
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Abstract

Norovirus and Rotavirus Prevalence in Pediatric Patients with Underlying Gastrointestinal Dysfunction in Atlanta, GA, 2012-2013

By Kelly N. Wahl

Norovirus (NoV) and Rotavirus (RoV) are the two major causes of acute viral gastroenteritis in children. Although the prevalence and symptomology of both viruses has been well-documented in pediatric populations, little is known about these viruses in vulnerable sub-populations such as children with underlying gastrointestinal dysfunction (G.D.) (e.g. Crohn's disease, intestinal malrotation, short gut syndrome).

To better understand the implications of G.D. on susceptibility to enteric viruses, stool samples submitted for bacterial stool testing at two large pediatric hospitals serving metropolitan Atlanta were prospectively collected between July 2012 and July 2013. RT-PCR was used to detect GI and GII NoVs and a commercially available EIA was used to detect RoV. All children had some type of underlying G.D. and symptoms of acute gastroenteritis (diarrhea and, or vomiting) for inclusion in this study.

Within the study, 22% (54/244) had a surgical and, or congenital condition of the gut, 7% (18/244) had inflammatory bowel disease (IBD), 49% (119/244) were tube fed, 19% (46/244) had both a surgical/congenital condition and were being tube fed, and 3% (7/244) had both a surgical/congenital condition and IBD. Sixteen percent (40/244) of samples were positive for RoV, 7.4% (18/244) samples were positive for GII NoV, and 1 sample was positive for GI NoV. There was a single NoV GII/RoV coinfection.

Children with a positive NoV/RoV stool sample were significantly younger (Mean: 4.2, 95% C.I.: 2.8, 5.6 years) than children with a negative stool sample (Mean; 6.1, 95% CI: 5.3, 6.9 years) (Wilcoxon T test, P = 0.0063). The duration of emesis was shorter in patients with NoV positive stool samples (Mean, 1.4 days) compared to RoV positive stool samples (2.5 days)(Wilcoxon T test, P= 0.0417). Rotavirus immunization was not protective against rotavirus infection when comparing full immunization vs. none (χ^2, P= 0.3228).

Additional studies are needed to determine the clinical impact of NoV gastroenteritis in this vulnerable subpopulation. It is unknown if children with specific types of G.D. experience more frequent illness and, or have more severe bouts of disease of longer duration.

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Literature Review

Norovirus

Noroviruses are a group of genetically diverse viruses that cause acute gastroenteritis in a number of animal species including humans. These viruses fall under the genus *Norovirus*, family *Caliciviridae*[1]. Noroviruses are heterogeneous with respect to their morbidity and epidemiology. Norovirus infections range in severity from asymptomatic to fatal, with most cases causing a self-resolving gastroenteritis of short duration. In addition, norovirus can be transmitted by a variety of mechanisms, including food, water, person-to-person, and surface contamination [2]. Without a way to culture human noroviruses, strains cannot be serotyped by biologically relevant criteria. Instead, strains are classified by genetics[1]. There are six genogroups (I-VI), and more than 30 genotypes [1, 3, 4]. Only genogroups I and II regularly cause illness in humans[1]. GI strains are generally associated with environmental contamination with foodborne outbreaks whereas GII strains tend to be transmitted by person-to-person contact [4]. As a result, GII strains are more often implicated in healthcare-associated outbreaks [5]. In the U.S. GI and GII noroviruses are responsible for approximately 19-21 million cases of sporadic and epidemic acute gastroenteritis annually[6]. Given the magnitude of this burden, additional research is needed to define the epidemiology of norovirus in potentially vulnerable sub-populations, such as children with underlying gastrointestinal dysfunction.

Burden

In the U.S., norovirus is the most common cause of epidemic gastroenteritis and consequently a major cause of morbidity. Between 2009 and 2010, norovirus was

responsible for 68% of single etiology outbreaks, and associated with 78% of cases, 46% of hospitalizations, and 86% of deaths reported to the National Outbreak Reporting System (NORS)[7]. NORS is a surveillance system that captures all outbreaks of acute gastroenteritis regardless of pathogen or mode of transmission [7, 8]. The burden of norovirus gastroenteritis in the U.S. can be described by the epidemiological iceberg. Annually, there are only an estimated 570-800 deaths attributed to norovirus out of 56,000-71,000 hospitalizations, more than 400,000 visits to the emergency department, 1.7-1.9 million outpatient visits, and 19-21 million episodes of norovirus gastroenteritis in the U.S[6].

Norovirus has a distinct pattern of temporality. Every 2-3 years new norovirus strains emerge causing distinctive peaks in norovirus activity [4, 9]. In addition to biennial and triennial peaks in norovirus activity, there are also striking annual peaks in norovirus activity during the winter months in the Northern Hemisphere giving norovirus gastroenteritis one of its older titles, Winter Vomiting Disease[10-12].

Noroviruses cause both sporadic cases and explosive outbreaks affecting hundreds of people within a few days. Cruise ships, banquets, music festivals, schools and other large gatherings have been implicated in norovirus epidemics [13-24]. Large norovirus outbreaks on cruise ships have given norovirus one of its other monikers, cruise ship virus[25].

Norovirus gastroenteritis is also an important hospital-acquired infection[26]. Noroviruses have been implicated in outbreaks in nursing homes, and acute and long term care facilities [26, 28-31]. Rhinehart et al. (2012) reviewed 386 outbreaks from 289 U.S. hospitals between 2008 and 2009 and found 18% of all outbreaks were attributed to

norovirus [32]. Given the vulnerable patient population in hospitals (the elderly and immunocompromised) norovirus in the healthcare setting can cause major morbidity and economic loss [31, 33]. After the introduction of a new norovirus outbreak surveillance system in English hospitals, Harris et al. (2013) found that norovirus outbreaks were annually associated with approximately 3,400 and 13,000 illnesses in staff and patients respectively, as well as 8,900 days of ward closure resulting in a loss of 15,500 bed-days per year [34].

Transmission and Stability in the Environment

Noroviruses are most commonly transmitted via the fecal oral route through contamination of food, water and the environment. After foodborne transmission, person to person transmission is most commonly implicated in outbreaks [5]. Disease transmission can also occur after projectile vomiting events when aerosolized emesis contaminates surrounding air and surfaces [35, 36].

A recent example of environmental transmission of norovirus was an outbreak in 2012 where a reusable shopping bag was implicated in a norovirus transmission among a traveling soccer team. The bag containing the team lunch was left in the bathroom where the primary case was actively vomiting and having diarrhea. The primary case did not remember touching the bags. It appears that the bag became contaminated via aerosolized emesis and stool from flushing the toilet. Two weeks later, during the outbreak investigation, norovirus was recovered from the implicated shopping bag [36].

Hospitals are also vulnerable to environmental norovirus contamination. Several studies have found norovirus contamination after patient areas were cleaned [26, 37]. Morter et al. (2011) found 31% of environmental swabs from areas housing infected

patients were positive for norovirus post routine cleaning [26]. Positive swab sites included; keyboards, soap and alcohol dispensers, and other reusable equipment housed within the ward and of course furniture and surfaces in the rooms and bathrooms of infected patients. A study by Liu et al (2013) showed that hands of human challenge patients had measurable levels of norovirus contamination. Patients hand contamination likely explains some of the environmental contamination found in the Morter et al. study[38].

Noroviruses' stability under a variety of conditions makes environmental contamination an effective transmission pathway. Controlled laboratory experiments, outbreak investigations, and environmental sampling surveys have demonstrated stability under a wide range of temperatures and exposure to a number of commonly used cleaning agents [2, 37, 39-41]. Liu et al. (2012) found that norovirus was stable for at least 28 days on steel coupons at temperatures between 4 and 37 °C [2]. Another study found that norovirus remained viable in groundwater for at least 61 days at room temperature [42].

In addition to stability in the environment, noroviruses are fairly resistant to many commonly used cleaning products. The nonenveloped norovirus virions are resistant to ethanol and quaternary ammonium disinfectants and hand sanitizers [39, 41]. Hand washing with soap and water still remains the best way to decontaminate hands [39, 43]. To the best of our knowledge, bleach effectively inactivates norovirus but given the nature of bleach, it is not always the most suitable product for cleaning carpets or hands.

Besides stability in the environment, noroviruses are highly contagious due to a low infectious dose and high viral shedding. The infectious dose for norovirus is

estimated to be 18 virions (95% CI, 1-4,350), calculated from human challenge studies using escalating challenge doses and mathematical models of human norovirus infection [44, 45]. In addition, norovirus infection (presence of virus in stool or seroconversion) and symptomology (diarrhea or emesis) follow separate positive dose response curves [44, 46].

Viral shedding occurs during symptomatic infection (10^8 genome copies or more per g of stool) and lasts for a substantial length of time post-infection [41]. Healthy adults asymptomatically shed norovirus in their stool for approximately 3 weeks post-infection. Children on the other hand shed asymptomatically much longer than adults. A study by Saito et al. found that 75% of children under 2 shed norovirus more than 30 days, and 18% shed for more than 60 days which is much longer than what is typical seen in adults[47]. Immunocompromised individuals and children can shed asymptomatically or symptomatically for months or even years [47-51].

Although asymptomatic shedding is concerning because of noroviruses' low infectious dose, symptomatic shedders have been found to be more of a concern for disease transmission than asymptomatic shedders because of higher viral loads [27, 48, 52, 53]. Nevertheless, immunocompromised individuals may be important reservoirs for nosocomial norovirus because of bouts of chronic diarrhea that may or may not be due to norovirus infection [27, 50, 54, 55]. In addition, there is evidence that immunocompromised persons, unable to clear prolonged norovirus infections are important sites of viral evolution due to antigenic drift [56, 57].

Symptomology

Noroviruses cause acute gastroenteritis in all age groups. In healthy adults, the disease is mostly self-limiting, with sudden onset of nausea, vomiting, watery diarrhea, abdominal pain, general malaise, and sometimes a low-grade fever. Stool does not generally contain blood [12, 46]. In healthy persons, the median incubation period is 24-48 hours, and symptoms last between 12 and 60 hours [5]. The frequency and severity of these symptoms varies by strain, dose and host factors [12, 44, 58]. For example, vomiting is less common in hospitalized patients and children under one year of age [46, 58].

The duration and severity of norovirus infection can be much greater in vulnerable persons such as the immunocompromised, the elderly, children under 5 years of age (<5) and individuals with underlying chronic illnesses [46, 59]. In immunocompromised individuals, clinical symptoms as well as chronic viral shedding can last for months or even years, increasing the risk of severe dehydration [49, 60]. In a study by Ludwig et al. (2008) the median duration of symptoms in pediatric cancer patients was 19 days, or 2-3 times longer than comparable immunocompetent children [49]. Adults over 65 are at increased risk of death because of chronic diarrhea, severe dehydration and comorbidities such as immunosuppression [7, 12, 46, 61]. Children <5 are the most likely of any age group to seek medical care for norovirus gastroenteritis, usually due to symptoms related to dehydration[12, 62]. Recently, two studies have found that norovirus infection in previously healthy children, was possibly associated with benign seizures within 3 days of the onset of symptoms without lasting neurological sequelae [60, 63].

Certain individuals may have underlying immunity to a particular strain as a result of previous infection or genetic immunity, reducing their odds of becoming infected and, or developing a symptomatic infection [44, 64]. Immunity as a result of previous norovirus infection was thought to be as short as 6-months based on early re-challenge studies but a recent study modeling immunity post-infection found that duration of immunity may be 4-8 years[65, 66]. Genetic immunity to norovirus infection is related to histo-blood group antigens and the presence of a non-functional α 1,2 fucosyltransferase *FUT2* gene. Humans produce histo-blood group on the surface of cells that noroviruses can bind to and potentially cause infection. It is thought that the B enzyme (related to the B blood group), alters the H type 1 antigen, preventing noroviruses from binding cells in the gut. Persons with B blood are less likely than persons with A or O blood types to become infected, or if infected, display symptoms. Individuals with a non-functional *FUT2* gene or "non-secretors" are resistant to symptomatic infection to GI and GII noroviruses. The virus cannot bind to host gut cells because they are missing α 1,2-linked fucose residue on their cell membranes[64].

The severity of acute viral gastroenteritis can be quantified using the Vesikari score. Originally developed for rotavirus vaccine trials as a way to compare the efficacy of vaccines against severe disease, this scoring method is also applicable for norovirus gastroenteritis in children [67, 68]. The Vesikari score takes into account the presence and duration of vomiting and/or diarrhea, fever, dehydration, and treatment status. Each factor is graded for severity on a scale of 1-3 for a total score between 0 and 20[68]. Scores ≥ 11 are considered severe, 7-10, moderate, and <7, mild. The modified Vesikari score uses the need for IV fluids instead of percent dehydration [69]. Records of vomiting

and diarrhea frequency and duration as well as measures of dehydration are often poor when conducting retrospective chart reviews. Either data is missing or records of the patients symptoms are not detailed enough to properly calculate Vesikari component scores. Consequently, Vesikari scores calculated from retrospective chart data can be unreliable[67].

Introduction of Rotavirus Vaccination and the Changing Epidemiology of Viral Gastroenteritis

Similar to norovirus, the burden of rotavirus annually peaks during the winter months and biennially as new strains emerge via antigenic shift and drift[70]. 2013 was a big year for rotavirus, in March of that year approximately 30% of cases reported to the National Respiratory and Enteric Virus Surveillance System (NREVSS) were positive for rotavirus antigen[71].

Prior to the introduction of rotavirus vaccines, rotavirus was responsible for 20 to 50% of severe, acute gastroenteritis in children globally [72]. By age 5, nearly 100% of children had at least one rotavirus infection, regardless of country of origin [72]. Unlike norovirus, rotavirus is responsible for the majority of childhood deaths due to diarrhea in low and middle income countries (LAMIC), mostly as a result of a lack of access to care [72]. In the U.S., approximately 40 to 50% of hospitalizations related to diarrhea in children <5 were due to rotavirus [72]. It was estimated that the total cost of rotavirus, including medical care, and lost productivity due to parents staying home to care for their children was ~ 1 billion dollars annually [72]. The development of a rotavirus vaccine was driven by mortality in LAMIC and the high cost of care in high income countries (HIC) [73].

Rotavirus vaccines are designed to protect against severe disease, not prevent infection entirely [73]. Like noroviruses there are multiple rotavirus genotypes requiring a vaccine to have cross-reactivity between multiple genotypes [70]. Currently there are two vaccines on the market in the U.S., RotaTeq and Rotarix. Both live vaccines are administered orally during the first year of life. RotaTeq is a 3-dose series given at 2, 4, and 6 months and Rotarix is a 2-dose series given at 2 and 4 months. RotaTeq is a bovine-human reassortant pentavalent vaccine containing a bovine surface protein (VP7) and four human rotavirus surface proteins (G1, G2, G3, and G4) [70, 73]. Rotarix is an attenuated strain of human rotavirus GIP [70]. RotaTeq and Rotarix were not actually the first rotavirus vaccines introduced in the U.S. The first rotavirus vaccine, RotaShield was introduced in 1998 but, was pulled from the market when post-licensure surveillance revealed a risk of intussusception, an unforeseen and serious complication where the intestine folds in on itself [72]. It was not until 2006 and 2008, rotavirus vaccines RotaTeq and Rotarix, respectively were introduced after extensive safety testing [72]. In 2006, the Advisory Committee on Immunization Practices (ACIP), recommended rotavirus immunization for infants and since then vaccine uptake has increased [74].

Since the introduction of rotavirus vaccination in the U.S., there has been a shift in the epidemiology of acute gastroenteritis. The rates of acute gastroenteritis and rotavirus-confirmed gastroenteritis have decreased sharply. The rate of hospitalization for all-cause acute gastroenteritis decreased 25 to 66% post-2006 depending on the season and age group studied [70]. The rates of rotavirus-specific hospitalization have decreased 60% to 100% depending on age group and season [70]. This translates into a 4 to 6% decrease in the rate of all cause hospitalizations in children <5[72]. Immunizing infants

seems to have also led to protection against rotavirus in older children and adults [72, 75]. Yi and Anderson (2013) found the prevalence of rotavirus in adult stool samples submitted for bacterial testing decreased 48% after the implementation of infant vaccination[75].

As a result of rotavirus immunization, norovirus has become the most common viral enteric pathogen in some hospital-based studies [76]. It was hypothesized that the burden of norovirus would rise as the burden of rotavirus decreases but studies to date have shown that norovirus rates have remained stable as the incidence of rotavirus has decreased [76].

While there has been a major decrease in the incidence of rotavirus in the U.S., vaccine uptake has remained low. A study of vaccine uptake for privately insured infants between 2006 and 2010 found that 20% had not received a single dose of rotavirus vaccine as of 2010[77]. With increasing vaccine uptake and decreasing incidence of rotavirus infection in the coming years there may be additional changes to rotavirus and norovirus epidemiology.

Underlying Gut Pathology

To date, there are no published studies describing the burden of norovirus infection in pediatric patients with underlying gastrointestinal dysfunction. Gastrointestinal dysfunction can be roughly divided into anatomic defects and surgery, and inflammatory bowel disorders. Anatomic defects include maladies such as Hirschsprung's disease, short gut syndrome, imperforate anus, and Berdon syndrome. Hirschsprung's disease is a gastrointestinal motility disorder resulting from the absence of nerves controlling intestinal peristalsis. Aganglionosis may extend only a few inches

from the rectum or, in rare cases, the entire length of the intestines. Most often, treatment includes resectioning the gut and removal of the nerveless segment of the colon. If there is no nervous tissue along the length of intestines, the whole length of the gut is removed and the patient must use an ileostomy bag. Imperforate anus, where the anus is malformed and may require a protective colostomy. Berdon syndrome, is a group of symptoms including reduce muscle tone in the bowel resulting in problems passing stool.

Hirschsprung's disease as well as other anatomical defects, cancer and infections resulting in severe enteritis and necrosis may require resectioning of the gut. Short gut syndrome is an extreme complication from resectioning the gut where there is not enough intestinal surface area left for uptake of proper nutrition and consequently children require parenteral nutrition. Short gut syndrome is the most common cause of pediatric intestinal failure [78]. The syndrome is characterized by malabsorption resulting from the surgical removal of large segments of the intestine and patients can require tubing feeding for proper nutrition. It is possible that these children are less susceptible to norovirus infection because they do not have the gut tissue required for infection.

The most common inflammatory bowel disorders leading to gastrointestinal dysfunction are Crohn's disease and ulcerative colitis. Some of these disorders are related to autoimmune issues which may have implications for mucosal immunity and viral enteric infection. Ulcerative colitis is a chronic inflammatory disease of the colon. Currently the etiology is unknown but it could be related to genetics, prior gastrointestinal infection, dietary, or other environmental factors. Symptoms include hematochezia (blood in stool), abdominal pain, and diarrhea. Severe norovirus infection in children is associated with pre-existing Irritable Bowel Disease and lead to

hematochezia, a symptom not associated with infection in previously healthy person [60, 79].

Genome

Noroviruses are positive-sense, single-stranded, non-enveloped RNA viruses with a linear genome containing 3 open reading frames (ORFs) [1, 80]. ORF1 encodes the RNA-dependent, RNA polymerase (RdRp) and five other non-structural proteins [81, 82]. ORF2 and ORF3 encode the major and minor capsid proteins VP1 and VP2 respectively [1, 60, 80, 81]. The genetic diversity of noroviruses appears to be the result genetic drift and occasionally recombination events [1, 4, 49, 58, 83, 84].

Currently, noroviruses are classified by sequencing ORF2 but it has become clear as the diversity and prevalence of norovirus strains had increased, this method is no longer specific enough to accurately genotype noroviruses[1, 85]. There has been confusion over the classification of some genotypes and lack of consensus on which regions of the genome are best for sequencing [1, 85]. Kroneman et al. (2013) suggested a standardized method of molecular genotyping and classification of human noroviruses that includes sequencing part of the RdRp on ORF1 (Region A) and part of ORF2 (Region C) [1]. Noroviruses tend to undergo recombination around the ORF1/ORF2 junction, thus a recombinant virus will have Region A from one strain and Region C from another[1]. If a recombinant is suspected, the intervening region between Regions A and C is sequenced to confirm the recombination. Adoption of this standardized sequencing protocol will increase recognition of recombinants and more accurately classify noroviruses[1].

Based on current genotyping methods, around the world, GII is by far the most common genogroup representing over 80-90% of strains recovered during surveillance studies testing for GI and GII strains [86-92]. In the U.S, GII strains were responsible for 89% of genotyped outbreaks between 2009 and 2013[4]. Within genogroup II, GII.4 strains are responsible for 60-80% of outbreaks worldwide[3].

Every 2-3 years a new GII.4 strain emerges, replacing the existing predominant GII.4 strain, and causing distinctive peaks in norovirus activity[4, 9]. In March 2012 the GII.4 Sydney strain emerged in Australia and quickly overtook the 2009 GII.4 New Orleans strain, to become the most prevalent strain[9, 83]. In the past, the incidence of norovirus peaked during the winter months, however, this pattern changed with the early emergence of the GII.4 Sydney [9]. In the U.S., GII.4 Sydney rapidly became the most commonly reported outbreak strain. From September to December 2012, the percentage of U.S. outbreaks attributed to GII.4 Sydney increased from 19 to 58%[9].

Diagnosis and Detection of Noroviruses

Sporadic norovirus cannot be reliably differentiated from other causes of viral gastroenteritis based on clinical presentation alone. During outbreaks, Kaplan's criteria can be used to determine with high specificity (99%) and reasonable sensitivity (68%), the occurrence of norovirus in otherwise healthy persons [59, 93]. The four components of Kaplan's criteria are; 1) the average duration of illness is between 12-60 hours, 2) the average incubation period is between 24-48 hours, 3) there is vomiting in more than half of suspected cases, and 4) no explanatory bacterial pathogen is found in patient samples [59]. However, Kaplan's criteria may not be appropriate for the diagnosis of norovirus outbreaks in persons with underlying illnesses such as the immunocompromised and

those with underlying gastrointestinal illness, where the mean duration of illness is much longer than 60 hours and vomiting may be less frequent[94].

Detection of norovirus has come a long way since the visualization of the Norwalk strain by electron microscopy in 1972[95]. This method was cumbersome and expensive, requiring careful sample preparation, an electron microscope and highly trained technicians[46]. Although highly specific, this method has <50% sensitivity and cannot differentiate between the diversity of strains present today [96](KOO 2011). Currently, genetic and immunoassays are preferred methods for the detection of Noroviruses.

Both enzyme-linked immunosorbent assays (ELISAs) and enzyme immunoassays (EIAs) are used to detect noroviruses. There are currently commercially available kits for both methods of detection [94, 97, 98]. Due to the diversity of noroviruses and their continued evolution of surface epitopes, these assays tend to have lower specificity and sensitivity than nucleic acid-based protocols [58, 94, 97]. With a short processing time and relatively low cost, immunoassays can be an effective way to determine whether a gastroenteritis outbreak is due to norovirus. However, they are not able to determine the norovirus genotypes and, therefore, are not commonly used for surveillance studies [58, 94, 97].

Real-Time Reverse Transcription Quantitative PCR (RT-qPCR) is currently the preferred method of detection because it has the highest sensitivity of all the available diagnostics[99]. Both RT-qPCR and conventional reverse transcription ($_C$RT-PCR) can be used to amplify and visualize viral RNA. RT-qPCR detects the presence and

amplification of viral cDNA through fluorescently tagged probes, whereas cRT-PCR products are visualized on an agarose gel.

Since 1992, a variety of cRT-PCR and RT-qPCR protocols and primer sets have been used to detect and genotype noroviruses [100, 101]. Until recently there was no consensus on which regions of the genome were the best targets for viral detection and sequencing[1]. Some protocols only used the sequence from a single region of ORF1 (polymerase) or ORF2 (capsid proteins) which can miss recombinants at the ORF1/ORF2 junction. Broadly reactive primers target ORF1 because it is conserved across strains. Type-specific primers target ORF2 because it is highly variable between strains [102].

Many PCR-based detection protocols use GI and GII specific primer sets because of high sequence diversity between genogroups[58]. It has been shown that the sensitivity of primers can vary across norovirus strains, complicating analysis of RT-PCR detection data. There are new primer sets with primers that can amplify GI, GII, and GIV viral genes in a single reaction [103]. In addition, as these viruses continue to evolve, new primer sets will need to be developed to account for ever-changing primer-target sequences [46, 104].

Outstanding Questions

The burden of norovirus gastroenteritis in children with underlying gastrointestinal dysfunction is unknown. The incidence of norovirus in this population could be higher if their underlying illness makes them more susceptible to norovirus infection. Conversely, some patients with underlying gastrointestinal dysfunction may be less susceptible to norovirus children than healthy children because their gut physiology prevents norovirus from infecting cells in the intestinal tract. For example, children with

ileostomies and colostomies may no longer have the gut tissue with the necessary receptors for norovirus infection. Without a firm grasp of norovirus infection at the molecular level it is impossible to know if this is true (but certainly plausible). Finding a way to culture norovirus will certainly be helpful.

It is also unclear if the distribution of norovirus strains differs between previously health children and children with underlying gastrointestinal dysfunction. GI strains tend to have lower morbidity than GII strains, specifically the frequency of painful. Consequently GI strains may be more common in these sick children because they are more vulnerable to severe infection and more likely to be admitted for care[48].

Underlying gastrointestinal dysfunction may modify the clinical picture of norovirus infection. It is unclear if these children present differently than children without underlying gastrointestinal dysfunction infected with norovirus, like hematochezia in children with irritable bowel disease[60]. Their symptoms may last longer and they may experience more severe symptoms, such as prolonged diarrhea and vomiting uncharacteristic of classic norovirus gastroenteritis. Modelling symptomology in these children could help define a combination of symptoms that suggest norovirus as a differential diagnosis when the cause of their gastroenteritis is unknown [105].

The prevalence of rotavirus in vulnerable pediatric subpopulations since the introduction rotavirus immunization is unknown. It is possible that rates of rotavirus gastroenteritis are lower in these children because of higher rates of rotavirus vaccine uptake.

Introduction

Noroviruses are a group of genetically diverse viruses that cause acute gastroenteritis in a number of animal species including humans. Only genogroups I and II regularly cause illness in humans[1]. Annually, noroviruses cause an estimated 19-21 million episodes of illness resulting in approximately 1.7-1.9 million outpatient visits, more than 400,000 visits to the emergency department, and 56,000-71,000 hospitalizations [6].

The burden of noroviruses peaks annually during the winter months and every 2-3 years when a new epidemic strain emerges [4, 9-12]. Noroviruses cause both sporadic cases and explosive outbreaks affecting hundreds of people within a few days. Cruise ships, banquets, music festivals, schools and other large gatherings have been implicated in norovirus epidemics [13-24]. Norovirus gastroenteritis is also an important hospital-acquired infection, causing major morbidity and economic loss due to the vulnerable patient population (the elderly and immunocompromised) [26-31, 33, 34].

Norovirus infections range in severity from asymptomatic to fatal, with most cases causing a self-resolving gastroenteritis of short duration. The frequency and severity of these symptoms varies by strain, dose and host factors [12, 44, 58]. In healthy adults, the disease is mostly self-limiting, with sudden onset of nausea, vomiting, watery diarrhea, abdominal pain, general malaise, and sometimes a low-grade fever. The median incubation period is 24-48 hours, and symptoms last between 12 and 60 hours [5]. The duration and severity of norovirus infection can be much greater in vulnerable persons such as the immunocompromised, the elderly, and children under 5 years of age (<5) [46, 59]. In immunocompromised individuals, clinical symptoms as well as chronic viral

shedding can last for months or even years, increasing the risk of severe dehydration[49, 60]. Children <5 are the most likely of age group to seek medical care for norovirus gastroenteritis, usually due to symptoms related to dehydration[12, 62].

To date, there are no published studies describing the burden of norovirus infection in pediatric patients with underlying gastrointestinal dysfunction. Gastrointestinal dysfunction can be roughly divided into anatomic defects and surgery, and inflammatory bowel disorders. Anatomic defects include maladies such as Hirschsprung's disease, short gut syndrome, and imperforate anus. Hirschsprung's disease as well as other anatomical defects, cancer and infections resulting in severe enteritis and necrosis may require resectioning of the gut. Short gut syndrome, characterized by malabsorption resulting from the surgical removal of large segments of the intestine, is the most common cause of pediatric intestinal failure and patients can require tubing feeding for proper nutrition [78]. It is possible that these children are less susceptible to norovirus infection because they do not have the gut tissue required for infection. The most common inflammatory bowel disorders leading to gastrointestinal dysfunction are Crohn's disease and ulcerative colitis. Severe norovirus infection in children is associated with pre-existing inflammatory bowel disease.

The burden of norovirus in children with underlying gastrointestinal dysfunction since the introduction of rotavirus immunization is unknown. It is unclear if the distribution of norovirus strains differs between previously health children and children with underlying gastrointestinal dysfunction. GI strains tend to have lower morbidity than GII strains. Consequently GI strains may be more common in these sick children because they may be more vulnerable to severe infection and more likely to be admitted for

care[48]. Underlying gastrointestinal dysfunction may also modify the clinical picture of norovirus and rotavirus infection. It is unclear if these children present differently than children without underlying gastrointestinal dysfunction. Their symptoms may last longer and they may experience more severe symptoms, such as prolonged diarrhea and vomiting uncharacteristic of classic norovirus gastroenteritis. Given the magnitude of this burden, additional research is needed to define the epidemiology of norovirus and rotavirus in this potentially vulnerable sub-population.

Methods and Materials

Inclusion/Exclusion Criteria

Residual stool samples submitted for bacterial stool testing were prospectively collected between July 9, 2012 and July 8, 2013 from two large tertiary care pediatric hospitals serving metropolitan Atlanta. The following inclusion criteria were used to create our cohort; children 0 to <18 years of age with vomiting and/or diarrhea. This includes both sporadic (no diarrhea in last 14 days) and nosocomial cases (onset of diarrhea ≥48 hours post admission). Underlying gastrointestinal dysfunction includes congenital malformation (e.g. Hirschsprung's disease, imperforate anus, Berdon syndrome), gut surgery (e.g. resection of the gut, short gut syndrome, ileostomy, colostomy), malignancy, inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis), and Celiac disease. Other diagnoses were included based on abstracting clinician's judgment (J. Yi). Exclusion criteria included: patients with chronic diarrhea (diarrhea lasting ≥2 weeks).

This study was approved by the Children's Hospital of Atlanta (#09-106) and Emory University (# 00062453) IRB committees.

Clinical Data

Demographic and clinical data was abstracted from electronic medical records for each stool sample. Abstracted data included admission/discharge dates, date of stool sample submission, basic demographic data (sex, age, race), symptomology (presence, duration, and frequency of fever, vomiting, and/or diarrhea), underlying gastrointestinal dysfunction, all underlying health conditions including health conditions causing immunosuppression (e.g., cancer, transplant). In addition, data was collected on current

and recent medication (e.g., antibiotics; past month, immunosuppressant; past year), rotavirus immunization status (2 or 3 shot series (Rotarix and RotaTeq respectively): full, incomplete, none), clinical laboratory testing results, and discharge diagnoses. For a full list of variables for which data was collected please see appendix for the clinical data abstraction form.

Symptomology was used to calculate Vesikari and modified-Vesikari scores. The scores represent clinical severity of illness and are based on the presence and duration of vomiting and diarrhea, fever, dehydration, and treatment status. Each factor is graded for severity on a scale of 1-3 for a total score between 0 and 20 [68]. Scores ≥ 11 are considered severe, 7-10 moderate, and <7, mild. The modified-Vesikari score uses the need for IV rehydration instead of percent dehydration because the degree of dehydration is not always recorded accurately. Based on the poor quality of dehydration data abstracted from electronic medical records, only the modified-Vesikari score was used in data analysis.

Stool sample submission date was used as a proxy for the onset of gastroenteritis symptoms. Time between admission date and stool sample submission was used to designate cases as community or hospital acquired, ≥ 48 hours between admission and order of stool sample was considered hospital acquired.

Stool Extraction and Real-Time RT-PCR

Stool was stored at -80 ˚C upon collection. A 20% stool suspension was prepared in RNAse/DNAse-free water. The lipids and other stool debris were removed by organic phase extraction using an equal volume of Vertrel XF (Dupont Chemicals, Wilmington, DE). After 2 hours at 4 ˚C, RNA was extracted from the aqueous phase using the

QiaAmp Viral RNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol.

GI and GII noroviruses were detected by real-time reverse transcription polymerase chain reaction (RT-qPCR) with the OneStep RT-PCR Kit (Qiagen, Valencia, CA) and genotype-specific, broadly reactive primers and probes developed by Kageyama et al. (2003).GI PCR reactions were comprised of 5 µL 5x buffer, 0.4 µM dNTPs, 0.4 µM COG1F, 0.4 µM COG1R, 0.3 µM Ring-1A (10 µM), 0.1 µM Ring-1B (10 µM), 0.25 µL RNAsin, 1 µL TQ mix, and 10 µL viral RNA for a total volume of 25 µL. GII PCR reactions were comprised of 5 µL 5x buffer, 0.4 µM dNTPs, 0.4 µM COG2F, 0.4 µM COG2R, 0.2 µM Ring-2, 0.25 µL RNAsin, 1 µL TQ mix, and 10 µL viral RNA for a total volume of 25 µL. Reactions were run in duplicate on a Stratagene MX 3000 sequence detection system (Agilent Technologies, Inc., Santa Clara, CA). Reaction conditions were as follows: reverse transcription 50 ˚C 32 min, polymerase activation 95 ˚C 10 min, 45 cycles of 94 ˚C 15 sec, 55 ˚C 15 sec, 60 ˚C 30 sec.

A full set of controls including a no template control, RNA from known positive and negative stool samples, and a GI or GII-specific standard (10^4 genomic copies) was run with every set of PCR reactions. A sample was considered positive if both reactions had Ct values less than 41. If a sample run in duplicate had a Ct value under 40 and a Ct value over 40, and the Ct value under 40 showed the potential for true amplification, the sample was repeated. If there was more than a 4 cycle difference between sample duplicate Ct values, the sample was repeated.

Conventional RT-PCR and Sequencing

All samples positive by RT-qPCR were re-extracted from stool and amplified using conventional reverse transcription polymerase chain reaction ($_C$RT-PCR) for sequencing. In an effort to better discriminate between norovirus strains, Region A (ORF1) and C (ORF2) were amplified. Region A was amplified using primers from Vennema et al. (2002) that result in a ~326 nucleotide amplicon for both GI and GII strains (Medici 2005). Region C was amplified using genotype-specific primer sets that amplify a ~330 (GI) or ~ 344 (GII) nucleotide amplicon (Kojima et al.; mattison et al.). Conventional RT-PCR reactions were run in duplicate on the CFX96 touch Real-Time PCR detection system (Bio-Rad Laboratories Inc., Hercules, CA) with the OneStep RT-PCR Kit (Qiagen, Valencia, CA). Reactions were run under the following reaction conditions: reverse transcription 50 ˚C 32 min, polymerase activation 95 ˚C 10 min, 35 cycles of 95 ˚C 20 sec, 37 ˚C (Region A) or 47 ˚C (Region D) 30 sec, 72 ˚C 40 sec, hold 72 ˚C 10 min. Region A PCR reactions were comprised of 6 µL 5x buffer, 0.4 µM dNTPs, 0.4 µM JV12Y, 0.4 µM JV13I, 1 µL TQ mix, and 6 µL viral RNA for a total volume of 30 µL. Region D PCR reactions were comprised of 6 µL 5x buffer, 0.4 µM dNTPs, 0.4 µM COG1F (GI) or COG2F (GII), 0.4 µM G1SKR (GI) or G2SKR (GII), 1 µL TQ mix, and 6 µL viral RNA for a total volume of 30 µL.

$_C$RT-PCR products with clear amplification products were purified with the QiaQuick PCR purification kit (Qiagen, Valencia, CA) per manufacturer's instructions. Samples with multiple amplification products were purified with Zymoclean Gel DNA Recovery kit (Zymo Research, Irvine, CA) per manufacturer's protocol. Samples were submitted to GeneWiz Inc. (Research Triangle Park, NC) for sequencing. Sample

sequences were classified using the Norovirus Genotyping Tool Version 1.0, developed by Kroneman et al. (2011) [106].

Rotavirus Testing

All stool samples were tested for rotavirus using the Premier Rotaclone (Meridian Bioscience Inc., Memphis, TN) qualitative EIA following manufacturer's instructions and read using a spectrophotometer at 450 nm. Samples were considered positive if they had an OD_{450nm} reading <0.15.

Data analysis

Data were analyzed using SAS (SAS Institute, Raleigh N.C.). Chi-square analysis, Fisher's exact and Wilcoxon t-tests were used to compare demographic characteristics and clinical symptoms between study groups using ($\alpha = 0.05$). Fischer's exact tests were used instead of Chi-square analysis when there were less than 5 observations per cell. Wilcoxon t-tests were used in lieu of student t-tests because the dependent variable is not assumed to be normally distributed.

In addition to basic descriptive statistics, several logistic regression models were run looking at symptomology and demographic variables modeling differences between NoV positive vs. negative stool, RoV positive vs. negative stool as well as NoV vs. RoV. Models were developed using a backwards elimination approach.

Results

Study Population

Two hundred and forty six children met the inclusion criteria, and 244 were included in the data analysis. Two were excluded because of missing rotavirus (RoV) testing results due to insufficient sample for testing. All children had some type of underlying gastrointestinal dysfunction and symptoms of acute gastroenteritis (diarrhea and, or vomiting) severe enough for a physician to order a stool culture. Within the study, 22% (54/244) had a surgical and, or congenital condition of the gut, 7% (18/244) had inflammatory bowel disease (IBD), 49% (119/244) were tube fed, 19% (46/244) had both a surgical/congenital condition and were being tube fed, and 3% (7/244) had both a surgical/congenital condition and IBD (Table 1). Thirty-six percent of cases (86/244) were considered hospital-acquired because the date of sample submission was >48hrs after hospital admission. Rotavirus accounted for the greatest number of viral detections, with 16.4% (40/244) of samples positive for RoV. Only 7.4% (18/244) of samples were positive for GII norovirus (NoV), and 1 sample (0.4%) was positive for GI NoV. There was a single NoV GII/RoV coinfection.

To evaluate whether viral gastroenteritis caused by NoV and RoV presents differently than other causes of acute gastroenteritis, as well as to determine whether cases of NoV and RoV acute gastroenteritis are distinguishable from one another, a series of comparisons were made for each demographic and clinical variable. To assess the characteristics of viral gastroenteritis due to NoV or RoV compared to any other cause, subjects with NoV or RoV positive stools (NoV/RoV positive) were compared to subjects whose stool samples were negative for NoV GI, NoV GII and RoV. To assess the

characteristics of acute gastroenteritis due to GII NoV, subjects with GII NoV positive stool samples (NoV GII positive) were compared to subjects whose stool samples were negative for GII NoV. This comparison group did include subjects with stool samples positive for GI NoV or RoV. To assess the characteristics of acute gastroenteritis due to RoV, subjects with stool samples that were positive for RoV (RoV positive) were compared to subjects with stool samples negative for RoV (which includes stool samples positive for NoV). Finally, to determine whether acute gastroenteritis due to NoV is different from that due to RoV, subjects with any NoV positive stool sample (GI and GII) were compared to subjects with stool samples positive for RoV. GI NoV infection was not independently assessed because only one sample was positive. In addition, a parallel analysis was conducted on just community acquired (sporadic) cases (n= 157, 64% of study group).

Demographics and Underlying Health Status

Children with a positive NoV/RoV stool sample were significantly younger (Mean: 4.2 years, 95% C.I.; 2.8, 5.6 years) than children with a negative stool sample (Mean: 6.1 years, 95% CI: 5.3, 6.9 years) (Wilcoxon T test, P = 0.0063) (Table 1). Although not statistically significant, this trend held for comparisons between subjects with NoV GII positive stools (Wilcoxon T test, P = 0.0793) and subjects with RoV positive stools (Wilcoxon T test, P = 0.0714). This was also true for community-acquired cases, where children with a NoV/RoV positive stool sample (Wilcoxon T test, P=0.00003), a RoV positive stool sample (Wilcoxon T test, P=0.0101), and a GII NoV stool sample (Wilcoxon T test, P =0.0483 respectively) were significantly younger than their respective comparison groups (Table 2).

RoV/NoV positive and RoV positive stool samples were more likely to be community acquired (χ^2, P= 0.0004, 0.0082 respectively). Level of care at admission (i.e. emergency department, intensive care unit, or floors inpatient) was not significantly different between children with positive and negative stool samples. There was no difference in the frequency of immunocompromising conditions or average absolute neutrophil count (ANC) between children with NoV/RoV positive and those with negative stool samples, however, children with NoV/RoV positive stool samples were significantly less likely to have used immunosuppressant medication in the past year (χ^2, P= 0.0300). This trend was also significant within the sporadic cases (χ^2, P= 0.0071). The use of antibiotics in the preceding month was not significantly different between patients with NoV/RoV positive stool samples and those with negative stool samples. Heart conditions were the only underlying disease that was distributed differently between children with NoV/RoV or RoV positive and their respective comparison groups (χ^2, P= 0.0238, 0.0562 respectively). Twenty two percent (4/18) and 23% (9/40) of children with GII NoV and RoV positive stool samples, respectively, had a heart condition compared with 10% (19/184) of children with NoV/RoV negative stool.

Symptomology

Compared to GII NoV negative stool samples, patients with GII NoV positive stool samples experienced a shorter period of emesis (Wilcoxon T test, P= 0.0527) but a higher number of emesis episodes per day (Wilcoxon T test, P= 0.0233) (Table 3). The duration of emesis was also shorter for patients with NoV positive stool samples (Mean, 1.4 days) compared to RoV positive stool samples (2.5 days)(Wilcoxon T test, P= 0.0417). The maximum number of diarrheal episodes within a 24 hour period was

significantly higher in patients with NoV positive stool compared to patients with RoV positive stool in the full study group. In the full study group (Wilcoxon T test, P= 0.0411), fever was less common in children with NoV GII positive stool samples compared to NoV GII negative stool samples (Fisher's exact test, P= 0.0288). Modified Vesikari scores were not significantly different between patients with NoV/RoV negative stool samples compared patients with positive stool samples. RoV positive vs. RoV negative stool samples also followed this trend (Wilcoxon T test, P= 0.004).

Within sporadic cases, duration of emesis was shorter for children with NoV GII positive stool compared to children with NoV GII negative stool samples (Wilcoxon T test, P= 0.0449) (Table 4). Children with a NoV/RoV positive stool sample also experienced more emesis episodes per day than children with a negative stool sample (Wilcoxon T test, P= 0.0239). The maximum number of diarrheal episodes within a 24 hour period was lower for patients with NoV positive stool (mean, 3.8 episodes) compared to patients with RoV positive stool (8.3 episodes) (Wilcoxon T test, P= 0.0216). Within sporadic cases, modified Vesikari scores were not correlated with either NoV or RoV positive stool samples.

Rotavirus Immunization

Rotavirus immunization was not significantly protective against rotavirus infection when comparing full immunization vs. none, or partial + full immunization vs. none in either the full study group (χ^2, P= 0.3228, 0.2639, full/none and partial + full/none respectively) or the sporadic cases (χ^2, P= 0.1767, 0.2062, full/none and partial + full/none respectively) (Table 5).

Modeling

Several logistic regression models were constructed to look at trends in symptomology and demographics between NoV positive vs. NoV negative stool, and RoV positive vs. RoV negative stool, as well as NoV positive vs. RoV positive stool. The only logistic regression model with a significant term was RoV positive stool sample by modified Vesikari score. A higher modified Vesikari score was predictive of a positive RoV stool. All other models had no remaining significant terms after backwards elimination.

Seasonality

Both NoV and RoV positive stool samples showed a strong seasonality, with the majority of positive samples occurring between December and April (Figure 1). RoV especially had a dramatic peak during March and April. It is important to note that between July 2012 and December 2012 only ~30% of stool specimens submitted to the CHOA labs were evaluated for inclusion in this study. From January 2013 to July 2013 ~80% of submitted tool specimens were evaluated for inclusion in this study.

Genotypes

Ten of 20 NoV positive samples could not be genotyped. For the remaining 10 samples, 9 were typed as GII.4 Sydney 2012 recombinant and 1 was typed as a GII.17.

Discussion

Overall, 16.4% (40/244) samples were positive for rotavirus (RoV), 7.4% (18/244) samples were positive for GII norovirus (NoV), and 1 sample was positive for GI NoV. There was a single NoV GII/RoV coinfection. Within the study, 22% (54/244) had a surgical and, or congenital condition of the gut, 7% (18/244) had inflammatory bowel disease (IBD), 49% (119/244) were tube fed, 19% (46/244) had both a surgical/congenital condition and were being tube fed, and 3% (7/244) had both a surgical/congenital condition and IBD (Table 1). Thirty-six percent of gastroenteritis cases (86/244) were considered hospital-acquired.

It is important recognize that all children in this study had some type of underlying gastrointestinal dysfunction and symptoms of acute gastroenteritis (diarrhea and, or vomiting) severe enough to seek care at a tertiary care facility and for a physician to order a stool culture. The underlying gastrointestinal dysfunction can cause symptoms of acute gastroenteritis. Given these factors, it is not surprising that there are not many significant differences in symptomology between positive and negative stool samples in this study.

Heterogeneity of Gastrointestinal Dysfunction

There was no difference in the distribution of underlying gastrointestinal dysfunction by stool sample test result. Specific types of gastrointestinal dysfunction were expected to lead to increased or decreased risk of infection but no significant relationship was observed. The lack of a net effect of gastrointestinal dysfunction on incidence or severity of disease could be a result of heterogeneity in underlying disease status. Given the range of diseases and disorders that fall under the label gastrointestinal

dysfunction, children were broadly divided up into 4 categories; surgical/anatomical conditions, inflammatory bowel disease, both, and other. Although these categories seem fairly straightforward, classifying gastrointestinal dysfunction is not that simple. For example, resectioning of the gut can occur for a variety of reasons, including an infection of the gut leading to necrotizing entercolitis, an anatomical defect such as Hirschsprung's disease, trauma from child abuse, or severe inflammation. While the main outcome is the same, a shorter gut, there may be small but significant differences in gut physiology and immunity post-resectioning that may lead to differences in susceptibility to infection and disease outcome. In addition, the effect of gastrointestinal dysfunction on mucosal immunity is not well studied. There is some evidence that long term parenteral nutrition leads to changes in immune function in the gut but this data has not been linked to increased risk of infection [107].

Another confounding factor related to the heterogeneity of gastrointestinal dysfunction was the severity of the underlying gut condition. Unfortunately there was no summary metric to categorize groups of diagnoses by severity. For example, length of gut removed during resection varies from patient to patient. Children with less tissue removed, who do not develop short gut syndrome may fare better than children with more gut tissue removed. In addition, two children may have the differing underlying conditions prompting resection but have the same length of gut removed, and these children could also fare differently. All of these confounding factors could explain why no relationship was observed between disease incidence, symptom severity and underlying gastrointestinal function.

Underlying health conditions and age

Similar to previous studies assessing the burden of RoV and NoV, children with RoV and NoV positive stools were on average significantly younger than children with negative stool. These findings are in line with age-specific trends of NoV and RoV in the U.S. Children under 5 years of age are the age group that experience the highest morbidity and are the most likely to seek medical attention for NoV and RoV gastroenteritis.

Few underlying health conditions were associated with either positive or negative NoV or RoV stool testing outcomes. In this study, there were no significant differences between children with positive and negative stool samples with regards to the presence of an immunocompromising condition or average ANC. Children with positive stool samples were actually less likely to be on immunosuppressant medication than children with negative stool samples. However, this result may be an artifact of study exclusion criteria. Children with chronic diarrhea (diarrhea lasting >14 days) were excluded. It is well known that immunosuppression has been linked to chronic, severe norovirus and rotavirus infection in adults and children that can last for months or even years [49, 50, 108, 109].

Cardiovascular disease was the only underlying condition associated with a positive stool sample. Children with positive stool samples were more likely to have a heart condition. This correlation has been reported previously; heart disease was found to be a risk factor of severe norovirus infection in a study of hospitalized patients as a result of decreased potassium levels [110].

Symptomology

Relative to other causes of acute gastroenteritis, the clinical presentation of acute gastroenteritis due to NoV and RoV are fairly similar. Since RoV and NoV were not compared separately to NoV/RoV uninfected cases, we expected to see smaller differences between GII NoV and RoV positive and negative stool samples. This was evident when comparing the symptomology of patients with a NoV or RoV positive stool sample. Duration of emesis and maximum number of diarrheal episodes per day were the only symptoms significantly different between patients with NoV and RoV positive stools. Duration of emesis was shorter in children with NoV positive stool samples. This trend of a shorter period of vomiting was also observed in children positive for GII NoV compared to GII NoV negative stool. These results support a previously observed finding that vomiting due to norovirus is less common in hospitalized patients and children under one year of age compared to otherwise healthy people with norovirus gastroenteritis [46, 58]. The modified Vesikari score tended to be higher for children with RoV positive stool samples. This result confirms previous findings that gastroenteritis due to rotavirus in children tends to be more severe than other types of gastroenteritis [72].

RoV immunization

Rotavirus vaccines are designed to protect against severe disease, not prevent infection [73]. Like noroviruses there are multiple rotavirus genotypes requiring a vaccine to have cross-reactivity between multiple genotypes [70]. Currently there are two vaccines on the market in the U.S., RotaTeq and Rotarix. Both live vaccines are administered orally during the first year of life. Rotavirus was not protective against rotavirus infection in this study, unlike the larger source study where the vaccine was protective. To date there have been no studies of vaccine effectiveness in children with

underlying gastrointestinal dysfunction. It is possible that the vaccine in not effective in these children as a result of gut physiology and, or immune function. Regardless, these children should still benefit from rotavirus immunization as a result of indirect vaccination effects [111]. It is also important to note that vaccine uptake was not high in this study group.

It is possible that some of the children with RoV positive stool samples are not experiencing rotavirus infection but are excreting vaccine virus as a result of recent immunization. The Rotaclone EIA detects surface antigens that are common to both wild-type and vaccine rotaviruses. Although this vaccine-virus shedding period lasts 3 to 9 days, in this study of children it could be responsible for a few false positive RoV positive stool samples [112]. Unfortunately the contribution of children with false-positive RoV stool samples could not be assessed as date of rotavirus immunization was not available.

Seasonality

Similar to norovirus, the burden of rotavirus annually peaks during the winter months and biennially as new strains emerge [70]. 2013 was a big year for rotavirus, in March of that year ~30% of cases reported to the National Respiratory and Enteric Virus Surveillance System (NREVSS) were positive for rotavirus antigen[71]. In this study both norovirus and rotavirus positive stool samples displayed their characteristic peaks during the winter months. Controlling for the winter peak in stool sample volume, 14 to 41% of samples were positive for norovirus or rotavirus between October 2012 and July 2013. Norovirus had a more gradual winter peak compared to rotavirus which had a large spike in March and April. It is possible that the smoother norovirus peak was due to the

early emergence of the epidemic GII.4 Sydney strain [9]. GII.4 Sydney was linked to outbreaks much earlier in the norovirus season than previous epidemic strains. The seasonal peaks observed in this study are similar to results of other studies examining the seasonality of these viruses.

NoV genotypes

NoV-positive samples were genotyped according to the standardized typing protocol suggested by Kroneman et al. (2013) that includes sequencing part of the RdRp on ORF1 (Region A) and part of ORF2 (Region C) [1]. Only 10 out of 20 NoV-positive samples could be genotyped using this protocol, 9 were categorized as GII.4 Sydney 2012 recombinant and 1 was typed as a GII.17. It appears that the new GII.4 Sydney recombinant emerged in December of 2012 [113]. The detection of GII.4 Sydney 2012 recombinants as opposed to the original GII.4 Sydney strain could be an artifact of our sample collection methods. Prior to January 2013 only ~30% of potentially eligible specimens were collected but after January 2013 ~80% of eligible specimens were collected. It is possible that the recombinant strain was detected exclusively in our study because of the shift in stool specimen recovery. Had there been higher recovery rates in the fall of 2012, we may have detected the original GII.4 Sydney strain.

Limitations

Due to the nature of this study, it was not possible to account for some potential biases in the data. For inclusion in this study, children had to have symptoms of diarrhea and, or vomiting severe enough for a physician to order to stool culture. However, children were excluded from this study if they had chronic diarrhea (diarrhea lasting >14 days). Chronic diarrhea was part of the exclusion criteria because noninfectious chronic

diarrhea is not uncommon in children with underlying gastrointestinal dysfunction and there was the risk of significantly expanding the study population if they were included. These inclusion/exclusion criteria most likely resulted in uncontrollable selection bias.

The inclusion/exclusion criteria in this study most likely altered the distribution of diarrhea and emesis symptoms in our study population which could have affected the symptomology analysis, as well as potentially affecting the outcomes of other variables in this study including the effect of immunosuppression on NoV and RoV infection. Chronic diarrhea is a well-known outcome of norovirus and rotavirus infections in immunocompromised patients [49, 50, 108, 109]. Had chronic diarrhea not been excluded in this cohort there may have been more NoV and RoV positive stool samples, and it may have been possible to detect an interaction between immune status and NoV and RoV infection.

In addition, the symptomology data was not robust. Retrospective chart review does not offer the most complete or accurate data for symptoms of gastroenteritis and dehydration. The abstracted data was dependent upon the parent or guardian's self-report of a child's symptoms prior to admission, which can be inaccurate.

It is important to note that between July 2012 and December 2012 only ~30% of stool specimens submitted to the CHOA labs were evaluated for inclusion in this study. From January 2013 to July 2013 ~80% of submitted tool specimens were evaluated for inclusion in this study. Although stool specimen collection does not seem to have suffered from systematic bias, the low rates of sample collection during the fall and early winter of 2012 could have lead us to miss some of the early emergence of GII.4 Sydney. This could explain the difference in detection rates between norovirus and rotavirus.

Strengths

A large sample size and in-house pathogen testing were the two major strengths of this study. This is the first study to look at children with underlying gastrointestinal dysfunction. The sample size was large enough to capture enough NoV and RoV cases to allow analysis of risk factors and symptoms. By performing NoV and RoV detection protocols in-house, samples with questionable test results could be rerun instead of labelling them inconclusive.

Future Works

Children with underlying gastrointestinal dysfunction are a heterogeneous population due the number and type of disorders and diseases that fall under its broad definition. More than likely, specific types of gastrointestinal dysfunction interact with NoV and RoV differently. Future studies are needed to elucidate specific mechanisms of gut dysfunction and immune function on enteric infections.

An area of norovirus epidemiology that needs further study is the role of norovirus in children with chronic diarrhea. At present no one is sure of the prevalence of norovirus in children with chronic diarrhea. Chronic norovirus infections may be overlooked in children with other conditions that make them prone to noninfectious diarrhea. There have been small studies looking at the duration of shedding and viral load over time in children with chronic norovirus gastroenteritis but no one has measured the proportion of chronic diarrhea that can be attributed to norovirus. Knowing the frequency of norovirus infection in children with chronic diarrhea could lead to more testing for norovirus in children with chronic diarrhea. Besides acting as potential reservoirs of

norovirus, it appears that persons with chronic norovirus infections are the site of norovirus evolution.

One aspect of norovirus epidemiology not addressed in this study was viral shedding. Without an exact date of onset for community-acquired cases there was no way to determine the stage of infection for given a patient. In addition, this study was limited to single samples from each subject and multiple samples over time are needed to determine the rates and dynamics of shedding in these patients. Future shedding studies would offer a view of the cause of norovirus infection in children with underlying gastrointestinal dysfunction.

Tables and Figures

Table 1. Demographic and Clinical Characteristics of Children with Underlying Gastrointestinal Dysfunction

Characteristic	Full Study[1] (n=244) No. (%)	NoV/RoV[2] (n=59) No. (%)	NoV GII[3] (n=18) No. (%)	RoV[4] (n=40) No. (%)
Gender				
Female	113 (46)		11 (61)	20 (47)
Race				
White	123 (50)		8 (44)	18 (45)
Black	73 (30)		3 (17)	16 (38)
Hispanic	33 (14)		6 (33)	4 (10)
Asian	8 (3)		1 (6)	1 (3)
Other	3 (1)		0 (0)	1 (3)
Unknown	4 (2)		0 (0)	1 (3)
Age (Year)				
<1	54 (22)		7 (39)	10 (25)
1-<5	86 (35)		8 (44)	20 (50)
5-10	44 (18)		1 (6)	2 (5)
>10	60 (25)		2 (11)	8 (20)
Mean (95% CI)	5.6 (5.0, 6.3)	*	3.6 (1.1, 6.1)	4.8 (2.8, 6.6)
Hospital-Acquired[3]				
Yes	87 (36)	*	3 (17)	**7 (18)**
Level of Care at Admission				
ED Outpatient	17 (7)		1 (6)	4 (10)
ICU Inpatient	52 (21)		4 (22)	6 (15)
Floors Inpatient	175 (72)		13 (72)	30 (75)
Previous Antibiotic Use[4]				
Yes	36 (15)		4 (22)	7 (19)
Immunosuppressant Medication[5]				
Yes	48 (20)	*	1 (6)	5 (13)
Immunocompromising Condition				
Yes	64 (26)		2 (11)	10 (25)
Degree of Infection Risk (ANC)[6]				
(≥1500)	172 (70)		13 (72)	30 (75)
Mild (1000 - <1500)	5 (2)		0 (0)	1 (3)
Moderate (500 - <1000)	1 (>1)		0 (0)	1 (3)
Severe (<500)	67 (27)		5 (28)	8 (20)
Gastrointestinal Dysfunction[7]				
Surgical/Congenital Condition	54 (22)		2 (11)	10 (25)
Inflammatory Bowel Disease	18 (7)		1 (6)	3 (8)
Tube Feeding	119 (49)		11 (61)	18 (45)
Surg./Cong. & Tube Feeding	46 (19)		4 (22)	9 (23)

Surg./Cong. & IBD	7 (3)		0 (0)	0 (0)
Additional Underlying Diseases				
Liver Disease	6 (2)		0 (0)	0 (0)
Prematurity[8]	35 (14)		4 (22)	4 (10)
Heart Disease	32 (13)	*	4 (22)	9 (23)
Lung Disease	47 (19)		1 (6)	5 (13)
Neurological/Developmental Conditions	128 (52)		9 (50)	18 (45)

[1]Full study includes all infected and uninfected patients excluding 2 observations where there was insufficient sample for rotavirus testing.

[2] Stool samples positive for GI or GII NoV or RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for both NoV and RoV.

[3] Stool samples positive for GII NoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for GII NoV. Note that this comparison group includes samples that were positive for RoV or GI NoV.

[4] Stool samples positive for RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for RoV. Note that this comparison group includes samples that were positive for NoV.

[3]Children with $\geq$ 48 hours between admission and stool sample submission were considered hospital acquired cases.

[4]Includes any antibiotic use in the past month.

[5]Includes any immunosuppressant medication in the past year.

[6]Absolute Neutrophil Count (ANC) was used as a proxy for infection risk, with lower counts indicating higher risk.

[7]Underlying gastrointestinal dysfunction was categorized as surgical and, or congenital (e.g. resection of the gut, Hirschsprung's disease, short gut syndrome), inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis), both (e.g. necrotizing entercolitis resulting in resectioning of the gut), or tube feeding.

[8]less than 37 weeks gestation.

Table 2. Demographic and Clinical Characteristics of Children with Underlying Gastrointestinal Dysfunction Classified as Sporadic Cases

Characteristic	Sporadic Only[1] (n=157) No. (%)	NoV/RoV[2] (n=49) No. (%)	NoV GII[3] (n=16) No. (%)	RoV[4] (n=33) No. (%)
Gender				
Female	63 (40)		8 (53)	14 (42)
Race				
White	80 (51)		7 (47)	14 (42)
Black	47 (30)		3 (20)	13 (39)
Hispanic	20 (13)		5 (33)	3 (9)
Asian	5 (3)		0 (0)	1 (3)
Other	3 (2)		0 (0)	1 (3)
Unknown	2 (1)		0 (0)	1 (3)
Age (Year)				
<1	31 (20)	*	**7 (47)**	**9 (27)**
1-<5	61 (39)		5 (33)	17 (52)
5-10	26 (17)		1 (7)	2 (6)
>10	39 (25)		2 (13)	5 (15)
Mean (95% CI)	5.6 (4.8, 6.5)		3.8 (0.8, 6.8)	3.9 (2.2, 5.7)
Level of Care at Admission				
ED Outpatient	17 (7)		1 (7)	4 (12)
ICU Inpatient	23 (15)		2 (13)	4 (12)
Floors Inpatient	177 (75)		12 (80)	25 (76)
Previous Antibiotic Use[4]				
Yes	33 (2)		4 (27)	7 (23)
Immunosuppressant Medication[5]				
Yes	29 (18)	*	1 (7)	**2 (6)**
Immunocompromising Condition				
Yes	37 (24)		1 (7)	7 (21)
Degree of Infection Risk (ANC)[6]				
(≥1500)	124 (79)		12 (80)	24 (73)
Mild (1000 - <1500)	4 (3)		0 (0)	1 (3)
Moderate (500 - <1000)	1 (1)		0 (0)	1 (3)
Severe (<500)	28 (18)		3 (20)	7 (21)
Gastrointestinal Dysfunction[7]				
Surgical/Congenital Condition	41 (26)		2 (13)	10 (30)
Inflammatory Bowel Disease	18 (11)		1 (7)	3 (9)
Tube Feeding	61 (39)		8 (53)	12 (36)
Surg./Cong. & Tube Feeding	32 (20)		4 (27)	8 (24)
Surg./Cong. & IBD	5 (3)		0 (0)	0 (0)
Additional Underlying Diseases				
Liver Disease	5 (3)		0 (0)	0 (0)
Prematurity[8]	23 (15)		4 (27)	4 (12)

Heart Disease	15 (10)	*	3 (20)	**7 (21)**
Lung Disease	25 (16)		1 (7)	4 (12)
Neurological/Developmental Conditions	77 (49)		8 (53)	14 (42)

[1]Only includes cases classified as sporadic

[2] Stool samples positive for GI or GII NoV or RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for both NoV and RoV.
[3] Stool samples positive for GII NoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for GII NoV. Note that this comparison group includes samples that were positive for RoV or GI NoV.
[4] Stool samples positive for RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for RoV. Note that this comparison group includes samples that were positive for NoV.
[3]Children with $\geq$ 48 hours between admission and stool sample submission were considered hospital acquired cases.
[4]Includes any antibiotic use in the past month.
[5]Includes any immunosuppressant medication in the past year.
[6]Absolute Neutrophil Count (ANC) was used as a proxy for infection risk, with lower counts indicating higher risk.
[7]Underlying gastrointestinal dysfunction was categorized as surgical and, or congenital (e.g. resection of the gut, Hirschsprung's disease, short gut syndrome), inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis), both (e.g. necrotizing entercolitis resulting in resectioning of the gut), or tube feeding.
[8]less than 37 weeks gestation.

Table 3. Symptomology of Children with Underlying Gastrointestinal Dysfunction

Characteristic	Full Study[1] (n=244) No. (%)	NoV/RoV[2] (n=59) No. (%)		NoV GII[3] (n=18) No. (%)	RoV[4] (n=40) No. (%)
Presence of Diarrhea					
Yes	208 (85)			17 (94)	34 (85)
Duration of Diarrhea (days)					
Med. (Max., Min.)	2 (1, 14)			2 (1,7)	2 (1, 7)
Max Diarrheal Episodes/Day[5]					
Med. (Max., Min.)	5 (1, 20)			2.5 (1, 10)	**7 (1, 20)**
Presence of Emesis					
Yes	134 (55)		*	13 (72)	27 (68)
Duration of Emesis (days)					
Med. (Max., Min.)	2 (1, 14)			**1 (1, 3)**	2 (1, 7)
Max Emesis Episodes/Day[6]					
Med. (Max., Min.)	2 (1, 20)		*	3 (1, 15)	4 (1, 20)
Fever[7]					
Yes	67 (27)			**1 (6)**	10 (25)
Modified Vesikari Score[8]					
Mild < 7	3 (15)			1 (50)	0 (0)
Mod. 7-10	12 (60)			1 (50)	3 (60)
Severe ≥ 11	5 (25)			0 (0)	2 (40)
Mean (95% CI)	8.7 (7.4, 10.0)			7.0 (0.0, 19.7)	10.4 (8.3, 12.5)

[1]Full study includes all infected and uninfected patients excluding 2 observations where there was insufficient sample for rotavirus testing.

[2] Stool samples positive for GI or GII NoV or RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for both NoV and RoV.

[3] Stool samples positive for GII NoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for GII NoV. Note that this comparison group includes samples that were positive for RoV or GI NoV.

[4] Stool samples positive for RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for RoV. Note that this comparison group includes samples that were positive for NoV.

[5]Maximum number of diarrheal episodes per 24 hour period

[6]Maximum number of emesis episodes per 24 hour period

[7]Fever classified as axillary temperature > 38 ◦C or 100.4 ◦F

8 [69]

Table 4. Symptomology of Children with Underlying Gastrointestinal Dysfunction Classified as Sporadic Cases

Characteristic	Sporadic Only[1] (n=157) No. (%)	NoV/RoV[2] (n=49) No. (%)	NoV GII[3] (n=16) No. (%)	RoV[4] (n=33) No. (%)
Presence of Diarrhea				
Yes	128 (82)		14 (93)	27 (82)
Duration of Diarrhea (days)				
Med. (Max., Min.)	2 (1, 14)		1.5 (1,7)	2 (1, 7)
Max Diarrheal Episodes/Day[5]				
Med. (Max., Min.)	5 (1, 20)		2 (1, 10)	7.5 (3, 20)
Presence of Emesis				
Yes	103 (66)		11 (73)	24 (73)
Duration of Emesis (days)				
Med. (Max., Min.)	2 (1, 14)		1 (1, 3)	2 (1, 7)
Max Emesis Episodes/Day[6]				
Med. (Max., Min.)	3 (1, 20)	*	5 (1, 15)	4 (1, 20)
Fever[7]				
Yes	43 (27)		1 (7)	8 (24)
Modified Vesikari Score[8]				
Mild < 7	2 (17)		1 (50)	0 (0)
Mod. 7-10	7 (58)		1 (50)	3 (60)
Severe ≥ 11	3 (25)		0 (0)	2 (40)
Mean (95% CI)	8.9 (7.0, 10.8)		7.0 (5.7, 19.7)	10.4 (8.3, 12.5)

[1]Only includes cases classified as sporadic

[2] Stool samples positive for GI or GII NoV or RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for both NoV and RoV.

[3] Stool samples positive for GII NoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for GII NoV. Note that this comparison group includes samples that were positive for RoV or GI NoV.

[4] Stool samples positive for RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for RoV. Note that this comparison group includes samples that were positive for NoV.

[5]Maximum number of diarrheal episodes per 24 hour period

[6]Maximum number of emesis episodes per 24 hour period

[7]Fever classified as axillary temperature > 38 ◦C or 100.4 ◦F

8 [69]

Table 5. Rotavirus Immunization in age eligible Children[1] with Underlying Gastrointestinal Dysfunction

		NoV/RoV[2]	NoV GII[3]	RoV[4]
Full Study	**(n=151) No. (%)**	**(n=45) No. (%)**	**(n=15) No. (%)**	**(n=30) No. (%)**
Full + Partial	62 (42)		8 (53)	10 (34)
None	84 (58)		7 (47)	19 (66)
Sporadic-Only Cases	**(n=98) No. (%)**	**(n=38) No. (%)**	**(n=12) No. (%)**	**(n=26) No. (%)**
Full + Partial	44 (46)		6 (50)	9 (36)
None	52 (54)		6 (50)	16 (64)

[1]Rotavirus immunization was not universally recommended in the U.S. until 2006. Only children young enough to have been eligible for rotavirus immunization were included in this analysis (>1 month and <6 Years of age).

[2] Stool samples positive for GI or GII NoV or RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for both NoV and RoV.

[3] Stool samples positive for GII NoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for GII NoV. Note that this comparison group includes samples that were positive for RoV or GI NoV.

[4] Stool samples positive for RoV, excludes GII/RoV coinfection. $\chi 2$, Fisher's exact, and Wilcoxon T tests were used to test for significant difference between this group and stool samples negative for RoV. Note that this comparison group includes samples that were positive for NoV.

Table 6. Distribution of Norovirus-Positive Samples by genotype

Genotype	(n=20) No.
GII.4 Sydney-recombinant	9
GII.17	1
Could not Type	10

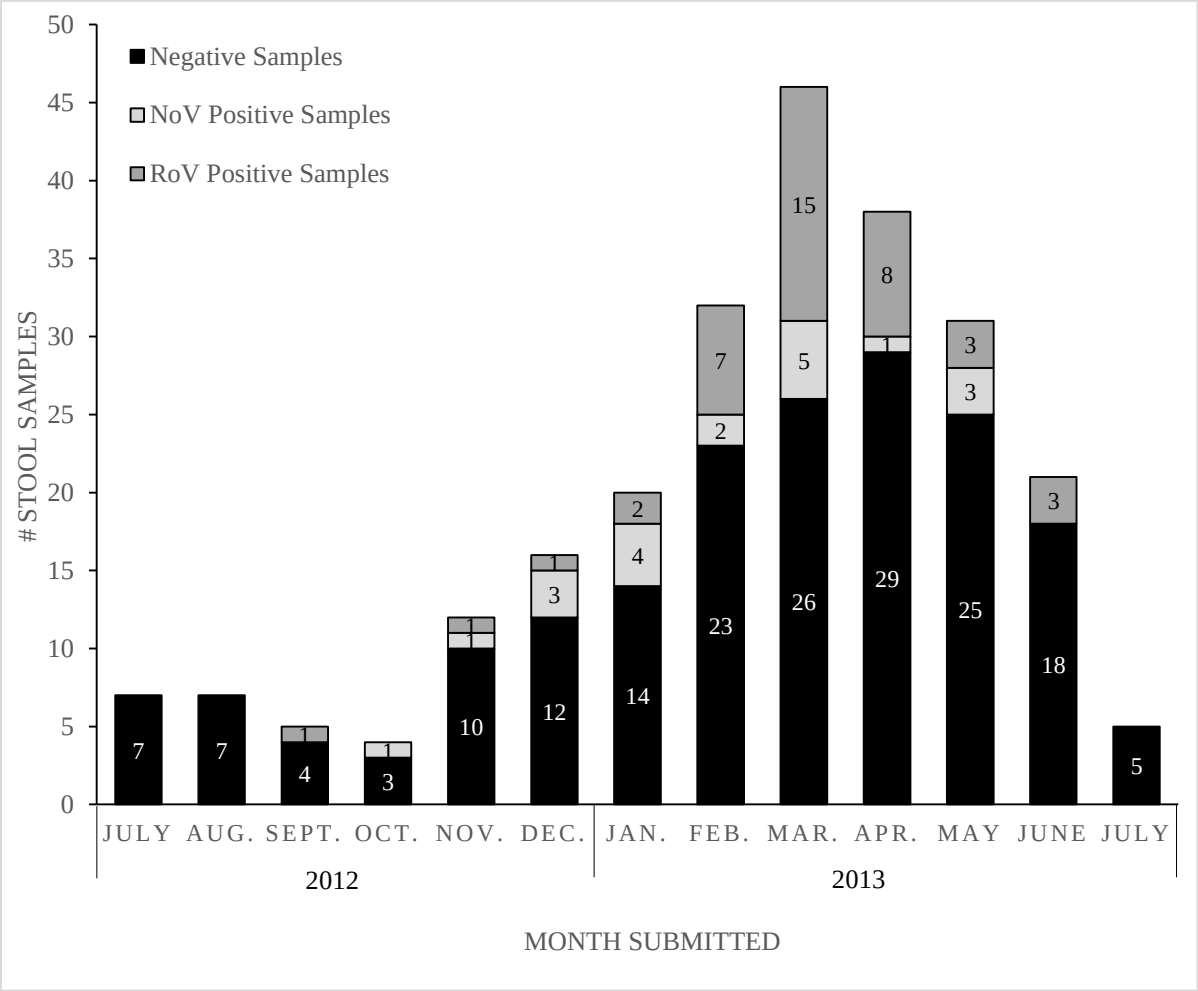

Figure 1. The distribution of stool samples by month of submission and test result. n_{total}= 244, $n_{uninfected}$ = 184, $n_{NoV\ pos.}$ = 20, $n_{RoV\ pos.}$ = 41.

Future Directions and Broader Implications:

Norovirus Immunization in Children with Underlying Gastrointestinal Dysfunction

In our study, rotavirus immunization was not significantly protective or harmful. Additional studies are needed to determine enteric virus vaccine effectiveness in children with underlying gut dysfunction. While children with underlying gastrointestinal dysfunction are a good group for norovirus immunization due to their potentially more vulnerable health status, these children may or may not be good candidates for a forthcoming norovirus vaccine because of immune system status.

Recently Takeda Pharmaceuticals finished a phase 1/2 trial of a bivalent norovirus virus-like particle (VLP) vaccine candidate[114]. VLPs are replicas of the viral capsid and stimulate a similar immune response to live virus but cannot replicate and cause infection because they do not contain genetic material. Results from the human challenge trial revealed that immunized persons experienced a reduction in the severity of disease including lower Vesikari scores, severity and duration of gastroenteritis symptoms. There was not a significant difference between the incidence of norovirus infection between the vaccine and placebo group. These results parallel the behavior of rotavirus vaccines. They lower the risk of severe disease but do not necessarily prevent infection. It will be interesting to see if children with underlying gastrointestinal dysfunction are protected directly by this norovirus vaccine candidate or when the vaccine becomes licensed, will experience indirect effects of norovirus immunization by reduced exposure to infected persons.

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Appendix

Appendix: Abstraction Form

Patient Information

MR#_____

Month and year of visit/admission: __/_____

Abstractioner initials:_____

Date of Abstraction:_____

Study #_____

Study #_____ Abstractioner initials:_____ Date of abstraction: ___/___/_____

DEMOGRAPHICS

Diagnosis:_____
_____
_____
_____
_____
_____

Age: __yr___mo **Sex:** __F __M

Patient status: ___inpatient ___outpatient (___ED ___clinic)

Level of care at admission if hospitalized: _____ inpatient unit _____intensive care unit

Transfer of service: ___no ___yes (which unit)_____

Hospital: ___Egleston ___Scottish Rite

Race/Ethnicity: __White __Black __Hispanic __American Indian/Alaska Native __Asian __ Native Hawaiian/Pacific Islander __Other __Unknown

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Gastroenteritis Classification

____ Nosocomial (onset of symptoms >72 hours of admission).

Time since admission (days, hours)_____

____ Sporadic/endemic (acute)

____ Chronic diarrhea (>14 days of symptoms)

(Check all that apply)

ROTAVIRUS VACCINATION: ____ never

Rotateq: ___partial (#doses:_____) ___complete (3 doses)

Rotarix: ___partial (#doses:_____) ___complete (2 doses)

Vesikari Score:_____

Modified Vesikari Score:_____

SIGNS/SYMPTOMS AT PRESENTATION

STOOL:

Diarrhea duration: ___ d ___hr **Frequency (max in 24 hr):** _____

Stool quality: __solid/pasty __ loose/formed elements __watery/liquid

Blood present? __yes __no **Mucus present?** __yes __no

EMESIS:

Vomiting duration: ___d ___hr **Frequency (max in 24 hr):** _____

Blood present? __yes __no **Bile present:** __yes __no

***Fever*: ≥38°C (≥100.4°F):** __no __subjective __measured (Tmax _____)

Nausea: __yes __no

Abdominal pain/cramping: **__**yes __no

Headache: __yes __no

Anorexia: __yes __no

Other/Complications (e.g-seizure, diaper rash, rectal problems, renal failure, bowel perforation, death):
_____
_____
_____

IMMUNOCOMPROMISING CONDITION:

___ None

___ Solid organ transplant
(___kidney____liver____heart___other:_____)

___ Cancer,
type:_____
_____

___ Bone Marrow Transplant (HSCT): ____allogeneic _____autologous

Study #_____ Abstractioner initials:_____ Date of abstraction:___/___/_____

___GVHD_____
_____

___ Other:
_____
_____

Time since transplantation or date of cancer
diagnosis:_____

Time since induction chemotherapy or
radiation:_____

Time since most recent chemotherapy or
radiation:_____

Immunosuppressive medications:

_____
_____
_____
_____
_____

PAST HISTORY AND RISK FACTORS

PMH: ___ previously healthy or

_____

Diet: ___breastfed ___bottlefed ____Tube feeding ___normal diet

Sick contact: ___no __yes (how long ago?)_____

Daycare/School: ___no ___yes

Travel: ____ no____yes (where, when, how long?)_____

Previous antibiotics indications and duration:___ no___ yes _____

Medications:

_____
_____
_____
_____
_____

HYDRATION STATUS

Current weight (kg): _____ Previous weight (kg:)_____ weight loss? ____yes_____ no (if yes, difference in kg):_____

Temperature: _____ Heart rate:_____ Blood Pressure: Hypotensive? ___no __yes, measurement ___/____

Breathing (breaths/min): ____, deep? __no ___yes

Urine output: ___normal ___decreased ___minimal

Tears: ___present ___decreased ___absent

Thirst: ___drinks normally, might refuse liquids ___thirsty, eager to drink ___drinks poorly, unable to drink

Mental status: ___ well, alert, normal ___fatigued or restless, irritable ___apathetic, lethargic, unconscious

Mouth and tongue: ___moist ___dry ___parched

Pulses: __normal __ normal to decreased ___ thready, weak, or impalpable

Capillary refill: ___normal ___about 2 sec/prolonged ___>3sec

Eyes: ___normal ___slightly sunken ___deeply sunken

Skin: ___instant recoil ___recoil <2sec ___recoild >2 sec

Extremities: ___ warm ___cool ___cold, mottled, cyanotic

Estimated percentage of dehydration:_____

Study #_____ Abstractioner initials:_____ Date of abstraction:___/___/_____

MANAGEMENT:

Rehydration: ___no ___ORT and discharged ___ IV rehydration and discharged ___ORT,IV rehydration and discharged

___ IV rehydration and hospitalized ___ORT and IV rehydration and hospitalized

If nosocomial: ____IV rehydration _____ORT rehydration

Diet: ___continuous, early feeding or regular diet ___modified diet ___other
(_____)

Total parenteral nutrition (TPN): ___no ____yes
(duration?)_____

Immunosuppressive medications (dose reduction):

1. _____(_____%)
2. _____(_____%)
3. _____(_____%)
4. _____(_____%)
5. _____(_____%)

Medications given during ED stay or hospitalization:

Antibioitic:_____

Antiemetic:_____

Antidiarrheal:_____

Probiotic: _____

SIGNS AND SYMPTOMS DURING HOSPITALIZATION

Days and hours hospitalized :___days ____hrs

Complications:_____
_____

LABS

STOOL STUDIES:

Test: stool culture: __/__/____ Result: _____

Test: ova and parasites: ___/___/____ Result:_____

Giardia EIA: __/___/____ Result:_____ Cryptosporidium Ag:___/___/____
Result:_____

Test: C. diff toxin PCR: ___/____/____ Result:_____ B027/NAP1/B1?:___no___yes

Test: adenovirus: __/__/____ Result: _____

Test: rotavirus at hospital: __/__/____ Result:
_____

Test: norovirus: __/__/____ Result: _____
Genogroup/Genotype:_____

Test: rotavirus in lab:__/___/____ Result:_____

Other positive stool: __/__/____ Result:
_____

WBC: _____ H/H:_____
Plt:_____DIFF:_____

Study #_____ Abstractioner initials:_____ Date of abstraction:___/___/_____

Electrolytes: Na:_____K:_____ Cl:_____HCO3:_____ BUN:_____Cr:_____Glu: _____ Other abn findings (LFTs,etc):_____

ESR:_____ CRP_____

Radiographic studies,pathology, and interventions:

____Abdominal XRay_____

____Abdominal Ultrasound_____

____CT_____

____Colonoscopy_____

Histopathologic
findings_____

_____CMV associated disease GI evaluation_____

_____GVHD (stage?)_____

____Surgery_____
