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Norovirus and Rotavirus Prevalence in Immunocompromised Patients and Nosocomial Infections in Egleston and Scottish Rite Children's Hospitals in Atlanta, GA

By

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Master of Public Health

Environmental Health

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# Norovirus and Rotavirus Prevalence in Immunocompromised Patients and Nosocomial Infections in Egleston and Scottish Rite Children's Hospitals in Atlanta, GA

By

Naeemah A. Munir

B.S.
Emory University
2011

Thesis Committee Chair: Pengbo Liu, PhD

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 Environmental Health
2012

## Abstract

Norovirus and Rotavirus Prevalence in Immunocompromised Patients and Nosocomial Infections in Egleston and Scottish Rite Children's Hospitals in Atlanta, GA

By

Naeemah A. Munir

*Background:* The introduction of the rotavirus vaccine in 2006 has decreased acute gastroenteritis (AGE) cases among children. The relative contribution of norovirus infections after the vaccine implementation has not been well described, particularly in immunocompromised patients and nosocomial AGE infections. Furthermore, immunocompromised patients may be a source of nosocomial infections due to these patients' lengthy hospital stays, prolonged viral shedding, and low infectious dose and environmental persistence of norovirus.
*Methods:* Stool specimens were collected between December 2009 and December 2010 from two children's hospitals: Egleston and Scottish Rite, in Atlanta, Georgia. We reviewed patients' electronic medical records for inclusion criteria of diarrhea, immunocompromised status, and/or nosocomial status. Our inclusion criterion for diarrhea was defined as ≥3 watery or looser-than-normal stools within a 24-hour period, or forceful vomiting with any loose stools. Immunocompromised patients were defined as having cancer, neutropenia, or transplant. Nosocomial cases were defined as diarrheal onset at least 48 hours after hospital admissions. Norovirus and rotavirus were tested in stool samples using RT-PCR and ELISA, respectively.
*Results:* A total of 111 patients were consisted in this study, with 59 (53.2%) immunocompromised, 31 (27.9%) nosocomial cases, and 21 (18%) both immunocompromised and nosocomial cases. We detected norovirus infections in 18 (16.2%) and rotavirus infections in 2 (1.8%) patients. All norovirus infections were GII, with 10 (55.6%) being GII.4 strain. Norovirus infections occurred between December and April. Among the enrolled patients, 20% received the rotavirus vaccine and 65% were born before the vaccine introduction, and were thus ineligible for rotavirus vaccination. There were no significant differences regarding the clinical symptoms between norovirus positive AGE and norovirus negative AGE. When analyzing the transmission source of norovirus nosocomial infection, there were no immunocompromised norovirus infections that preceded nosocomial cases, suggesting that immunocompromised norovirus infections are not the source of nosocomial norovirus infections.
*Conclusion:* Norovirus was the most prevalent etiology in immunocompromised AGE patients and nosocomial AGE infections in two pediatric hospitals in Atlanta. Our findings provide supporting evidence for a norovirus vaccine, development of a rapid norovirus assay, and enforcement of stricter appropriate hygiene policies to decrease nosocomial infections.

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**Acknowledgements**

This study was supported by a Children's Healthcare of Atlanta Friend's Research Fund award to Dr. Andi Shane (2009-2010). We thank Avigail Banguilan, Calista Fchenck, Sepehr Hosseini, and Lisa Spitz from the Children's Healthcare of Atlanta (CHOA) microbiology laboratories for collection and separation of fecal specimens. We would also like to thank several advisors who contributed throughout the study: Dr. Pengbo Liu, Stefanie Sarnat, Julia Sobolik, and Dr. Paul Gastañaduy. Additionally, we thank Vijay Ram for assisting with rotavirus detection and data entry, Jeffery Bale for assisting with norovirus detection, and Sereena Gillani for assisting with data entry.

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## Introduction

Acute gastroenteritis is a major cause of morbidity and mortality among children worldwide. In the United States, acute gastroenteritis accounts for more than 1.5 million outpatient visits, 200,000 hospitalizations, and estimated 300 deaths each year [1]. Before a rotavirus vaccine was developed, rotavirus was the leading cause of acute gastroenteritis hospitalizations among pediatric populations, followed by norovirus [2].

Globally in the pediatric population, rotavirus was responsible for 111 million episodes of acute gastroenteritis that required home care, 25 million episodes that required clinical visits, and 2 million episodes that required hospital visits, annually. Approximately 440,000 deaths due to rotavirus infection occurred annually in children under the age of 5 years. Previous studies indicated that nearly every child would have an episode of rotavirus acute gastroenteritis by age 5 [3] . Norovirus has been less of a burden compared to rotavirus, yet remains a common cause of gastroenteritis. In the United States, roughly 21 million norovirus cases occur each year, with 25% being foodborne. In developed and developing countries, norovirus infections are responsible for 10-15% of severe gastroenteritis cases in children under the age of 5 years [4].

A rotavirus vaccine, RotaTeq®(Merck) was introduced to the market in 2006. During the first randomized clinical trial, hospitalizations and emergency department visits were reduced by 94.5% and the vaccine was 98% effective against severe acute gastroenteritis caused by rotavirus [5]. In 2010, RotaTeq® received WHO prequalification status and is now administered in Africa, Asia, Europe, Latin America, and the United States [6]. Currently, there is no commercial norovirus vaccine available.

After the implementation of the rotavirus vaccine in the US, the dynamics and prevalence of the two leading pediatric acute gastroenteritis agents are changing. Two years after introduction of the vaccine, a 50% decline in the magnitude of acute gastroenteritis due to rotavirus was reported by the Centers for Disease Control and Prevention [7].

Previously in Atlanta, GA, we assessed the prevalence of norovirus and rotavirus infections in previously healthy pediatric patients admitted to the emergency department or outpatient clinic for acute gastroenteritis. Of the previously healthy population, 22.5% of diarrheal cases were caused by norovirus, while only 5.4% of diarrheal cases were caused by rotavirus [personal communication with Paul Gastañaduy]. The results from this previous study reflect the viral dynamic shift between norovirus and rotavirus. In the current study, we follow-up on our previous work to examine the infection status of rotavirus and norovirus in two subpopulations: immunocompromised patients and nosocomial diarrheal patients.

Rotavirus and norovirus are important pathogens of nosocomial (hospital-acquired) gastroenteritis infections. Several biologic traits make norovirus a successful nosocomial pathogen: a low infectious dose (10 to 100 particles), relative resistance to disinfectants, and prolonged survival in the environment [8]. Few studies have assessed the role of norovirus as a cause of healthcare-acquired gastroenteritis among US pediatric patients. In a study performed in United Kingdom, enteric viral pathogens were identified in 53% of nosocomial infection: 31%-rotavirus, 16%-norovirus, and 15%-adenovirus [9]. Recent surveillance data from six European countries has shown that rotavirus is the main cause of nosocomial pediatric diarrhea, with a prevalence ranging between 31% and

87%, followed by norovirus, accounting for 17-46% of the diarrheal cases [10]. These two studies were completed before the introduction of the rotavirus vaccine.

A possible source of norovirus nosocomial infections may derive from patients with norovirus infection and underlying conditions such as impaired immunity, which result in lengthy hospital stays and prolonged norovirus excretion. A recent report described a chronic diarrhea case and prolonged norovirus excreting during a 114-day period in a 10-month old baby after combined liver, pancreas, and small bowel transplant [11]. Similarly, a recent publication describes nine pediatric cancer patients with prolonged norovirus shedding ranging from 22-433 days after the onset of symptoms; the symptoms for all but one ceased well before the viral shedding ceased [12]. More importantly, three immunocompromised chronic norovirus shedders have recently been shown by molecular techniques to be the source of hospital outbreaks in a large tertiary care hospital in the Netherlands [13].

Given the high prevalence of norovirus infections, the increasing population of immunocompromised patients in the US, and a decrease in the contribution of rotavirus to nosocomial disease due to vaccine effects, the relative contribution of norovirus to nosocomial diarrhea may increase. Since there is limited information regarding the prevalence of norovirus among immunocompromised patients and nosocomial cases in pediatric populations, we aimed to examine the clinical and molecular epidemiology of norovirus-associated gastroenteritis among immunocompromised patients and norovirus nosocomial infection in the pediatric hospital system of Atlanta. Specifically, our current project has two main objectives:

1) To determine the prevalence of norovirus and rotavirus among immunocompromised and nosocomial gastroenteritis infections in a pediatric population.

   Hypothesis: Norovirus is more prevalent than rotavirus among immunocompromised gastroenteritis patients and nosocomial gastroenteritis cases.

2) To identify immunocompromised patients as a source of nosocomial infections by spatial, temporal, and genomic factors.

   Hypothesis: Norovirus infections among immunocompromised patients are a source of norovirus nosocomial infections.

## Methods

### *Stool Sample Collect and Clinical Informatics Acquirement*

Residual stool specimens submitted for clinical diagnostic purposes were prospectively collected from December 2009 to December 2010 in the laboratories of two large pediatric hospitals in metropolitan Atlanta: Egleston and Scottish Rite. Stool samples were collected and stored at -80°C until tested. Clinical information for each patient was abstracted from electronic medical records including admission/discharge dates, room number, basic demographics (age, sex, race), current health condition (transplant patient, cancer patient), severity of diarrhea (duration, maximum episodes of stool and vomit in 24 hours, presence of blood or mucous), medications/treatment plans, and additional clinical test results. Rotavirus immunization information was obtained from Georgia immunization registry (GRITS) for Georgia residents.

### *Inclusion Criteria*

Electronic medical records of each patient were reviewed for inclusion and exclusion criteria. Inclusion criteria for immunocompromised diarrhea in this study were children with acute gastroenteritis (defined as ≥3 watery or looser-than-normal stools within a 24-hour period, or forceful vomiting with any loose stools), less than 18 years, and an immunocompromising condition (e.g. cancer and associated neutropenia, or solid organ or bone marrow transplant). Inclusion criteria for nosocomial diarrhea was defined as diarrheal onset at least 48 hours after hospital admission in children less than 18 years.

### *Stool Suspension Preparation*

Briefly, a 20% stool suspension was prepared in RNAse- and DNAse-free water (wt/vol). After thoroughly vortex, the solution was mixed with equal volume of Vertrell

(Dupont Chemicals, Wilmington, DE). The final solution was incubated for 2 hours at 4°C.

*Viral RNA Extraction*

RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA.). Briefly, the mixture of stool suspension and Vertrell was first centrifuged at 10,000R RPM for 10 minutes at 4°C. A 140-µl supernatant was removed and mixed with lysis buffer with carrier RNA added. After a 10-minute room temperature incubation, 560 µL 100% ethanol were added to the solution. The solution was then transferred to a QIAamp spin column. After centrifugation, buffers AW1 and AW2 were added to the spin column to wash the column, respectively. After spinning the column at 13,000 RPM) for 1 minute, 50 µL of Buffer AVE was added to the column and incubated for 5 minutes at room temperature and was subsequently centrifuged at 9,800 RMP for 1 minute, producing 45 µL of RNA.

*Real Time RT-PCR*

Norovirus genogroups I and II were separately tested by Qiagen OneStep RT-PCR Kit (Qiagen, Valencia, CA) incorporated with our in-house primers/probes [COG1 F (sequence: CGY TGG ATG CGN TTY CAT GA), COG1 R (CTT AGA CGC CAT CAT CAT TYA C), COG2 F (CAR GAR BCN ATG TTY AGR TGG ATG AG), COG2 R (TCG ACG CCA TCT TCA TTC ACA)] and probes [RING1 (A)-TP (d FAM- AGA TYG CGA TCY CCT GTC CA-TAMRA), RING1 (B)-TP (d FAM- AGA TCG CGA TCT CCT GTC CA-TAMRA), RING2-TP (d FAM- TGG GAG GGC GAT CGC AAT CT-TAMRA)] [14]. Two PCR mixtures were prepared for detecting GI and GII noroviruses, respectively. For GI norovirus, a PCR mixture including 4.75 µL of RNAse-

free water, 5.0 µL of 5x Qiagen oneStep buffer, 1.0 µL of dNTP, COGIF, and COG1R, 0.75 µL of RING1 (A)-TP, 0.25 µL of RING1 (B)-TP and RNAse inhibitor, and 1 µL of Qiagen RT-PCR enzyme mixture of HotStart Taq DNA polymerase and reverse transcriptase was created and 10 µL of RNA were subsequently added to the mixture. For GII norovirus, 5.5 µL of RNAse-free water, 5.0 µL of 5x Qiagen oneStep buffer, 1.0 µL of dNTP, COG2F, and COG2R, 0.5 µL of RING2-TP, 0.25 µL RNAse inhibitor, and 1 µL of Qiagen RT-PCR enzyme mixture of HotStart Taq DNA polymerase and reverse transcriptase were thoroughly mixed to create a 15 µL PCR solution. 10 µL of viral RNA was added to the 15 µL PCR mixture. The RT-PCR was conducted on the Stratagene MX 3000 sequence detection system. Reverse transcription was performed at 50°C for 32 minutes; then polymerase activation and reverse transcriptase inactivation was performed at 95°C for 10 minutes; finally, 45 cycles of amplification were carried out at 95°C for 15 seconds and 56°C for 1 minute (for each cycle). Each sample was tested twice and concordant detection less than 38 cycles was considered positive.

*Conventional RT-PCR and Electrophoresis*

Subsequently, norovirus positive samples were re-amplified via conventional RT-PCR. Since there were only GII positive norovirus samples detected, only one PCR mixture was prepared including 6.0 µL of 5 x buffer, 1.2 µLs of dNTP, COG2 F, G2 SKR (sequence: CCRCCNGCATRHCCRTTRTACAT) [15], and Qiagen RT-PCR enzyme mixture of HotStart Taq DNA polymerase and reverse transcriptase, 0.3 µL of RNAse inhibitor, and 12.9 µL of RNAse-free water, producing a total volume of 24.0 µL A total of 6.0 µL of RNA was added to the mixture. Conventional RT-PCR occurred in a thermocycler at 50°C for 32 minutes during reverse transcription; then 95°C for 15

minutes for polymerase activation and reverse transcription deactivation; followed by 35 cycles of amplification were carried out at 95°C for 20 seconds, 50°C for 30 seconds, and 72°C for 40 seconds; and finally, ending the program at 72°C for 7 minutes. The finished conventional RT-PCR product was resolved on a 2% agarose gel stained with ethidium bromide and visualized under ultra-violet light to detect sufficient amplification for sequencing (Figure 1).

*Sequence Analysis*

Norovirus sequences were aligned with reference sequences from the GenBank database using Clustal W program (MEGA version 4, Tamura, Dudley). Genotypes were identified using the neighbor-joining method of phylogenetic analysis (Figure 2).

*ELISA*

Rotavirus was detected by a commercial enzyme -linked immunosorbent assay (ELISA) kit (Premier™ Rotaclone, Meridian Bioscience, Cincinnati, OH). This monoclonal antibody assay rapidly detected the product of the sixth viral gene (VP6), a specific antigen that is in all known human rotaviruses. A 10% stool suspension was added to the wells with anti-rotavirus monoclonal antibody conjugated to horseradish peroxidase and incubated for 60 minutes at room temperature. After incubation, the wells with samples were washed 5 times with de-ionized water to remove unbound enzyme labeled antibodies. Urea peroxide and 3,3', 5,5'-Tetramethylbenzidine (TMB) were added to the wells. After a 10-minute incubation at room temperature, the enzyme bound wells converted to colorless-indicating no rotavirus antigens present- or blue color- indicating presence of rotavirus antigen.

*Statistical Analysis*

Data was entered twice in Epi Info™ (version 3.5.1, August 13, 2008) and cleaned in Microsoft Excel 2010. Epi Info™ and SAS® (version 9.3) were used for statistical analysis. To address objective one, we calculated and compared the prevalence of norovirus and rotavirus infections in the immunocompromised and nosomcial patient groups. To address objective two, we graphed norovirus nosocomial infections spatially, by hospital department, and temporally, by onset of symptoms and hospital stay duration. We then added to this graph all immunocompromised norovirus infections and other norovirus infections that we had information on from our prior study of previously healthy patients. Using this graph, we assessed whether the nosocomial infectious could be attributed to specific known preceding norovirus infections. Further analysis was conducted to determine significant differences in diarrheal severity between norovirus positive and norovirus negative acute gastroenteritis. We used two sample t-tests with an alpha level of 0.05. Variances were not equal based on the graph of the distribution of continuous variables and therefore we reported the Satterthwaite method of unequal variances. A p value $<0.05$ was considered to be statistically significant.

## Results

*Study Population*

A total of 111 patients met our study criterion and their respective stool samples were tested. The majority of patients were immunocompromised (72%), yet there was some overlap between immunocompromised patients and nosocomial cases (Table 1). Two-thirds of the patients were enrolled from the Egleston Hospital (Table 1). 46.8% of the study population was female and the same percentage of patients was under the age of 5 (Table 2). The mean age for the study population was 6.52 (±5.45) years, with a mode of 2 years of age (n=14, 12.6%).

Among the entire study population, 61 (55%) patients were currently or previously taking antibiotics. A smaller proportion of the study population, 34.2%, was currently on immunosuppressive medication (Table 2). 65 subjects (58.6%) were born before 2006 and thus were ineligible for the rotavirus vaccine. Of the remaining 46 patients, 22 (19.8%) received the rotavirus vaccine (Table 2).

Of all immunocompromised patients, 44 (54.3%) were cancer patients; and 11 cancer patients received bone marrow transplants and 1 received a solid organ transplant (Table 3). Among the immunocompromised patients who had nosocomial diarrhea, 17 (81%) were cancer patients. Of those cancer patients, 47.1% also had bone marrow transplants. 14.3% of immunocompromised and nosocomial patients had solid organ transplants.

Most patients stayed in the hospital for less than two weeks, however the majority (38.1%) of immunocompromised patients with nosocomial gastroenteritis stayed in the hospital between one and two months (Table 4). The gastroenteritis symptoms among

immunocompromised patients mainly lasted three days or less (33.9%) whereas the majority of nosocomial cases persisted between 4 to 8 days (58.1%). Approximately half of immunocompromised and nosocomial patients did not have episodes of emesis during their gastroenteritis illness. A quarter of nosocomial cases acquired fever whereas 61% of immunocompromised and 52.4% of immunocompromised patients with nosocomial AGE had fever (Table 4).

*Norovirus and Rotavirus Prevalence*

A total of 18 (16.2%) norovirus infections and 2 (1.8%) rotavirus infections were detected among the study population (Table 5). 14 norovirus infections were among immunocompromised patients and 4 were among nosocomial cases, and 1 was an immunocompromised, nosocomial diarrhea case (Table 5). The two patients with rotavirus infections had nosocomial diarrhea and one of which was also immunocompromised. The 18 norovirus infections were classified as GII genogroup and the majority (55.6%) was GII.4 (Table 7, Figure 2). Two thirds of the norovirus infections occurred in children under the age of 5 years. Of the patients with norovirus infections, one third was on immunosuppressive medication during the infection and two thirds received antibiotics prior to the onset of symptoms. 7 norovirus positive patients were cancer patients, 2 of whom were also bone marrow transplant patients; 6 received solid organ transplants; and 1 received a bone marrow transplant only. All norovirus infections occurred between December 2009 and April 2010, with the majority of infections occurring in February and March (Figure 3). The 2 rotavirus infections occurred in December and January.

Followed by norovirus, *Clostridium difficile (C. diff)* was the 2nd most prevalent etiology in the study affecting 11 patients including 10 immunocompromised patients and 1 nosocomial AGE patient. Two patients with *C. diff* had previously received antibiotics and 1 was on immunosuppressive medication. 81 (73%) of collected specimens had stool culture results, with 4 having positive growth. All bacterial caused diarrhea occurred in immunocompromised patients, with 1 being a nosocomial case as well. The detected enterophathogens were *Campylobacter spp.* and *Staphylococcus aureus* (Table 5). The bacterial infections occurred in the later months of the year (Figure 3).

There were no significant differences between the diarrheal severity between the 18 norovirus positive cases and the remaining 93 norovirus negative diarrheal cases. A higher percentage of norovirus positive patients had chronic diarrhea (diarrheal symptoms persisting more than 2 weeks) compared to norovirus negative patients (27.8 % and 15.1%) (Table 5). Norovirus infected persons were slightly younger than non-norovirus infected persons (5.22 years vs. 6.77 years) (Table 6).

*Immunocompromised patients as a source of nosocomial norovirus infection*

Of the five norovirus nosocomial cases, two occurred in Egleston hospital and three occurred in Scottish Rite. Four patients were one year old or younger. The other patient was a 17 year old who also had Acute Lymphoblastic Leukemia. The diarrheal onset of these cases ranged from 5 days after admission to 1 month after admission. In contrast to our hypothesis, no immunocompromised norovirus infections preceded the nosocomial norovirus infections (Figures 4, 5). However, we did have information on three norovirus infections from previously healthy patients in the previous study that preceded nosocomial infections in Scottish Rite and two infections from previously

healthy patients that preceded nosocomial infections in Egleston. In Egleston, one nosocomial case occurred in the cardiac department and no other norovirus positive infections occurred in that department. In the 4-West department, a previously healthy patient with a GII.4 norovirus strain was discharged on December 25th 2009 and a nosocomial infection of the same strain occurred on January 30th 2010 after being admitted on January 24th (Figure 4). In Scottish Rite, two nosocomial cases occurred in the intensive care unit. The two patients stay overlapped for three weeks; however, one strain was unable to be sequenced and thus it is unknown whether they infected with the same genotype. In 1-East West department, a previously healthy patient was discharged on December 21st 2009 with a GII.4 strain and on January 13th 2010, symptoms began for a nosocomial norovirus infection in a patient who was admitted on January 8th (Figure 5).

## Discussion

*Study Population*

Our study assessed the prevalence of norovirus and rotavirus infections among immunocompromised patients with acute gastroenteritis (AGE) and patients with nosocomial AGE in two pediatric hospitals (Egleston and Scottish Rite) in Atlanta, Georgia. 111 subjects, either with an immunocompromising condition (cancer, transplant, or neutropenia), and/or a nosocomial infection (diarrheal symptoms occurring at least 48 hours after hospital admission), met our study criteria of AGE (≥3 looser than normal stools in 24 hours or 1 emesis episode accompanied by at least 1 looser than normal stool) and were enrolled in this study.

Although the two hospitals where we collected samples from have similar capacities for inpatient care along with the same annul admissions rate, 67% of patients included in this study were admitted to Egleston. The Egleston site is more specialized compared to its counterpart with an expanded intensive care unit and a new cancer center [16]. Thus immunocompromised patients may tend to visit Egleston more often than Scottish Rite.

Although our study was mainly comprised of children under the age of 5, younger children, especially under the age of 3, tend to have a higher attack rate for acute gastroenteritis compared to older children [17]. The majority (57.7%) of all nosocomial cases, regardless of immunocompromising condition, were under the age of 5. This age distribution is consistent with other studies assessing nosocomial infections in pediatric populations. A Brazilian study assessing the risk factors of nosocomial infections in pediatric populations found that incidence in nosocomial infections was significantly

higher in younger children (particularly under the age of 1) compared to older children (over the age of 5) [18].

Since the rotavirus vaccine was introduced to the American public in 2006, only 46 (41.4%) patients were eligible for the vaccine in this study. The Advisory Committee on Immunization Practices (ACIP) recommends that the first dose of the vaccine is administered between 6 and 12 weeks, with optimal administration at 2, 4 and 6 months. Of the 46 patients who were eligible, 22 (47.8%) received the vaccine. According to the Immunization Information Systems (IIS) in the United States, the mean vaccine coverage was 72%, ranging from 48-86% by mid-2009 [19]. The vaccine coverage in this study was slightly less than the average coverage from the IIS data. There were 11 patients whose immunization information could not be found using GRITS, either because they were not registered or they resided outside of Georgia. In addition, this vaccine coverage remains to be 10% lower than other common infant immunizations such as Diphtheria-Tetanus-acellular Pertussis (DTap) and pneumococcal vaccines. 6 of the 13 patients who did not receive the rotavirus vaccine were immunocompromised patients. Physicians suggest that vaccines be administered at least 3 months after chemotherapy cessation and 6 months after solid organ transplant operations to reduce the risk of adverse effects and impaired vaccine efficacy. Consequently, the rotavirus vaccine may not have been administered to immunocompromised patients, depending on the timing of their transplant operations and chemotherapy [20].

Within each subgroup of our study population, previous antibiotic use was observed more often than not. Antibiotic associated diarrhea is usually caused by *C. diff.* (10-20% of cases), other bacterial enteric pathogens (*Salmonella* and *Staphylococcus*

*aureus*), adverse effects of antibiotics on intestinal mucosa, and/or consequences of reduced of fecal flora [21].

38 patients were taking immunosuppressive medication during the onset of their diarrheal symptoms. All but one patient were transplant patients, either bone marrow or solid organ. Transplant patients are usually given immunosuppressive medication in order for their bodies to be more receptive and not reject the foreign organism. Immunosuppressed children have an increased risk for various infections [22] and gastrointestinal complications are a common side effect of immunosuppressive medication [23]. The incidence of diarrhea during immunosuppression therapy can vary depending on the type of transplant, the specific drug, and the dose of drug. Tacrolimus- a specific immunosuppressive medication, which 16 patients were taking at the time of their AGE, had the highest incidence of diarrhea (72%), compared to other drugs (incidence ranging from 14% to 72%) [23]. The side effects of antibiotics and immunosuppressive medications suggest that patients in our cohort had drug-induced diarrhea. Additionally this may explain the large percentage of unknown etiology.

*Norovirus and Rotavirus Prevalence*

Our overall prevalence of norovirus infections was 16.2% among all the subjects. The prevalence in each subpopulation- immunocompromised only, nosocomial only, and immunocompromised and nosocomial- was 22%, 12.9%, and 4.8%, respectively. This prevalence was higher than the estimated prevalence of 12% from a global meta-analysis pre-rotavirus vaccine implementation [24]. In addition to the higher norovirus prevalence, our rotavirus prevalence of 1.8% was drastically lower than the pre-vaccine prevalence of

50% [7] and also lower than the reported post-vaccine prevalence of 6%. These data support our hypothesis that the prevalence of norovirus caused gastroenteritis has surpassed that of rotavirus caused gastroenteritis. Our current findings suggest norovirus-rotavirus dynamic changes are consistent with the partner study, which assessed the AGE etiologies in previously healthy patients. Although both the prevalence of norovirus and rotavirus was lower in the current study compared to the previous study (16.2% vs. 22.5% and 1.8% vs. 5.5%, respectively), the trend among etiologies was similar. These data also support the efficacy of the rotavirus vaccine.

Despite the fact that only 20% of our cohort received the rotavirus vaccine, the low rotavirus infection prevalence in our data appear to support the theory that this vaccine has induced herd or community immunity [7, 25]. The two patients who were infected with rotavirus in this study had not received the vaccine previously; one was 14 years of age at the start of the vaccine implementation and thus was ineligible.

The seasonality of norovirus positive cases identified in this study, with all infections occurring between December and April and peaking in January and February, is concordant with the national, verified seasonality [26, 27]. Of the two rotavirus infections, one occurred in December, during the seasonality of the winter months [28], and the other occurred in June, outside of the expected seasonality. Additionally, our *C. diff* seasonality trends resembled those of other studies [29], with the majority (73%) occurring in the winter months.

The noroviral genotypic profile is concordant with that of the partner study and other studies [4, 27, 30, 31], suggesting that GII is the more common genogroup and

GII.4 is currently the most common strain. GII.12 was the second most common strain in both this study and the partner study.

We expected to see more cases of *C. diff* among nosocomial cases in this study. 10 (19.2%) nosocomial infections were not tested for *C. diff* which may explain a lower than expected prevalence. CDC surveillance during the mid-1980s determined that *C. diff.* was the cause for 45.1% of nosocomial diarrhea with known etiologies [32]. *C. diff* spores are quite stable in the environment and are relatively resistant to disinfectants. Consequently the toxin has a contamination rate as high as 58% on hospital surfaces such as bedpans, furniture, and blood pressure cuffs. *C. diff.* has also been detected on the hands of healthcare workers, as well [33].

*Immunocompromised patients as a source of nosocomial norovirus infection*

There were a total of 5 nosocomial norovirus cases, yet no immunocompromised norovirus infections occurred before nosocomial cases. These data do not support our hypothesis that immunocompromised patients may be a source of nosocomial infections. However, there were several norovirus infections among previously healthy patients that we had information on from our prior study that did precede the nosocomial infections. The two previously healthy patients preceding nosocomial infections in the same department were discharged at least 20 days prior to the onset of symptoms in nosocomial patients. There are, however, several possible explanations for the five norovirus nosocomial cases in our study. Norovirus transmission due to aerosolized vomitus particles and environmental surface contamination could be possible sources of nosocomial infections. Norovirus particles have been shown to be persistent on

environmental surfaces between 7 to 35 days [34, 35]. The environmental persistence of norovirus suggests that the previously healthy patients may have been the source of the nosocomial infections.

During a Norwalk-like virus outbreak in a hospital setting, proximity to vomiting was the most significant risk factor for infected staff, which suggests that viral transmission was due to aerosolized viral particles and subsequent surface contamination [36]. Furthermore, in one of the largest documented Norwalk-like virus hotel outbreaks, viral detection on various surfaces- including carpets, toilets, door handles, and curtains- suggest that contaminated surfaces from aerosolized viral particles played an important role in the persistence of this 5 month outbreak [37]. In addition to environmental surfaces and aerosolized virus particles, hospital workers could be another source of norovirus nosocomial infection. Human challenge studies suggest that fingertips can be contaminated with norovirus from contaminated surfaces. There have also been studies of healthcare providers becoming infected who did not have direct contact with infected patients, suggesting that staff were infected by contaminated surfaces [38]. Outside visitors and asymptomatic norovirus infections are potential contributors to nosocomial infections as well.

*Limitations*

There were several limitations associated with this study. The first is that this study was a hospital-based study. We were dependent solely on clinical information captured within the electronic medical records. The clinical data present in the records

tended to be incomplete and unstandardized. There were several risk factors and dehydration specifics that were inconsistently recorded among the records. In addition, our collection of residual stool in this study was dependent on the ordering of stool sample analysis by the patient's physicians. For example, diarrhea is a side effect of some chemotherapy treatments [39] and some physicians may not have ordered testing a patient's stool because he/she assumed the diarrhea was caused by chemotherapy. Therefore we may not have captured all the patients that fit our criteria. Furthermore, etiology detection for samples- other than norovirus and rotavirus- were also dependent on the physician's discretion. Not all stool samples were tested for *Clostridium difficile* and bacteria (87.4% and 73%, respectively) by the hospital and thus we may have underestimated the prevalence of these etiologies.

The stool samples were only collected at one time during each patient's course of clinical symptoms; thus we were unable to determine the noroviral shedding persistence and if immunocompromised patients viral shedding was longer than that of healthy patients. In addition, we did not have information on asymptomatic infections, which may be approximately 30% of all norovirus infections are asymptomatic [4]. As such, we may have not captured some norovirus positive patients in our study because they did not present with diarrhea.

We were unable to successfully genotype two norovirus positive strains probably due to two reasons. One reason could be that the sample with low titer of virus may be difficult to amplify sufficient amount of PCR products for sequencing. The other reason could be that very rare strains of norovirus and the primers that were used were unable to

be sufficiently bound for amplification. Since the two non-genotyped strains were from a nosocomial case, we were completely unable to identify a possible source.

Lastly, since this was a retrospective study, we were unable to conduct any environmental sampling of the hospital to detect noroviral surface contamination as a potential source of nosocomial cases. Additionally, we did not interview hospital workers or outside visitors if they had diarrheal symptoms and contact with the five patients with nosocomial diarrheal infection.

## Conclusion

The results from this study have several public health implications for gastroenteritis in the pediatric population. Norovirus appears to now be the leading viral etiology for gastroenteritis in children. Our data provide supporting evidence for the development and administration of an effective norovirus vaccine in order to prevent future gastroenteritis cases and subsequent adverse effects among immunocompromised patients. Additionally, our data support the development of a rapid norovirus test in order to detect norovirus infections and potentially prevent hospital outbreaks. The two children's hospitals included in this study do not test for norovirus in their respective laboratories. Hospitals should enforce stricter hygiene strategies to decrease nosocomial diarrhea infections. Healthcare workers should continually and consistently practice appropriate hand-washing technique. Future studies may want to collect stool samples in a longitudinal fashion to assess the length of noroviral shedding among immunocompromised patients. Also, environmental sampling and hospital worker interviews may want to be included to further assess the source of nosocomial norovirus infections.

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## Tables and Figures

Table 1. Patient status and sample source

| | Number (n=111) | % |
|---|---|---|
| **Patient Status** | | |
| Immunocomp only | 59 | 53.2 |
| Nosocomial only | 31 | 27.9 |
| Both immunocomp and nosocomial | 21 | 18.0 |
| **Hospital** | | |
| Egelston | 74 | 66.7 |
| Scottish Rite | 37 | 33.3 |

Table 2. Demographic characteristics of immunocompromised and nosocomial patients with AGE

| | Immuno (n=59) N (%) | Nosocomial (n=31) N (%) | Noso and Immuno (n=21) N (%) | Total (n=111) N (%) |
|---|---|---|---|---|
| **Hospital** | | | | |
| Egelston | 45 (76.3) | 12 (38.7) | 17 (81) | 74 (66.7) |
| Scottish Rite | 14 (23.7) | 19 (61.3) | 4 (19) | 37 (33.3) |
| **Gender** | | | | |
| Female | 22 (37.3) | 21 (67.7) | 9 (42.9) | 52 (46.8) |
| Male | 37 (62.7) | 10 (32.3) | 12 (57.1) | 59 (53.2) |
| **Age** | | | | |
| <5 | 22 (37.3) | 22 (71) | 8 (38.1) | 52 (46.8) |
| 5-10 | 22 (37.3) | 6 (28.6) | 5 (23.8) | 33 (29.7) |
| >10 | 15 (25.4) | 3 (14.3) | 8 (38.1) | 26 (23.4) |
| **Rotavirus Vaccine** | n=55 | n=26 | n=19 | n=111 |
| Yes | 9 (15.3) | 10 (32.2) | 3 (14.3) | 22 (19.8) |
| No | 8 (13.6) | 5 (16.1) | 0 | 13 (11.7) |
| Ineligible* | 38 (64.4) | 11 (35.5) | 16 (76.2) | 65 (58.6) |
| **Previous Antibiotic Use** | n=51 | n=23 | n=20 | n= 94 |
| Yes | 30 (59.5) | 14(45.2) | 17 (80.1) | 61 (55) |
| No | 21 (30.4) | 9(29) | 3 (14.3) | 33 (29.7) |
| **Immunosuppressant Medication** | | | | |
| Yes | 30 (50.8) | 1 (3.2) | 7 (33.3) | 38 (34.2) |
| No | 29 (49.2) | 30(96.8) | 14 (66.7) | 73 (65.8) |

* Ineligible indicates that patients were born before the rotavirus vaccine implementation in 2006

Table 3. Immunocompromising Conditions (N=80)

| Condition | N (%) |
|---|---|
| Cancer | 32 (40) |
| w/ bone marrow transplant | 11 (13.8) |
| w/ solid organ transplant | 1 (1.3) |
| Solid Organ Transplant | 30 (37.5) |
| Bone Marrow Transplant | 5 (6.3) |
| Neutropenia | 1 (1.3) |

Table 4. Clinical symptoms of immunocompromised and nosocomial patients with AGE

| | Immuno (n=59) N (%) | Noso (n=31) N (%) | Immuno and Noso n=21 N (%) |
|---|---|---|---|
| **Hospital Stay** | | | |
| ≤14 | 48 (81.3) | 12 (38.7) | 3 (14.3) |
| 15-30 | 7 (11.9) | 9 (29) | 8 (38.1) |
| ≥ 31 | 4 (6.8) | 10 (32.3) | 10 (47.6) |
| Mean | 10.56±14.8 | 35.61±42.5 | 33.57 ±19.42 |
| **Duration of Symptoms** | n=57 | | |
| ≤3 | 20 (33.9) | 7 (22.6) | 9 (42.9) |
| 4-8 | 16 (27.1) | 18 (58.1) | 5 (23.8) |
| 9-14 | 9 (15.3) | 4 (12.9) | 2 (9.5) |
| >14 | 12 (20.3) | 2 (6.5) | 5 (23.8) |
| Mean | 9.28 ± 8.95 | 7.97 ± 12.31 | 10.43 ± 13.32 |
| **Max Diarrheal Episodes** | n=53 | n=30 | |
| <3 | 6 (10.2) | 4 (12.9) | 2 (9.5) |
| 4-6 | 20 (33.9) | 9 (29.0) | 7 (33.3) |
| ≥7 | 27 (45.8) | 17 (54.8) | 12 (57.1) |
| Mean | 7.42 ± 4.2 | 7.03 ± 2.62 | 7.33 ±2.99 |
| **Max Emesis Episodes** | | | |
| 0 | 33 (55.9) | 17(54.8) | 8 (38.1) |
| 1 | 6 (10.2) | 7 (22.6) | 5 (23.8) |
| 2-4 | 18 (30.5) | 6 (19.4) | 6 (28.6) |
| ≥5 | 2 (3.4) | 1 (3.2) | 2 (9.5) |
| Mean | 2.92 ±2.1 | 2.14±1.51 | 2.46±2.26 |
| **Fever** | | | n=20 |
| Yes | 36 (61) | 8 (25.8) | 11 (52.4) |
| No | 23(39) | 32 (74.2) | 9 (42.9) |
| **Presence of Blood in Stool** | n=51 | n=30 | n=20 |
| Yes | 10 (16.9) | 4 (12.9) | 7 (33.3) |
| No | 41 (69.5) | 26 (83.9) | 13 (61.9) |

Table 5. Etiologies of AGE among immunocompromised and nosocomial patients

| | N | Norovirus (%) | Rotavirus (%) | Bacteria (%) | C. Diff (%) | Unknown (%) |
|---|---|---|---|---|---|---|
| Immuno | 59 | 13 (22) | 0 (0.0) | 3 (5.1) | 10 (16.9) | 33 (55.9) |
| Noso | 31 | 4 (12.91) | 1 (3.2) | 0 (0.0) | 1 (3.2) | 25 (80.6) |
| Immuno and Noso | 21 | 1 (4.8) | 1 (4.8) | 1 (4.8) | 0 (0.01) | 18 (85.7) |
| Total | 111 | 18 (16.2) | 2 (1.8) | 4 (3.6) | 11 (9.9) | 76 (68.4) |

Table 6. Acute gastroenteritis (AGE) severity between norovirus positive and negative cases.

| | NoV* Positive (%) N=18 | NoV* Negative (%) N=93 | P-Value |
|---|---|---|---|
| **Duration of symptoms** | n=16 | | |
| ≤7 | 7 (38.9) | 64 (68.8) | 0.17 |
| 8-14 | 4 (22.2) | 15 (16.1) | |
| >14 | 5 (27.8) | 14 (15.1) | |
| **Max Diarrheal Episodes** | n=16 | n= 88 | |
| ≤6 | 6 (33.3) | 42 (45.1) | 0.65 |
| >6 | 10 (55.6) | 46 (49.5) | |
| **Max Emesis Episodes** | | | |
| ≤1 | 12 (66.7) | 64 (68.8) | 0.86 |
| ≥2 | 6 (33.3) | 29 (31.2) | |
| **Fever** | | n=92 | |
| Yes | 7 (38.9) | 44 (47.3) | 0.3 |
| No | 11 (61.1) | 48 (51.6) | |
| **Age** | 5.22 ± 6.31 | 6.77 ± 5.26 | 0.33 |

*NoV indicates norovirus

Table 7. Norovirus Genotypes among immunocompromised and nosocomial patients

| | | | GII | | | |
|---|---|---|---|---|---|---|
| | N | NoV Pos | GII.3 | GII.4 | GII.12 | GII.13 |
| Immuno | 59 | 13* | 2 | 7 | 2 | 1 |
| Noso | 31 | 4 | 0 | 3 | 1 | 0 |
| Immuno and Noso | 21 | 1* | 0 | 0 | 0 | 0 |

*Two were not successfully sequenced.

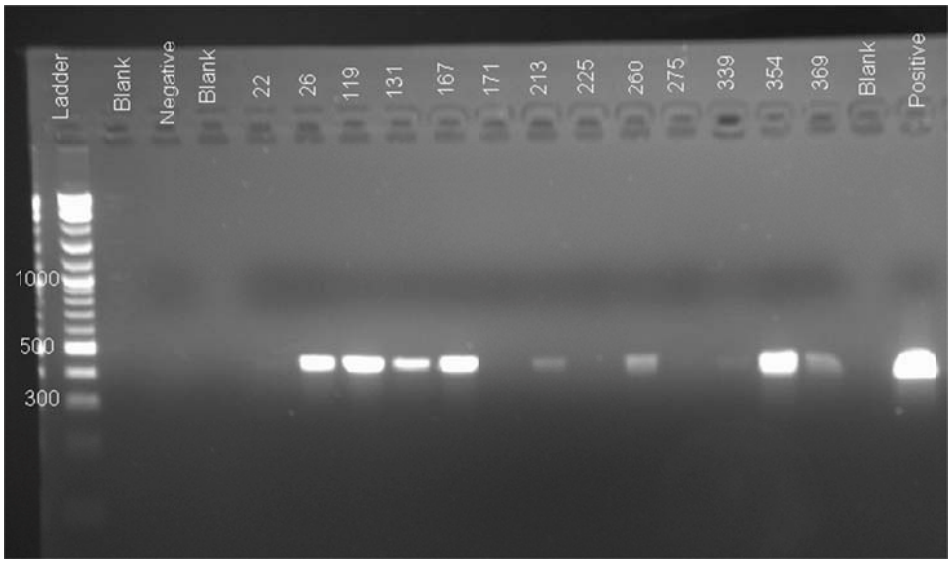


Figure 1. Norovirus PCR results in 2% agarose gel stained with ethidium bromide and visualized under UV light. Negative: PCR negative control; Positive: PCR positive control; Blank: nothing in well to minimize cross contamination. The numbers 22 to 369 represent 13 RNA samples extracted from the stool of patients at Egleston and Scottish Rite Hospitals.

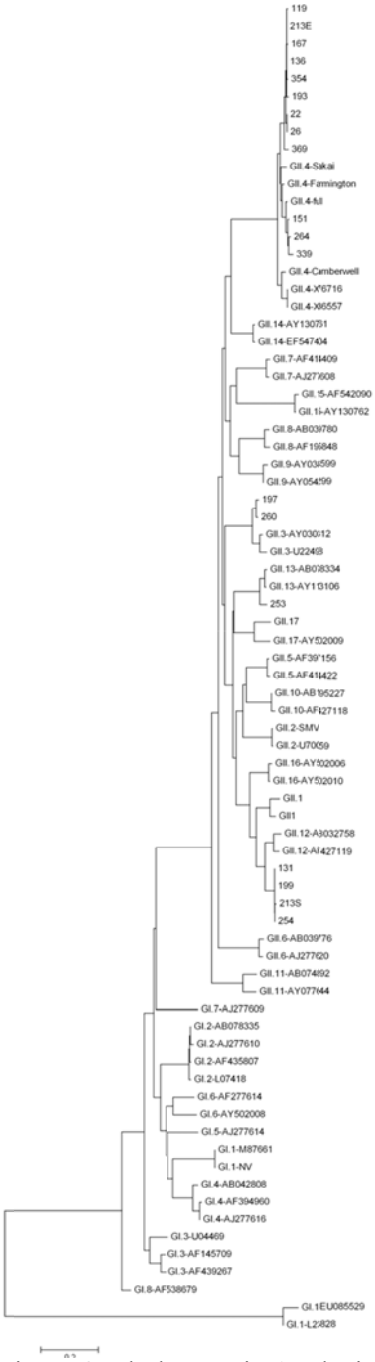


Figure 2. Phylogenetic Analysis of Positive Noroviruses. Norovirus GII reference sequence codes: **GII.1:** Hawaii (U07611); **GII.2:** Snow Mountain Virus (SMV); U70059; **GII.3:** AY030312; U22498; **GII.4:** Sakai; Farmington; Camberwell; X76716; X86557; **GII.5:** AF397156; AF41442; **GII.6:** AB039776; AJ277620; **GII.7:** AF414409; AJ277608; **GII.8:** AB03978; AF19548; **GII.9:** AY038599; AY054299; **GII.10:** AB195227; AF427118; **GII.11:** AB074892; AY077644; **GII.12:** AB039776; AJ277608; **GII.13:** AB078334; AY113106; **GII.14:** AY130761; EF547404; **GII.15:** AF542090; AY130762; **GII.16:** AY502006; AY502010; **GII.17:** AY502009

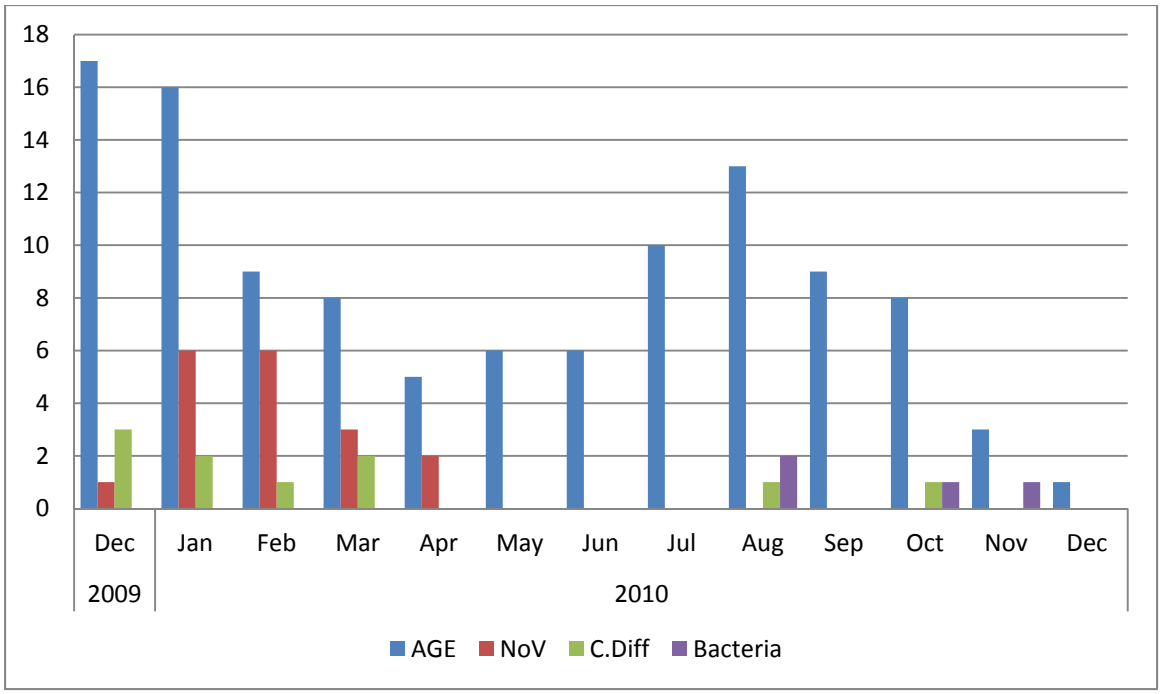


Figure 3. Seasonality of acute gastroenteritis (AGE) and norovirus (NoV), *Clostridium difficile* (C. Diff), and bacterial (Bacteria) infections

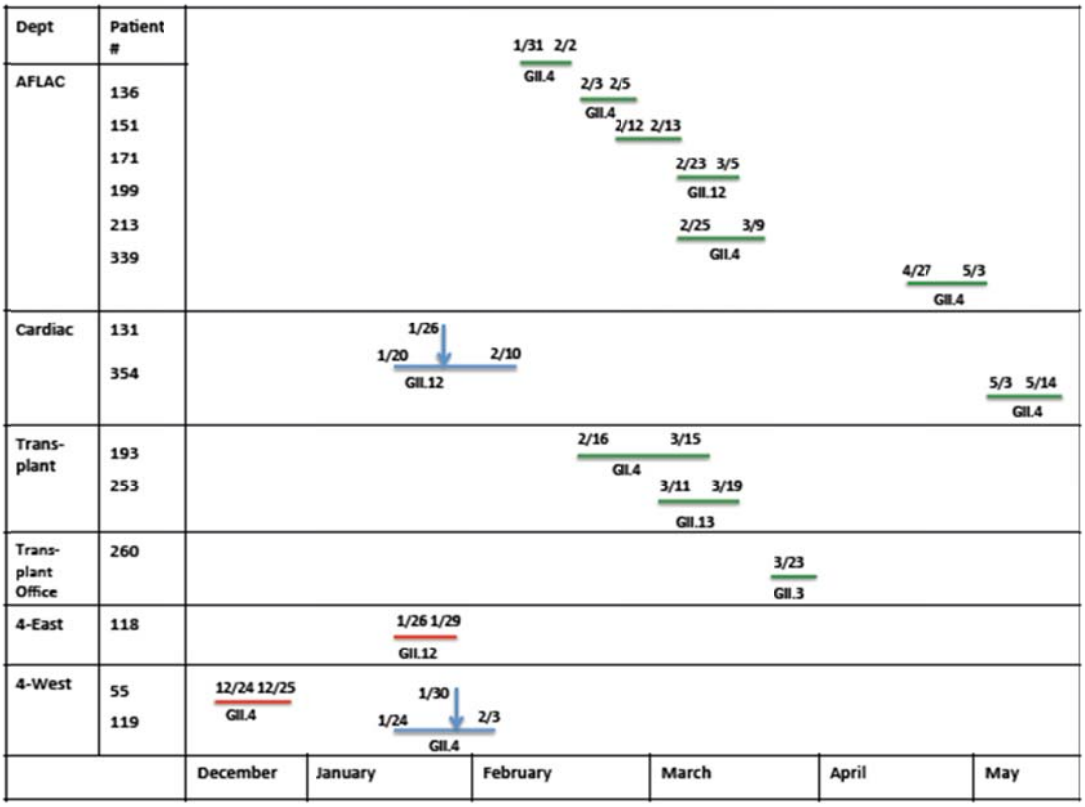


Figure 4. Immunocompromised, nosocomial, and previously healthy- only those preceding nosocomial infections- norovirus infections in Egleston Hospital. Lines in green indicate immunocompromised infections; lines in red indicate previously healthy patients from partner study that precede nosocomial cases; lines in blue indicate nosocomial cases. Arrow above line and date indicate onset of diarrheal symptoms, suggesting a nosocomial infection

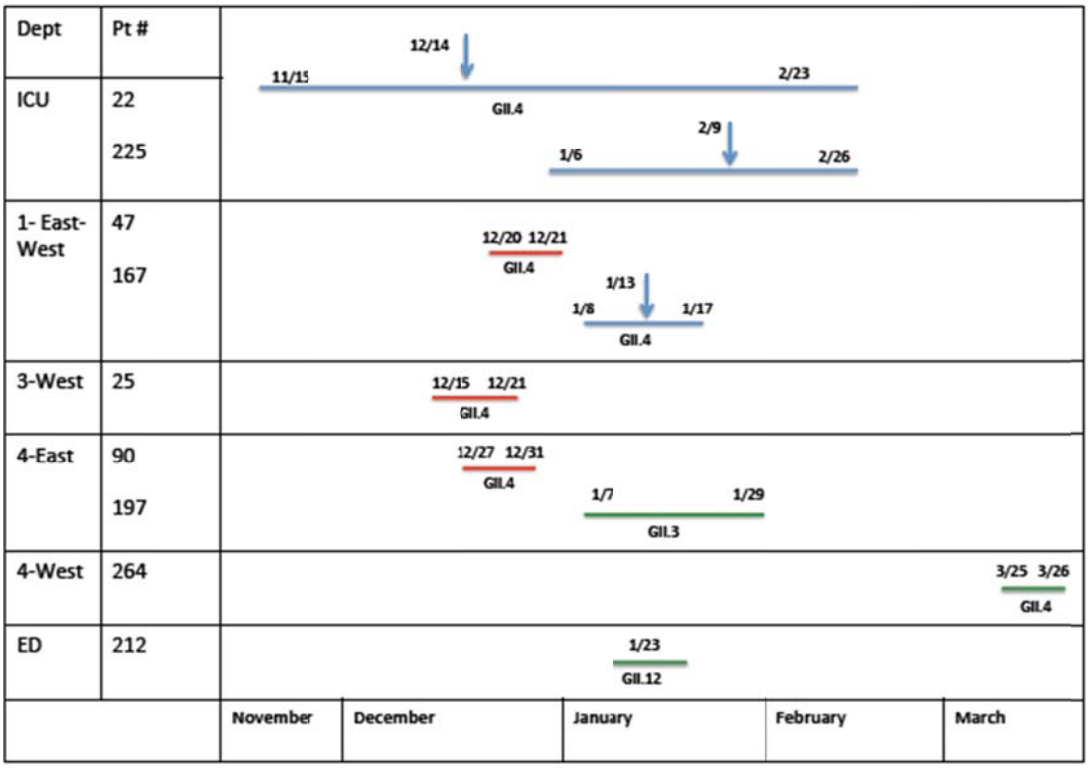


Figure 5. Immunocompromised, nosocomial, and previously healthy- only those preceding nosocomial infections- norovirus infections in Scottish Rite Hospital. Lines in green indicate immunocompromised infections; lines in red indicate previously healthy patients from partner study that precede nosocomial cases; lines in blue indicate nosocomial cases. Arrow above line and date indicate onset of diarrheal symptoms, suggesting a nosocomial infection

# Appendix

## Background

Norovirus and rotavirus are major contributors to acute gastroenteritis, especially in children. This review describes the clinical manifestation, epidemiology, genome, diagnosis, prevention, and vaccine details on the two viruses. Additionally the viruses' role in immunocompromised persons and nosocomial infections are addressed.

### Norovirus

#### *Clinical Manifestation*

The symptoms of norovirus infection include nausea, vomiting, diarrhea, abdominal pain, myalgia, headache, malaise, and fever, usually lasting between 24 and 48 hours. The general incubation time is 24 to 48 hours, ranging between 10 and 50 hours. There is no treatment for norovirus infections and thus managing the symptoms is the typical response. This may include rehydration via oral or intravenous methods and replenishing electrolytes and glucose. For children under the age of 5 years, 20 mg of elemental zinc per day for 10-14 days is also a common treatment [40]. The viral dose needed to cause an infection is approximated 10-100 viral particles. During the peak of viral shedding, each gram of feces contains approximately 5 billion infectious viral particles. The viral shedding peak is usually 2 to 5 days after infection [4]. Noroviruses are quite stable in the environment, able to survive in temperatures ranging from freezing to 60°C and survive in 10ppm chlorinated water [41]. 30% of norovirus infections are asymptomatic yet the virus can be detected in feces up to four weeks after infection [4].

*Epidemiology*

In the United States, roughly 21 million norovirus related illnesses occur each year, with 25% being foodborne [4]. In developed and developing countries, norovirus infections are responsible for 10-15% of severe gastroenteritis cases in children under the age of 5 years [4]. Norovirus is the most common cause of gastroenteritis outbreaks, causing at least 50% of outbreaks worldwide [4]. Outbreak settings include hospitals, nursing homes, cruise ships, schools, and early childcare facilities [4]. Humans are the only reservoir for norovirus and transmission typically occurs from person-to-person via fecal-oral route. Contact with fomites or contaminated surfaces are also transmission routes for norovirus. Foodborne outbreaks usually occur from infected food handlers or when crops are in contact with contaminated water (from human waste). Waterborne transmission occurs from exposure to/ contact with human waste contaminated water, such as septic tanks and well water, or in recreational water when there is a failure in disinfectants, such as chlorine [4].

Most norovirus infections occur during the wintertime (October-April) with specific peaks in February and March. Outbreaks are known to occur during summertime (May-September) as well. Crowding and overpopulated areas, such as cruise ships and long term hospital facilities, are associated with increased transmission and incidence of infections. This may explain season independent outbreaks [26].

*Virus Genome*

Norovirus is a non-enveloped, single-stranded RNA virus and is a member of the *Caliciviridae* family [4]. The various norovirus strains were only recently classified, with proposed nomenclature. After sequencing the amino acids of the major capsid protein of

163 strains, Zheng *et al* determined that noroviruses can be classified on three levels: strain, cluster, and genogroup (Figure 6).

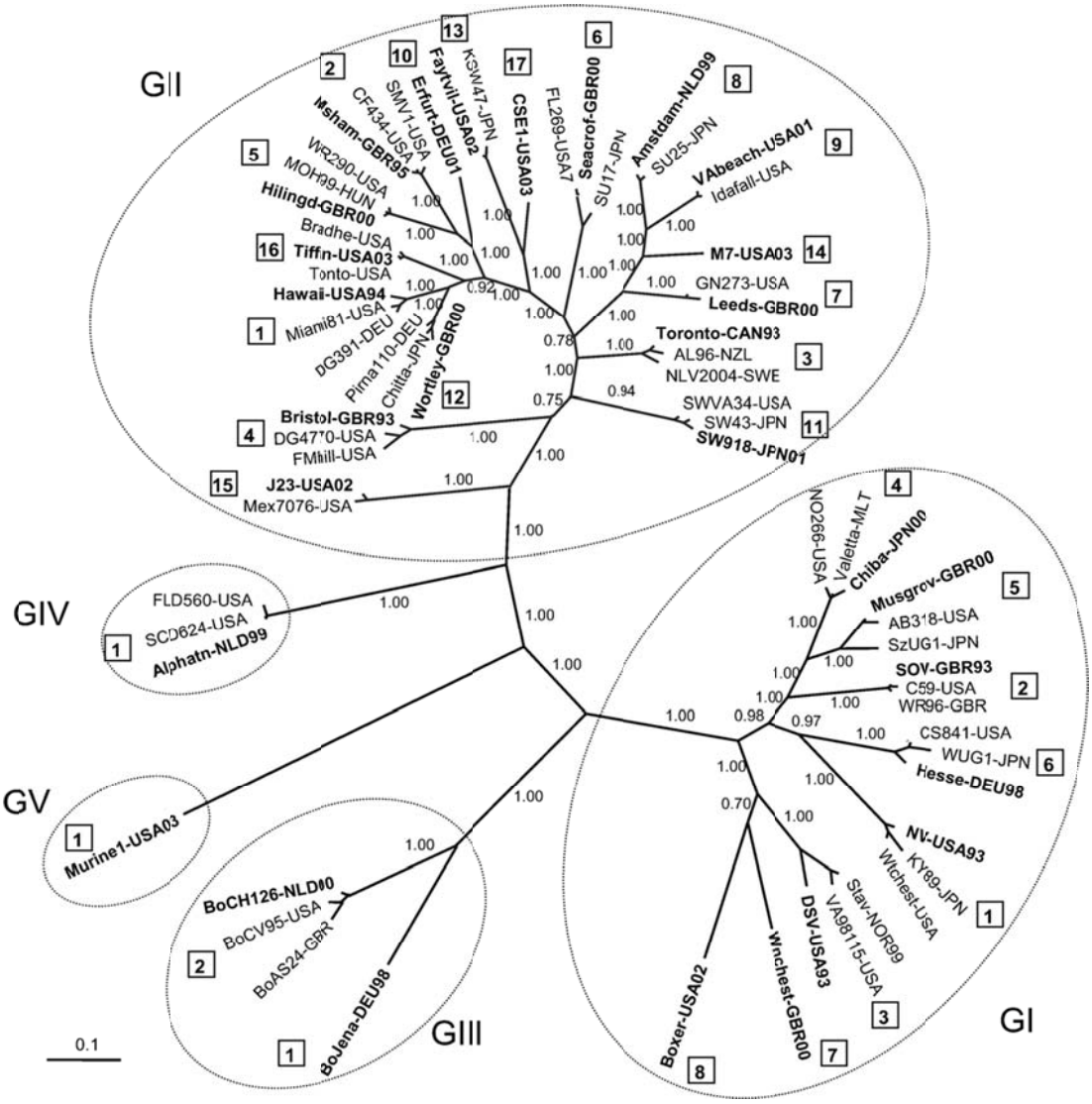


Figure 6: Norovirus classification and nomenclature [42].

There were 29 clusters: 8 in GI, 17 in GII, 2 in GII, 1 in GIV, and 1 in GV. GI, GII, and GIV are all found in humans, except for GII.11, which is found in pigs. The two largest genogroups, 1 and 2, encompass the most common strains and contain the most diverse strains [42]. Noroviruses have great diversity with sequences varying by up to 60% between each genogroup and 57% between human norovirus strains. This is a higher

level of diversity compared to other viruses (poliovirus – 20%, and rubella 10%) [42]. The high diversity suggests that the genogroups may be individual species. However a lack of serotyping data on the correlation of phylogenetic and antigenic characterizations prohibits this theory to be further validated [42]. Such diversity is caused by the accumulation of point mutations from error prone RNA replication and the recombination of related viruses [27].

The positive-sense single stranded RNA is roughly 7,700 base-pairs which encodes 3 open reading frames (ORF) [43]. All three ORFs encode for different proteins that allow the virus to successfully survive and infect [44]. ORF1 encodes non-structural proteins, processed co- and post-translationally. The exact number of non-structural proteins is unknown. The order of ORF1 is p48, NTPase, p22, VPg, $3CL^{PRO}$, and RdRp (Figure 7).

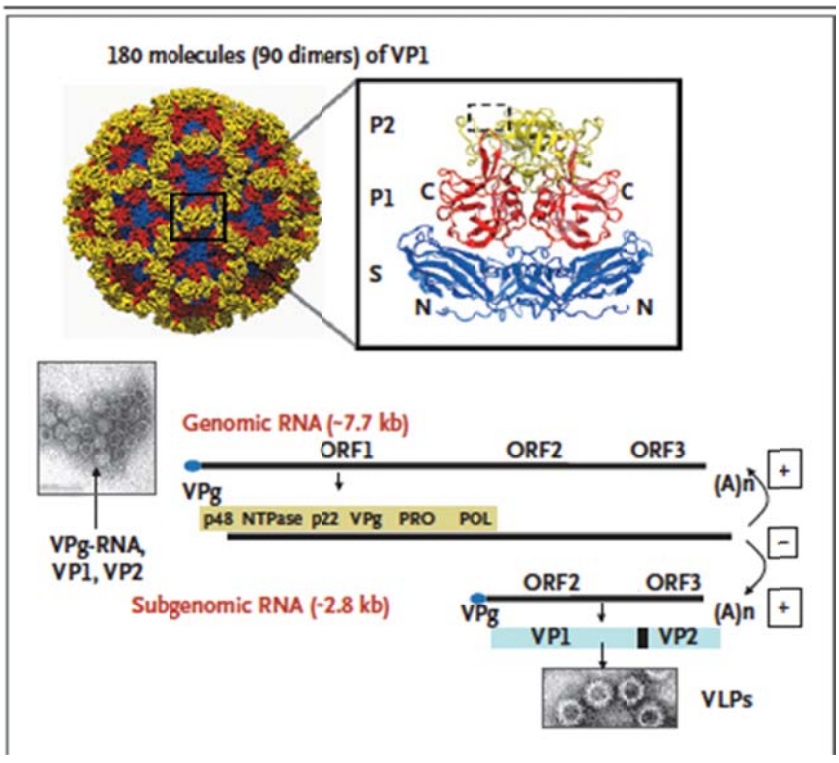


Figure 7: Structure of norovirus capsid and genome [27].

The p48 region may regulate cell proliferation and also may help to anchor membrane-bond replication complexes, acting as a scaffolding protein for replication complex assembly. NTPase is associated with RNA helicase. There is no specific data on the function of the p22 region of the ORF1. VPg is covalently linked to genomic and sub-genomic mRNA (messenger RNA). $3CL^{PRO}$ encodes for a single protease that digests proteins, and lastly, RdRp may function in potential stabilization of primers to initiate RNA synthesis [43].

The other two ORFs encode for structural proteins: the major capsid protein, VP1, and the minor structural protein, VP2, respectively.VP1 is approximately 530-555 amino acids and folds into two major domains: shell domain, S, and protruding domain, P (Figure 7). S is comprised of the N-terminal 225 amino acids, which contain elements for formation of the icosahedron, the shape of the virion. P is comprised of the remaining amino acids in ORF1 and has two subdomains, P1 and P2. The two domains interact with each other in order to increase the stability of the capsid and form the virion protrusions. The P2 domain is inserted in between the P1 domain and may be associated with receptor binding and immune reactivity [43].

VP2 encoded by ORF3 is shorter than VP1, with an approximate length of 208-268 amino acids. This protein is quite variable between strains and its role in the replication cycle remains unclear. There tend to be one or two copies of VP2 per virion. The VP2 may function as RNA genome packaging, since VP1 lacks N-terminal basic RNA binding domain found in other similar capsid proteins [43].

*Diagnosis*

There are three methods for detecting norovirus: transmission electron microscopy, antigen- enzyme-linked immunosorbent assay, and reverse transcriptase polymerase chain reaction. All three tests have both advantages and disadvantages with regards to time, accuracy, and cost [45].

Transmission Electron Microscopy: Experts use electron microscopes at 50,000-100,000-fold magnification to detect viral particles in fecal samples. Particles are identified by the size (diameter between 27-40 nm) and shape (spherical and no envelope). TEM is a rapid test, producing results within three hours, and can also detect other potential viral particles; however, the electron microscope is quite expensive, requiring a trained expert to identify norovirus particle, and the process will only allow speciation to genus level [45]. TEM was mainly used prior to the sequencing of the norovirus genome. Additionally, TEM lacks sensitivity, with a detection threshold of $10^6$ viral particles [46].

Enzyme-linked Immunosorbent Assay: ELISA diagnostic tests detect norovirus specific antibody-antigen interactions. ELISA tests are easy, allowing large quantities to be tested simultaneously, and is relatively fast, producing results within six hours. This antigen detection test however lacks specificity and is likely to produce false positives, requiring an additional alternate test to confirm results [45]. This specific test lacks specificity because there is a lack of sufficient quantities and quality of norovirus antigens currently available [46].

Reverse Transcriptase- Polymerase Chain Reaction: RT-PCR detects norovirus by amplification of certain regions in the viral RNA with region specific primers and probes.

There are two types of RT-PCR: conventional and real time. Conventional amplifies the RNA of interest and the amplified product is subsequently visualized after gel electrophoresis, which indicates the size of the amplified region. The product of this assay can then be sequenced. Real time RT-PCR utilizes florescent probes in order to determine the cycle number at which sufficient amplification has been reached. This assay produces a quantitative measurement of viral load. RT-PCR is the most sensitive and specific of the three tests but also requires expensive equipment and a skilled technician; RT-PCR produces results in eight hours [45]. A recent study compared the sensitivity and specificity between conventional RT-PCR and ELISA, finding that the ELISA tests were 36 and 38% sensitive and 96% and 88% specific [46]. They concluded that RT-PCR should remain the gold standard test until higher quality and quantity norovirus antigens are developed for ELISAs [46].

*Prevention and Control*

The best method to control and prevent norovirus infections is proper hand hygiene. Washing hands for 20 seconds under running water with antiseptic soap is the most effective method. The effectiveness of alcohol-based hand sanitizers continues to be disputed and thus normal hand washing is the recommended method. With regards to surface disinfectants, 5% bleach solutions- if used within 24 hours or 10% for storage of up to 30 days- and 70% ethanol are recommended to control current and prevent future norovirus infections [4].

*Vaccine*

Multiple pharmaceutical companies are in the process of developing a safe and effective norovirus vaccine; however, currently no vaccine is available to the public.

Since human norovirus cannot be grown in vitro, virus like particles (VLPs) are used to better understand the virus and to develop a vaccine. There are several VLP-vaccines that are Food and Drug Administration approved and widely available (eg vaccines against hepatitis B virus and human papillomavirus) [47]. Recently, a double-blinded randomized clinical trial tested efficacy and safety of a new norovirus VLP vaccine, derived from the norovirus genogroup GI.1. In order to enhance the efficacy of the vaccine, monophosphoryl lipid A and mucoadherent chitosan were added to the VLP. After vaccine administration, no adverse events occurred and recipients secreted VLP specific antibodies [48]. This clinical trial study is the first to illustrate adequate safety and immune response from norovirus VLP vaccine administration [47].

## Rotavirus

### *Clinical Manifestation*

Rotavirus symptoms include vomiting, fever, and watery diarrhea. The general incubation time is 24-72 hours with symptoms lasting 4-6 days. The sole treatment for rotavirus infections is managing symptoms. Managing symptoms may involve rehydration, either oral hydration or intravenous hydration if symptoms are severe and replenishing electrolytes and sugars [40].

### *Epidemiology*

Before 2006 and the implementation of a novel vaccine, rotavirus was responsible for 111 million episodes of acute gastroenteritis that required home care, 25 million episodes that required clinical visits, and 2 million episodes that required hospital visits, annually. Approximately 440,000 deaths due to rotavirus infection occurred annually in

children under the age of 5 years. Rotavirus prevalence was the same in developed and developing countries but poor developing countries made up 82% of rotavirus deaths. It was estimated that by age 5 nearly every child will have an episode of rotavirus acute gastroenteritis [49].

Rotavirus transmission is mainly person-to-person via fecal oral route. In developing countries, transmission may occur from rotavirus fecal water contamination. Individuals may also become infected by contact with contaminated surfaces or fomites. Transmission and outbreaks usually occur in childcare centers or long-term health facilities. Rotavirus may survive days to weeks on surfaces, four hours on hands, and weeks in recreational and drinking water [50].

Rotavirus infection incidence peaks during the winter season in the Americas, however peaks in spring and fall seasons in other parts of the world. There is no seasonal trend in tropical areas [28]. In the United States, the rotavirus season has been defined as November to May, usually beginning in south western region of the US and spreading to the north east [50].

*Virus Genome*

Rotavirus is a double stranded RNA, non-enveloped, icosahedral virus. The rotavirus capsid contains 11 segments, each coding for a single protein except for segment 11, which codes for two proteins. Of the 12 proteins, half are structural and the other half are nonstructural [51]. The virus resembles a wheel with short spokes and well-defined rim (Figure 8).

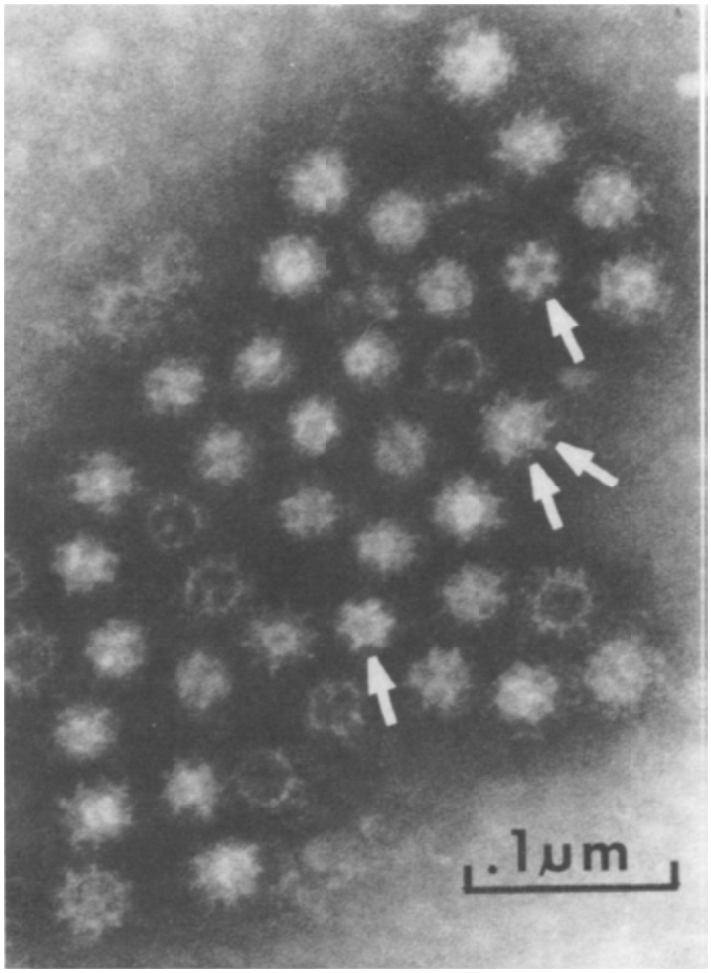


Figure 8: Electron micrograph of rotavirus particle[52]

Three types of particles are usually detected when looking at the virus: double-shelled, single-shelled, and core. Chemical disruptions of the double and single shelled particles produce single-shelled and core particles, respective. The entire genome is approximated 18,522 base pairs. There are 6 structural proteins, one of which cleaves into two more structural proteins: VP1, VP2, VP3, VP4 (cleaving into VP5 and VP8), VP6, and VP7. VP1, VP2 and VP3 are structural core proteins; VP2 is the most abundant and also assists in RNA binding and might be a nucleocapsid protein, bound tightly to RNA segments. VP3 is the minor core protein and may co-migrate with the outer capsid protein VP4 [53].

VP4 is a surface protein and is cleaved into VP5 and VP8 in the presence of trypsin. This cleave has been shown to enhance penetration of virus into susceptible cells. It is also involved in restricting viral growth in tissue and associated with protease-enhanced plaque formation. VP6 is a major structural protein on the outer surface of the single-shelled particles and is associated with the formation of tubules. Lastly, VP7 makes up the majority of the virion outer capsid of purified particles and is consequently the target for antibody reactivity. The remaining proteins are non-structural proteins and their functions are not as well known compared to their counterparts [53].

The rotavirus genus includes at least seven serogroups (A-G). RVA, RVB, and RVC infect humans and the remaining serogroups infect animals, mainly birds. RVA is the most important serogroup with regards to human infectivity, morbidity and mortality [54]. The RVA serogroup has been further categorized in four ways:

1. Antigenic properties of VP6, VP7, and VP4 (subgroups, G-serotypes, and P-serotypes, respectively)
2. Migration patters of RNA genome segments after gel electrophoresis (long, short, super-short, or atypical)
3. Whole genome RNA hybridization patterns (genogroups)
4. Nucleotide sequence analysis (genotypes) [54].

*Diagnosis*

Rotavirus is usually detected in stool samples by either electron microscopy (EM) or enzyme-linked immunosorbent assay (ELISA). Similarly to norovirus diagnostic techniques, EM is a more rapid test whereas ELISA is a more sensitive and specific test. EM detects rotavirus by assessing the shape and size of viral particles. This technique

may identify viral particles within as little as a few minutes. Additionally, EM can detect other viral particles in a specimen in the case of co-infection; however, the technique can only address one specimen at a time. On the other hand, ELISA protocols can test large quantities of samples in one test. The ELISA technique detects rotavirus antibodies present in stool specimens. ELISA techniques usually identify a higher percentage of rotavirus infections compared to the EM technique [55].

*Prevention and Control*

Since rotavirus is rather resistant to commonly used disinfectants, stronger and more effective agents are necessary to control transmission and potential outbreaks. Such effective agents include chlorhexidine gluconate (active ingredient in Hibiclens) and quaternary ammonium when in high alcohol concentration solutions. 95% ethanol solutions are also effective in disinfecting surfaces. With regards to hand washing, there is no soap or disinfectant that is truly effective against rotavirus; however, when in contact with infected individuals, it is suggested to use a waterless alcohol hand sanitizer [50].

*Vaccine*

The first rotavirus vaccine, Rotashield®, RRV-TV (Wyeth Lederle Vaccines and Pediatrics, Marietta, PA, USA) was introduced to the public in October 1998. Within the first 10 months of the vaccine introduction, 15 cases of intussusception were reported following vaccine administration. The rare intestinal disorder developed between 3-14 days following vaccine intake. The majority of cases occurred among children who received the first dose after 3 months of age. In September 1999, the US Advisory

Committee on Immunization Practices withdrew its recommendation for Rotashield®, and the drug company soon voluntarily withdrew the vaccine from the market [56].

The withdrawal of Rotashield® and its association with intussusception were controversial, however, in 2006 a new vaccine, RotaTeq ®(Merck) was introduced. 70,000 infants were recruited for a randomized clinical trial to test the efficacy and safety of this new vaccine. There was a slightly higher risk for intussusception between vaccine recipients and placebo recipients (1.6, with a 95% confidence interval of 0.4-6.4). After vaccine administration, hospitalizations and emergency department visits were reduced by 94.5% and the vaccine was 98% effective against severe acute gastroenteritis caused by rotavirus [5]. In 2010, RotaTeq® received WHO prequalification status and is administered in Africa, Asia, Europe, Latin America, and the United States [57].

Nosocomial Diarrhea

Rotavirus and norovirus are important pathogens to nosocomial (hospital-acquired) gastroenteritis infections among US pediatric patients. In a study performed in United Kingdom, enteric viral pathogens were identified in 53% of nosocomial infection: 31%-rotavirus, 16%-norovirus, and 15%-adenovirus [9]. Recent surveillance data from six European countries has shown that rotavirus is the main cause of nosocomial pediatric diarrhea, with a prevalence ranging between 31% and87%, followed by norovirus, accounting for 17-46% of the diarrheal cases [10]. Nosocomial gastroenteritis can be a great economic burden on healthcare facilities. Surveillance of three major hospitals in England determined that the economic loss of gastroenteritis nosocomial infections was

roughly $184 million [58]. The two studies assessing the nosocomial etiologies were completed before the introduction of the rotavirus vaccine.

Diarrhea in Immunocompromised Patients- Prolonged Viral Shedding

A possible source of norovirus nosocomial infections may derive from patients with norovirus infection and underlying conditions that may result in impaired immunity, lengthy hospital stays, and prolonged norovirus excretion. A recent report described a chronic diarrhea case and prolonged norovirus excreting during a 114-day period in a 10-month old baby after combined liver, pancreas, and small bowel transplant [11]. Similarly, a recent publication describes nine pediatric cancer patients with prolonged norovirus shedding ranging from 22-433 days after the onset of symptoms; the symptoms for all but one ceased well before the viral shedding ceased [12]. More importantly, three immunocompromised chronic norovirus shedders have recently been shown by molecular techniques to be the source of hospital outbreaks in a larger tertiary care hospital in the Netherlands[13].

Prolonged viral shedding of rotavirus has also been documented in immunocompromised patients. The normal rotavirus shedding in healthy patients is usually 10 days. Rotavirus particles were detected for 34 days in an elderly woman with impaired immunity due to decreased natural killer cell activity. Viral shedding from 1 month to a full year was detected in RotaTeq® vaccine recipients who had severe combined immunodeficiency. Also, human immunodeficiency virus and cancer patients expressed viral shedding up to 6 weeks [59].

Summary

Norovirus and rotavirus are two similar agents that cause acute gastroenteritis, yet they have respective distinctions (Table 8). They have similar routes of transmission; however norovirus is more prominent in foodborne outbreaks, compared to rotavirus. Rotavirus has a longer incubation and symptomatic duration, yet their treatments are the same of managing symptoms and hydration. The two viruses can be detected in the same manner, but ELISA is the preferred method of rotavirus detection while RT-PCR remains the preferred detection method for norovirus. Additionally, norovirus and rotavirus have varying hand and surface disinfectant specifics.

Table 8: Summary of Norovirus and Rotavirus

| | Norovirus | Rotavirus |
|---|---|---|
| Incubation | 24-48 hours | 24-72 hours |
| Duration | 24-48 hours | 4-6 days |
| Prevalence, children | 10-15% | 40%* |
| Treatment | Manage symptoms/rehydrate | Manage symptoms/rehydrate |
| Vaccine | No | Yes |
| Transmission | Person-to-person via fecal-oral route; food; water | Person-to-person via fecal-oral route; water |
| Detection | ELISA, EM, RT-PCR | ELISA, EM, RT-PCR |
| Rapid Test | No | Yes |
| Hand Disinfectant | Running water with antiseptic soap | Waterless alcohol hand sanitizer |
| Surface Disinfectant | 10% bleach; 70% ethanol | 95% ethanol |

* This prevalence was before the rotavirus vaccine implementation

## Clinical Abstraction Form

Form used to abstract clinical information about each subject from electronic medical records



**NoV-Imm Chart Abstraction Form**

*From Medical Chart*

Date Of Abstraction: __/__/____ (month/ day/ year)
By (initials): __

*Into Electronic Database*

Date Of Abstraction: __/__/____ (month/ day/ year)
By (initials): __

**Patient Information:**

Patient # _ _ _ _
MR # _____
Patient status: o In-patient o Out-patient (○ ED ○ Clinic)
Hospital: o Eg o SR
Room #_____
Date of visit/admit: __/__/____ (month/ day/ year)
Discharge date: __/__/____ (month/ day/ year)

**Demographics:**

Date of birth: __/__/____ (month/ day/ year)
Sex: o Female o Male
Race-Ethnicity: o White o Black o Hispanic o American Indian or Alaskan Native o Asian o Native Hawaiian or Other Pacific Islander o Other o Unknown

**Immunocompromising Condition:**
o Solid Organ Transplant
o Kidney o Liver o Heart oOther: _____
o Bone Marrow (HSCT)
Type: oAllogeneic or o autologous
o Cancer, type: _____
o Other: _____
Date of transplantation or date of cancer diagnosis:
__/__/____ (month/ day/ year)
Date of induction chemotherapy or radiation:
__/__/____ (month/ day/ year)

**Gastroenteritis Classification:**
o Nosocomial (onset of symptoms >48h after admission or <48h after discharge)
o Sporadic/Endemic (acute)
o Chronic diarrhea (>14 d of symptoms)
(may check more than 1 if it applies)

**Signs at Presentation:**
**Diarrhea:** o No o Yes
*Duration: ___d
OR Date of onset: __/__/____ (month/ day/ year)
*Frequency (max # in 24 hr): _____
Stool quality: o Solid, pasty o Loose, formed elements o Watery, liquid
Presence of blood: oNo o Yes
Presence of mucus: oNo o Yes
**Vomiting:** o No o Yes
*Duration: ___d
*Frequency (max # in 24 hr): _____
Presence of blood: o No o Yes
Presence of bile: o No o Yes
***Fever** [>38°C (100.4°F)]: o No o Yes
○ Subjective ○ Measured,
Tm @ Home ___.__;
Tm @ ED, Clinic or Hospital ___.__
**Nausea:** oNo o Yes
**Abdominal pain/cramping:** o No o Yes
**Headache:** o No o Yes
**Anorexia:** o No o Yes
**Other**/Complications (e.g., URI Sxs, seizures, diaper rash, rectal prolapsed, renal failure, bowel perforation, death):
1._____
2._____
3._____

**Hydration Status:**

Current weight (kg): ____
Previous weight (kg): ____ (from current illness)
Weight loss (kg): ____

**Mental Status:** o Well, alert, normal o fatigued or restless, irritable o apathetic, lethargic, unconscious

**Thirst:** o Drinks normally, not thirsty o Thirsty, eager to drink o Drinks poorly, unable to drink

Eyes: o Normal o slightly sunken o deeply sunken

**Capillary refill:** o Normal o Prolonged ≈2 sec o >3sec, prolonged, minimal

**Extremities:** o Warm o Cool o Cold, mottled, cyanotic

**Past History and Risk Factors:**

Travel: oNo o Yes: _____

Dates: __/__/____ to __/__/____ (month/ day/ year)

Pets (e.g., turtles): oNo o Yes:
_____

Rotavirus vaccination: o Yes o Not given
Rotateq o Partial (#doses__) o Complete (3 doses)
Rotarix o Partial (#doses__) o Complete (2 doses)

Sick contact: oNo o Yes: Who?
_____
_____
Day care or School: o No o Yes

Previous Antibiotics: o No o Yes
Indication: _____

Immunosuppresive medications (and doses):
1._____
2._____
3._____
4._____

**Management:**

*Hydration: o No intervention o ORT and discharged o IV rehydration and discharged o ORT, IV rehydration and discharged o IV rehydration & Hospitalized o ORT, IV rehydration & Hospitalized

If nosocomial: o IV rehydration o ORT rehydration

Total parenteral nutrition (IPN) o No o Yes

Immunosuppresive medications (dose ***reduction***)
1._↓_____(____%)
2._↓_____(____%)
3._↓_____(____%)
4._↓_____(____%)

o Immunoglobulin:
_____

Other: _____

Medications for gastroenteritis (check all that apply):
o Antibiotic:
_____
o Antiemetic:
_____
o Antidiarrheics:
_____
o Probiotics:
_____

**Labs:**

**Stool Studies:**
Test: Rotavirus
Date: __/__/____ Result: o Negative o Positive

Test: Adenovirus
Date: __/__/____ Result: o Negative o Positive

Test: Stool Culture
Date: __/__/____ Result: o Negative o Positive:
_____

Test: Norovirus RT-PCR
*1.* Date: __/__/____ Result: o Negative o Positive
Noro Genogroup/Genotype: __/__ Ct____

*2.* Date: __/__/____ Result: o Negative o Positive
Noro Genogroup/Genotype: __/__ Ct____

*3.* Date: __/__/____ Result: o Negative o Positive
Noro Genogroup/Genotype: __/__ Ct____

Test: C. difficile (toxin assay A/B)
Date: __/__/____ Result: o Negative o Positive

Test: Ova and parasite
Date: __/__/____ Result: o Negative o Positive
_____
Other stool studies
Date: __/__/____
Result: _____
Date: __/__/____
Result: _____

Stool WBCs o Negative o Positive
Stool RBCs o Negative o Positive, &/or
Occult blood o Negative o Positive

**Other labs:** (at onset of gastroenteritis)
WBC _____
H/H ___/___
Plts ____
Diff S___ B___ L___ M___ Eo___ Ba___
Electrolytes: Na___ K___ Cl___ Bicarb___ BUN___
Cr___ Gluc___
Other Abnormal (e.g., LFTs, etc.):
_____
_____

ESR ____ CRP ____

**Colonoscopy:**
Endoscopic findings:
_____
_____
Histopathologic findings:
_____
_____

CMV associated GI disease evaluation: o No o Yes:
Results_____
_____
**Graft versus host disease: o No o Yes**
Stage _____
Details_____
_____
**CT abdomen findings: (closest to illness)**
_____
_____

**Additional Comments:**