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Epidemiology and risk factors for transmission of norovirus in resource-limited communities in Guatemala

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Bachelor of Science, Biology
Bachelor of Arts, Environmental Studies
Certificate in Field Epidemiology
University of North Carolina at Chapel Hill
2008

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Abstract

Epidemiology and risk factors for transmission of norovirus in resource-limited communities in Guatemala

By Jaymin Patel

Background: Noroviruses (NoVs) are the most common cause of epidemic, non-bacterial gastroenteritis globally. They result in 900,000 clinic visits among children and cause 64,000 episodes of diarrhea resulting in hospitalizations in industrialized countries. In developing countries, they result in over 1 million hospitalizations and up to 200,000 deaths among children under the age of five. Despite the development of sensitive diagnostic methods, there is little knowledge on risk factors associated with endemic norovirus transmission in developing countries.

Methods: We analyzed diarrhea cases from a population based surveillance system in the Department of Santa Rosa, Guatemala from June 2007 through November 2010. Enrolled patients who provided whole stool samples were tested for norovirus via real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Samples were cultured for common bacterial pathogens and tested by enzyme immunoassay for rotavirus. Information regarding water sources and water treatment methods along with clinical and demographic data were collected through interviews. Multivariate analysis was conducted by performing logistic regression to study the effects of multiple risk factors on the outcome using a hierarchical backwards elimination approach.

Results: Out of the 3508 eligible patients, 3048 (86.9%) provided stool samples, which included 2089 patients who provided whole stool samples for norovirus testing. Norovirus was detected in 306 (14.7%) patients, of which 55 (17.6%) were from genogroup I (GI), 258 (82.4%) from genogroup II (GII) and seven cases were mixed infections. An overwhelming majority of cases was presented in children as 70.9% of GI cases (n=39) and 86.8% of GII cases (n=224) were detected in children under five. Children under five also made up 95.4% of hospitalized and 77.3% of ambulatory cases. Filtering water was identified as a risk factor for norovirus infection after controlling for age, sex and assumed severity of illness (aOR: 2.44 [95% CI, 1.68-3.56]).

Conclusion: Norovirus is an emerging public health concern especially in developing countries. Children under five are at higher risk for experiencing severe clinical manifestation of diarrheal illness due to norovirus infections. Access to safe water sources and appropriate water treatment methods are critical to reduce waterborne transmission of endemic norovirus in rural communities.

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Table of Contents

BACKGROUND AND LITERATURE REVIEW

Introduction

This background and literature review provides a comprehensive summary of norovirus epidemiology as well as important topics such disease pathogenesis, transmission, infection, treatment and immunology. The research will also highlight gaps in our understanding of norovirus epidemiology especially in developing countries. The study conducted and presented in subsequent sections of this thesis has tried to address some of those gaps and provided recommendations for future research questions and studies.

Background

Diarrheal illnesses rank among the top five leading causes of mortality and account for 4-6 million deaths annually world wide [1]. According to the World Health Organization (WHO), this problem is exacerbated in children; it is the second leading cause of death in children less than five years of age, and is attributed to 1.5 million deaths in that age category [2]. Diarrhea is also the leading cause of malnutrition among children under five years which is a significant contributor to childhood morbidity and mortality worldwide. Diarrheal infections are caused by various bacterial, viral and parasitic pathogens and are commonly spread through the fecal-oral route [2].

Noroviruses are the most common cause of epidemic, non-bacterial gastroenteritis in the world. They are responsible for 50% of gastroenteritis outbreaks globally and cause 21 million cases and 300 deaths annually in United States [3, 4]. Previous studies have helped us understand the transmission of norovirus in developed countries but the epidemiology of noroviruses in the developing world remains largely unknown. A recent systematic review by Manish Patel *el al.* reported that norovirus infections result in 900,000 clinic visits among children and cause 64,000 episodes of diarrhea resulting in hospitalizations in developed countries every year [5]. The review estimates that norovirus infections also cause over 1 million hospitalizations and up to 200,000 deaths among children under the age of 5 in developing countries [5]. Yet little is known about the true disease burden and risk factors that influence transmission of the norovirus in resource poor settings [5, 6].

History

Noroviruses were indentified as etiologic agents of acute gastroenteritis in the 1970's. The first ever documented outbreak of norovirus took place at an elementary school in Norwalk, Ohio in 1968. During this outbreak, 116 out of 232 students presented signs of sudden onset of vomiting, diarrhea and other flu like symptoms [7]. It was not until 1972, when Albert Kapikian discovered the etiology of the disease associated with this outbreak [8]. Through Immune Electron Microscopic (IEM) procedure, he examined stool samples of volunteers challenged with fecal filtrate from the elementary school students who were affected by the outbreak and discovered small, round structured viruses [8]. This was the first time a virus was characterized as the etiologic agent of epidemic gastroenteritis.

Even though the discovery of norovirus via IEM in 1972 was novel and groundbreaking, the use of electron microscopy to visualize filtered and concentrated noroviruses had several limitations; it was expensive, time consuming and rarely available outside of research settings. This methodology of diagnosis also had low sensitivity, involved tedious procedures and was unable to classify viruses [9]. Since 1972, there have been major advances in developing more effective tools for detecting norovirus which include radio-immunoassays, enzyme-immunoassays, but the most robust of them all has been Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) [9, 10]. The use of RT-PCR is considered to be the gold standard in detecting norovirus due to its high sensitivity and specificity levels along with its ability to classify viruses [11, 12]. There still exists a lack of standardized set of primers and protocols for norovirus testing through RT-PCR [13]. Standardization of this technique should help increase the reliability of these tests and enable the laboratories to detect a wide range of strains.

Structure and Classification

Noroviruses are non-enveloped, single stranded RNA viruses. Their genome is 7.7 kb in length and contains three Open Reading Frames (ORFs). ORF1 encodes for an RNA-dependant RNA polymerase, proteases, and several proteins important to the viral replication process [14]. ORF2 and ORF3 code for structural proteins which are responsible for the self assembling capsid [15, 16]. The capsid protein contains a hypervariable region which is thought to determine the strain specificity and cellular binding. This region on the capsid protein is the targeted region for RT-PCR to detect and classify the viruses by its strain, cluster, or genogroup [17].

Noroviruses belong to the *Caliciviridae* family and the *norovirus* genus [18]. There was no official classification scheme for noroviruses until 2006 and were identified under several names, including Norwalk-like Virus, Small Round Structured Virus (SRSVs), and Winter Vomiting Disease [18]. Since the 2006, noroviruses have been classified into five distinct genetic classifications called genogroups (GI-V) [18]. The five genogroups are further divided into 29 clusters, each categorized with a number and name of the prototype strain [19]. Each cluster is comprised of unique strains detected and indentified during outbreaks in humans and animals. Genogroups I, II and IV have been known to cause infections in humans [18, 19]. It is not possible to determine the exact number of strains in each cluster and genogroup because new strains continue to be added. However, the most commonly observed norovirus strains belong to GI and GII genogroups [20]. Interspecies transmission of noroviruses have not been observed yet but recently strains that infect pigs were found to be part of the GII genogroup and a GIV strain was found to be the cause of diarrhea in dogs, suggesting that there is a possibility of zoonotic transmission [3].

Pathogenesis

The pathogenesis of noroviruses is not well understood and is limited due to the lack of a good animal model and the fact that they are difficult to culture in laboratories [21]. Murine norovirus has been used in the past to study norovirus infection in humans, but there has been a debate in the academic world with regards to the similarity between the two types of norovirus and its applicability to norovirus infections in humans [22]. Efforts to culture the virus have met unsuccessful results in the past as well [23].

Recently researchers have started using gnotobiotic pigs to study norovirus pathogenesis, which shows some potential [24]. Due to these limitations researchers have only been able to study norovirus either by conducting human challenge experiments or by studying naturally occurring outbreaks. Noroviruses in humans are transmitted through the fecal-oral routes which bind to the gastroduodenal epithelial cells in the intestine [25]. Once inside the human body, the mechanism by which the virus replicates is not well known. There is some evidence from murine norovirus studies that viral replication occurs in macrophages and dendritic cells [26, 27]. This might hold true for human infections as well but human cell culture systems would have to confirm these findings. Prospective and retrospective studies along with case control studies have provided a wealth of information to our understanding of the pathogen and they have the added benefit of results being more representative of real conditions than laboratory studies.

Transmission

Noroviruses are highly infectious, very stable and infect all age groups, although young children, elderly and people with chronic illness are at a higher risk of contracting the disease [28-30]. One gram of stool from an infected person may contain anywhere between 10^8 - 10^{11} viral copies [31]. They have a low infectious dose (<100 viral particles), which allows for successful transmission with just few virus particles [31-33]. Coming in contact with infected stool, vomit, saliva or aerosolized particles puts a susceptible individual at high risk of infection. Noroviruses are very stable and persistent pathogens. They have shown to survive in the presence of chlorine, alcohols, and ammonium compounds [34, 35]. The combination of high infectivity, prolonged duration of viral shedding, and persistence in the environment facilitates their transmission especially during outbreaks.

Transmission of norovirus can occur in several ways, which include contaminated water, food, surfaces, and direct contact with infected persons [35-37]. Waterborne outbreaks of norovirus can occur in a variety of ways which involve faulty and contaminated water sources where chlorination fails as well as contamination of recreational water systems [38-43]. Foodborne transmission usually occur in food service settings such as catered events, restaurants, and private homes where the food items have been handled by infected individuals or washed with contaminated water [34]. Person-to-person and environmental transmission frequently occur in health care settings such as hospitals, nursing homes, daycare centers, cruise ships, and other settings where people work or live in close quarters via the fecal-oral route, ingestion of aerosolize particles or exposure to contaminated surfaces [35, 44, 45].

Clinical characteristics and Treatment

Norovirus infections have an average incubation period of 24-48 hours and not all infections lead to clinical manifestation of the disease [33, 46, 47]. The presence and severity of disease depends on the individual and the specific strain that is causing the infection. Clinical symptoms include acute onset of diarrhea, vomiting, nausea, fever, myalgia, chills and abdominal cramps [5, 19, 46]. Virus shedding may last for as long as 3 weeks leading to transmission from people who have recovered. Diarrhea stools are watery, non-bloody, and do not contain mucous. Dehydration is the most common result of norovirus infection and may lead to death among young children and the elderly [3].

Symptoms typically resolve within 12-72 hours but recent studies have shown longer mean duration of illness (4-6 days) among high risk populations such as children less than 11 years and patients affected during hospital outbreaks [3, 36, 48]. Infections among the elderly and in hospital settings have also been reported as more severe especially among those with chronic medical conditions than in otherwise healthy individuals. There is currently no effective vaccine in place and treatment for norovirus infections includes effective case management through oral rehydration solutions that provide electrolyte replacement and sugar [19, 49]. Antimotility medications (i.e. diphenoxylate or loperamide) have proved to be ineffective in curbing fluid loss and are not recommended for use especially among children younger than 3 years of age [19, 37].

Immunology

There has been a big push to study and understand the human immunological response to norovirus infection. Some volunteers studies have shown that infection with one strain has resulted in short term immunity (<6 months) against the same strain [48]. Long term immunity is difficult to maintain and has not been observed yet [50]. Due to the genetic variability of norovirus, immunity from one strain does not translate into immunity of other strains in the same or different genogroup [51]. As a result there are a constant high number of susceptible individuals and a large human reservoir from which the disease can spread

Susceptibility to norovirus infections is influenced by genetic variability and resistance along with acquired immunity [37]. Studies have shown that genetic resistance to norovirus to be associated in individuals who are homozygous recessive for the α(1,2)fucosyltransferase gene. This gene facilitates the process of cell surface carbohydrates attachment, which is thought to influence genetic resistance [52]. Some studies have also concluded that susceptibility is based on an individual's histo-blood group antigens [53]. Norovirus infections in humans lead to seroconversion, specifically there is an increased production of IgG and IgA antibodies [54, 55]. Further in depth studies need to be carried out to truly understand the role of genetics in the immunological response to norovirus infections [56].

Transmission route and outbreak settings

There have been specifics routes of transmission identified for norovirus infections (i.e. waterborne, foodborne, person-to-person) but there are not many studies that look at how distribution of cases and persons at risk differ by transmission type. One study in 1982 found "common source" outbreak to have more cases than those attributed to person-to-person transmission [57]. Other studies suggest that person-to-person transmission present with more opportunities for the spread of the disease than foodborne transmission as it is dependent on the level of distribution for that particular food item [44, 58]. They further go on to say that in public settings such as schools, hospitals and residential-care facilities person-to-person transmission facilitate transmission much better than in any other settings [44]. None of these studies looked to see if the differences observed were statistically significant, hence it is difficult to draw any solid conclusions. Majority of the investigations that look at association between settings, transmission routes and norovirus infections have been in developed countries. The lack of good quality data is evident when trying to study norovirus epidemiology in developing countries. The systematic review by Patel *et al.* cites this as a reason for being unable to make "firm estimates" about the disease burden of norovirus in the developing world. Further investigation is needed to thoroughly study how norovirus infections differ by settings and transmission in developing countries [5].

Genogroup and transmission

Another relationship of interest is to look at the association between genogroups and transmission of norovirus in different settings (i.e. which genogroups are commonly associated with different outbreak settings?) No single study focuses just on this question entirely but some studies do touch upon this issue briefly in their discussions. A study done in 2005 found that GI noroviruses were more frequently seen in school settings, while GII noroviruses were more commonly associated in outbreaks in nursing homes and hospitals. It also showed that GI strains were more frequently observed in environmental outbreaks than GII strains [59]. The authors also did not look at the differences to see if they were statistically significant, hence reliable conclusions cannot be drawn. However, Blanton *et al.* found that GII/4 strains were found more commonly in person-to-person outbreaks than foodborne outbreaks [60]. There is still a considerable debate over which genogroups are associated with which transmission type as there have been studies that present contradictory conclusions. Bon *et al.* study found that GI strains were more commonly associated with waterborne outbreaks than GII strains but Maunula *et al.* concluded the opposite [38, 59]. Again all of these studies were conducted in developed countries and the conclusions that hold true in these settings may or may not hold true for developing countries. More efforts need to be undertaken to study these associations in developing countries.

Conclusion

Noroviruses are highly infectious, very stable and a critical emerging disease globally. Since the discovery of norovirus in Ohio, we have made significant progress in studying the epidemiology of the disease pathogen. However, several questions still remain unanswered that will give us a more comprehensive understanding of how the disease interacts with people and its environment. There is a substantial gap of knowledge in transmission type, setting and risk factors that are associated with increased transmission specifically in developing countries. Many studies have failed to look at these questions in depth and usually focus primarily on identifying norovirus as the etiologic agent. Of the studies that have tried to focus on these relationships, most of them have either discussed it in passing or failed to see if the observations they recorded were statistically significant. This has limited our ability to infer these relationships and associations with any validity.

The geographic scope of past norovirus studies have also posed as a barrier to our understanding of the relationship between different exposures and disease outcome. Most studies look at outbreaks from a particular country or region of the world. While this may be the most effective way to investigate the outbreak in that particular setting, studies are largely ignoring the relationship of norovirus transmission across borders. A US study showed the global circulation of a single Norovirus strain. This demonstrated that globalization does not only apply to people only but pathogens as well [61].

It is clear that our understanding of the epidemiology of norovirus is much more comprehensive in developed countries than in developing countries but we cannot generalize that the epidemiologic characteristics are same in both settings. Since the developing world experiences the majority of the disease burden, there is a clear need for studies which analyze norovirus transmission in these countries.

Significance and Goals

As norovirus continues to emerge and pose a grave public health threat around the world, this investigation will help provide critical information to public health officials who are involved in controlling and curbing the transmission of norovirus. The study presented here seeks to address some of the gaps in our understanding of the norovirus epidemiology in developing countries and attempts to answer some of the questions described above in the background and literature review section. The study's focus on risk factors will provide information that will compliment with our current knowledge of norovirus to enhance our efforts decrease the burden of norovirus disease and respond to control outbreaks promptly.

The goal of the study is to gain a better understanding of the epidemiology of noroviruses in the Department Santa Rosa, Guatemala as diarrhea is a major cause of morbidity and mortality in the country. Specifically, we want to identify risk factors at a population level that are associated with increased transmission of the disease. We also want to identify areas where interventions can be implemented to reduce the risk of norovirus especially among the most vulnerable populations.

MANUSCRIPT

Abstract:

Background: Noroviruses (NoVs) are the most common cause of epidemic, non-bacterial gastroenteritis globally. They result in 900,000 clinic visits among children and cause 64,000 episodes of diarrhea resulting in hospitalizations in industrialized countries. In developing countries, they result in over 1 million hospitalizations and up to 200,000 deaths among children under the age of five. Despite the development of sensitive diagnostic methods, there is little knowledge on risk factors associated with endemic norovirus transmission in developing countries.

Methods: We analyzed diarrhea cases from a population based surveillance system in the Department of Santa Rosa, Guatemala from June 2007 through November 2010. Enrolled patients who provided whole stool samples were tested for norovirus via real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Samples were cultured for common bacterial pathogens and tested by enzyme immunoassay for rotavirus. Information regarding water sources and water treatment methods along with clinical and demographic data were collected through interviews. Multivariate analysis was conducted by performing logistic regression to study the effects of multiple risk factors on the outcome using a hierarchical backwards elimination approach.

Results: Out of the 3508 eligible patients, 3048 (86.9%) provided stool samples, which included 2089 patients who provided whole stool samples for norovirus testing. Norovirus was detected in 306 (14.7%) patients, of which 55 (17.6%) were from genogroup I (GI), 258 (82.4%) from genogroup II (GII) and seven cases were mixed infections. An overwhelming majority of cases was presented in children as 70.9% of GI cases (n=39) and 86.8% of GII cases (n=224) were detected in children under five. Children under five also made up 95.4% of hospitalized and 77.3% of ambulatory cases. Filtering water was identified as a risk factor for norovirus infection after controlling for age, sex and assumed severity of illness (aOR: 2.44 [95% CI, 1.68-3.56]).

Conclusion: Norovirus is an emerging public health concern especially in developing countries. Children under five are at higher risk for experiencing severe clinical manifestation of diarrheal illness due to norovirus infections. Access to safe water sources and appropriate water treatment methods are critical to reduce waterborne transmission of endemic norovirus in rural communities.

Introduction:

Diarrheal illnesses rank among the top five leading causes of mortality and account for 4-6 million deaths annually world wide [1]. According to the World Health Organization (WHO), this problem is exacerbated in children; diarrheal illnesses are the second leading cause of death in children less than five years of age, and cause 1.5 million deaths in that age category [2]. Noroviruses are the most common cause of epidemic, non-bacterial gastroenteritis in the world and are responsible for 50% of gastroenteritis outbreaks globally [3, 4]. Norovirus infections result in 900,000 clinic visits among children and cause 64,000 episodes of diarrhea resulting in hospitalizations in industrialized countries. In developing countries, noroviruses result in over 1 million hospitalizations and up to 200,000 deaths among children under the age of five [5]. Noroviruses are an emerging public health threat that contribute significantly in terms of morbidity and mortality and pose a considerable challenge for healthcare providers.

Very few developing countries have the resources to make sensitive and specific diagnostic methods such as real-time reverse transcriptase polymerase chain reaction (rRT-PCR) readily available, hence limiting our ability to study the epidemiology of norovirus and its influence as an etiologic agent of diarrheal illness in resource poor settings. Most studies in the past have focused on characterizing the relationship between noroviruses and epidemic gastroenteritis. Studies analyzing differences in norovirus genogroups by transmission and settings indicate the GI strains are more commonly associated with environmental and waterborne outbreaks than GII strains. However the vice versa has also been observed making it difficult to draw a strong conclusion [38, 45, 58-60].

Recent studies have indicated that noroviruses are an important cause of mild and severe endemic gastroenteritis around the world, especially among children [5]. Very few studies have looked at the epidemiology of norovirus in developing countries in an effort to identify risk factors that are associated with increased transmission of the disease. It is clear that our understanding of the epidemiology of norovirus is much more comprehensive in developed countries than in developing countries but we cannot generalize that the epidemiologic characteristics are same in both settings. Since the developing world experiences the majority of the disease burden, there is a clear need for studies which analyze norovirus transmission in these countries especially with regards to different transmission settings. A study from Mexico found that domestic hygiene measures, type of room used for cooking, having a dog in or near the home and mother's involvement in agricultural activities were risk factors for seroresponse [55]. But there exists a gap in understanding the role of water quality and water source in facilitating the spread of norovirus.

Although it is hard to make firm conclusions, limited data indicate that noroviruses play an important role in acute gastroenteritis in Central America [55]. The goal of this study is to gain a better understanding of the epidemiology of noroviruses in Santa Rosa, Guatemala by analyzing active population based surveillance data in Santa Rosa, Guatemala. Specifically, we wanted to identify risk factors at a population level that are associated with increased transmission of the disease. We were also interested in comparing and contrasting factors associated with infection of norovirus and rotavirus as both cause significant morbidity in children. This comparison would help us better understand the dominant transmission pathways for these pathogens. In addition, we wanted to highlight areas where interventions can be implemented to reduce the risk of norovirus especially among the most vulnerable populations.

Methods:

Study Site

The study was conducted in the southern administrative department of Santa Rosa in Guatemala, approximately 50km from Guatemala City (Figure 1). Santa Rosa has an area of 3,164 km^2, divided into 14 municipalities (municipios) with a population of approximately 319,963 and altitude range from sea level to 1,945 m [62]. The majority of the population is Ladino and lives in rural areas and the most common language spoken is Spanish. The temperature in Santa Rosa remains stable throughout the year between 23° and 26°C, but the rainfall varies substantially with majority of the rainfall experienced between May and September.

There are four different levels of healthcare services provided by the national government in Santa Rosa. These include one regional referral hospital in Cuilapa, 14 health centers (one in each of the municipalities), 56 health posts, and convergence centers. Each of the 14 health centers is attended by a physician and several nurses whereas all of health posts are staffed by a nurse and primarily serve the smaller communities. The convergence centers provide healthcare services through contracted non-governmental organizations. There are other sources of healthcare in Santa Rosa, which include private hospitals and clinics, pharmacies and traditional healers.

Surveillance system

The population based surveillance system has been in place in the Department of Santa Rosa since June 2007. The study site is participating in the *Vigilancia Comunitaria* (ViCo) project, which is a population based surveillance system implemented by the Center for Health Studies of Universidad del Valle de Guatemala (CHS-UVG) and the

International Emerging Infectious Program (IEIP) of the Centers for Disease Control and Prevention's Regional Office for Central America and Panama (CDC-CAP). The ViCo site at Santa Rosa is a well equipped population based surveillance system with the appropriate epidemiological, clinical, and laboratory-based capacity for multiple syndromes – including laboratory proven diarrheal illnesses.

Diarrheal surveillance was carried out at the hospital in Cuilapa, and at all of the governmental healthcare facilities in the municipality of Nueva Santa Rosa, which included one health center and five health posts. Patients who presented with clinical symptoms of diarrhea at the health facilities or were admitted to the hospital with a diarrhea-related diagnosis were eligible for enrollment in the surveillance system. Diarrhea case was defined as ≥3 loose or liquid stools within a 24 hour period during the last seven days in a resident of Santa Rosa. Patients who reported an episode of diarrhea in the week prior to onset of the present episode were excluded from the study. Patients at the hospital, health centers and health posts meeting the case definition were asked to participate in the study. If agreed to participate, written informed consent was asked from the patients. Written, informed consents were sought from parents or caregivers of children under 18 years, and children between 7-17 years were also asked for their written informed assent.

Once the consent was provided, surveillance nurses conducted standardized patient interviews and reviewed medical charts for hospitalized patients to collect relevant clinical and epidemiological data regarding their current episode of diarrheal illness. Data were collected using hand held Personal Digital Assistants (PDAs), and managed and stored using Microsoft 2008 SQL Server (Redmond, VA).

Stool samples were collected as either whole stool samples or via cary blair rectal swabs from diarrhea eligible patients who were enrolled in the surveillance system. Samples were collected and stored at 4°C and transported to the International Emerging Infections Laboratory at the Universidad del Valle de Guatemala (UVG). Whole stool samples were tested for norovirus genogroups I and II via rRT-PCR. The stool samples were also cultured for common bacterial pathogens known to cause gastroenteritis (i.e. *Salmonella spp., Shigella spp.* and *Campylobacter spp)* and tested for rotavirus by enzyme immunoassay.

Statistical Analysis

All cases of diarrhea from June 2007 through November 2010 were considered in the study. Data analysis was conducted using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Hospitalized cases were assumed to be severe cases of diarrhea whereas ambulatory cases presenting to the health center or health posts were assumed to represent mild to moderate cases of diarrhea. Descriptive and univariate analysis were performed to determine the distribution of norovirus by genogroup and age. Norovirus and rotavirus distribution was also analyzed by hospitalized and ambulatory cases in the 14 municipalities of Santa Rosa.

Bivariate analysis consisted of characterizing relationships between norovirus infection, defined as laboratory confirmed case and potential risk factors for waterborne transmission including methods for treatment of drinking water and water sources. Differences in water treatment methods and water sources among norovirus and non-norovirus cases as well as rotavirus and non-rotavirus cases were analyzed using chi-square tests for significance. As many water treatment techniques and water sources were similar, we combined them together into new variables, for example piped water shared with another family and municipal piped outside the house were combined together as piped water-public. Unadjusted odds ratios were calculated along with their respective 95% confidence intervals.

Multivariate analysis was conducted by performing logistic regression to study the effects of multiple risk factors on the outcome using a *hierarchical backwards elimination approach*. Variables included in the multivariate models were determined *a priori* based on significant p values ($p<0.10$) Separate logistic models were fit on norovirus and rotavirus outcomes with the following exposure variables included as predictors: any treatment of drinking water, filtering water, boiling water, chemically treating water, storing drinking water, piped private water, piped public water, bottled water, water from public laundry, well water and surface water. Age, sex and health facility type were kept in the model as they showed strong associations with both norovirus and rotavirus. Multicollinearity was assessed using condition indices (CIs) and variance decomposition proportions (VDPs) by running a COLIN macro in SAS. If a condition index was found to be above the cutoff value of 30, terms containing a VDP value of greater than 0.5 were removed in descending order until all condition indices were below the cutoff value. Two-way interaction was assessed using likelihood ratio test and all variables were assessed for confounding using data-based criterion, which was defined as a 10% change in the odds ratio of the primary exposure variable in the full model. The likelihood ratio test was statistically insignificant, so no interaction terms were included in the final model. In the model for norovirus, all variables were found to be within the 10% range but were still retained in the final model because of evidence presented in previous studies. In the rotavirus model, all variables except healthcare facility type were found to be potential confounders, and therefore all variables were kept in the model. Adjusted odds ratio and 95% confidence intervals were calculated for all multivariate analyses.

The study was approved by institutional review board at the Centers for Disease Control and Prevention (Atlanta, GA), Emory University, and the UVG (Guatemala City, Guatemala).

Results:

Patient characteristics

A total of 3508 patients were eligible for the study, out of which 3048(86.9%) patients provided stool samples for testing for diarrheal pathogens. Of the 2089 patients who provided whole stool samples for rRT-PCR testing, norovirus was detected in 306 (14.7%) patients. Among the positive norovirus cases, there were 55 (17.6%) cases that belonged to genogroup I (GI), 258 (82.4%) cases were determined to be of genogroup II (GII) and seven cases were diagnosed as mixed infections with both genogroups (Table 1). Rotavirus was tested on 2312 samples and 307 (13.3%) diarrhea cases were found to be positive for the pathogen (Table 2). An overwhelming majority of norovirus cases was presented in children as 70.9% of GI cases (n = 39) and 86.8% of GII cases (n = 224) were detected in children under five. The same was observed with rotavirus distribution where 84.4% (n = 259) of the rotavirus cases were seen in children under five (Table 2).

To determine the geographic distribution of norovirus, we analyzed hospitalized cases of norovirus by municipalities in the Department of Santa Rosa. The majority of the norovirus cases came from 3 municipalities; Barberena, Cuilapa, and Oratorio accounted for 63.8% of the hospitalized cases of norovirus (Table 3).

Overall hospitalized cases experienced a higher severity of clinical symptoms than ambulatory cases for both norovirus and rotavirus but this was especially evident in children. Among hospitalized and ambulatory cases we saw that children under one were more likely to experience a severe manifestation of the disease than children over one. There was also a higher proportion of hospitalized norovirus (67, 62.0%) and rotavirus (106, 67.5%) cases that were male compared to less severe, ambulatory norovirus (101, 51.0%) and rotavirus (73, 48.7%) cases (Table 4). Vomiting was observed in 63% of the hospitalized norovirus cases as compared to 46.6% in ambulatory norovirus cases. In rotavirus cases, vomiting was recorded in 69% of the hospitalized patients and 55% in ambulatory cases (Table 4). In ambulatory clinics, 132 norovirus patients (66.7%) had stool with mucous whereas only 57 (52.8%) of the hospitalized cases recorded the same. This difference was absent among rotavirus cases as 57.3% of hospitalized cases and 58.7% of ambulatory cases observed stool with mucous (Table 4). While more hospitalized patients (25%) with norovirus experienced watery stools than ambulatory cases (18.7%), the reverse was observed among rotavirus cases (42% in hospitalized cases and 50.7% in ambulatory cases) (Table 4). Fever was reported more often among hospitalized norovirus cases (31.5%) than ambulatory cases (14.1%) but only 26.8% of hospitalized rotavirus cases reported fever when compared to 33.3% of ambulatory cases (Table 4). A higher proportion of both norovirus and rotavirus hospitalized cases showed signs of dehydration indicated by drier oral mucosa and sunken eyes than their ambulatory counterparts (Table 4).

Risk factors for norovirus and rotavirus transmission

Risk factors for waterborne transmission showed some association with norovirus infection. The bivariate analysis showed that sex (male) was significantly associated with norovirus. Treatment of drinking water was not significantly associated but filtering water, boiling water, chemically treating water, and storing drinking water were all significant risk factors for norovirus (Table 5). Looking at different water sources, private piped water was significantly associated with norovirus and while there were some indication of an association between public piped water, bottled water, water from public laundry, well water and surface water and norovirus, these associations were not statistically significant (Table 5). When contrasting with rotavirus, sex (male) again was a significant risk factor for the disease. While treatment of drinking water was not associated significantly, filtering water, boiling water and chemically treating water along with storing drinking water were all identified as having a protective effect (Table 6). Private piped water was a significant risk factor for rotavirus and the associations between rotavirus and public piped water, bottled water and surface water were statistically insignificant. Water from public laundry and well water showed protective effect which was significant (Table 6).

While bivariate analysis of risk factors provided some information on waterborne transmission of these diseases, we were interested in multivariate modeling to control for potential confounders and calculate effect of several factors simultaneously. Our final multivariate model revealed that filtering water was a risk factor for norovirus transmission while controlling for age, sex and assumed severity of illness (aOR: 2.44 [95% CI, 1.68 – 3.56]) (Table 7). In the case of rotavirus, our final model identified filtering water as having a protective effect (aOR: 0.48 [95% CI, 0.33 – 070]) while private piped water was a significant risk factor (aOR: 2.17 [95% CI, 1.33 – 3.54]) when age, sex and assumed severity of illness were controlled for (Table 7).

Discussion:

To our knowledge, this is the first study to investigate risk factors associated with norovirus infections in Central America. The primary goal of this study was to investigate and identify risk factors that facilitate waterborne transmission of endemic norovirus. The results presented here begin to give us an insight into how waterborne routes affect transmission in developing countries such as Guatemala where access to safe water and water quality is a constant concern. We analyzed norovirus data from a population based surveillance system in the Department of Santa Rosa, Guatemala using logistic regression to adjust for potential confounders. Based on our results, we found that filtering water was a significant risk factor for norovirus when controlling for age, sex and assumed severity of illness. When compared to rotavirus, filtering water showed to have a protective effect whereas private piped water was identified as a significant risk factor controlling for age, sex and assumed severity of illness. These findings suggest that water treatment methods and water sources play a significant role in transmission of norovirus but the effect of this set of risk factors might be different for norovirus and rotavirus. We also found that children under five experienced substantially higher morbidity due to norovirus and rotavirus infections than other age categories.

In general, our analysis showed that the GII genogroup was the dominant strain in circulation among residents of Santa Rosa (Table 1). Results from previous studies have shown that GI strains were more commonly associated with waterborne outbreaks than GII strains. Other studies have also indicated that that GII/4 strains were found more commonly in person-to-person outbreaks than foodborne outbreaks [38, 59, 60]. This suggests that person-to-person transmission might be the primary route of transmission of norovirus in this particular setting. Our findings also indicated that children under five were more severely affected than any other age group. The cumulative distribution curves showed that between 70-87% of the cases in both genogroups occurred by age five (Figure 2). A similar trend was seen in cumulative distribution of rotavirus cases. Since these cases were from either the regional hospital or ambulatory clinics, we found that children under five saw a higher rate of clinical manifestation of norovirus and rotavirus infections than children above five years and adults. We found that a greater proportion of males (62%) with norovirus were hospitalized than females (38%) and the same was seen for rotavirus (males: 67.5%, females: 32.5%), suggesting that females had a better immune response to infection than males (Table 3).

When considering potential risk factors for waterborne transmission in bivariate analysis, we found that storing water, filtering water, boiling water and chemically treating water were identified as significant risk factors for norovirus. Piped private water was also found to be associated with norovirus in this population (Table 5). For rotavirus storing water, filtering water, boiling water, chemically treating water and well water were found to have a significant protective effect whereas piped private water was identified as a risk factor (Table 6). These crude associations gave us an idea of how each set of potential risk factors might affect the disease outcomes when controlling for confounders such as age, sex and assumed severity of illness and adjusting for other exposures.

Overall, multivariate modeling revealed that only filtering water was a significant risk factor when controlled for age, sex and assumed severity of illness. This finding is of concern as it suggests that filtering water is not effective in preventing the transmission of norovirus. The way the residents filter their drinking water might eliminate other diarrhea causing pathogens but is ineffective in removing norovirus. For rotavirus, filtering water had a protective effect while piped private water was identified as a risk factor. The only water source that was found to have a significant association with rotavirus after controlling for age, sex and assumed severity of illness was piped private water. It showed that improving quality of drinking water may reduce rotavirus in this setting while it might not affect diarrhea incidence where the etiologic agent is norovirus. It also suggested that there are possibly different set of risk factors for norovirus and rotavirus when looking at waterborne transmission. One possible hypothesis from this analysis, given the distribution of norovirus strains in Santa Rosa: waterborne transmission compliments person-to-person transmission of norovirus, which might be the more dominant route of transmission, such that a person might get infected from contaminated water but transmission is facilitated through person to person contact.

This study had a number of strengths. It employed rRT-PCR as the diagnostic method for norovirus in a population based surveillance system, an extremely sensitive technique. The study also differentiated between the different strains that were prevalent in Santa Rosa. It also characterizes the relationship between risk factors for waterborne transmission of norovirus while controlling for potential confounders to get an accurate estimation of effect. There are a number of limitations to this study as well. First, information was not collected on all possible routes of transmission for norovirus (i.e. foodborne, person-to-person), which made it difficult to identify a comprehensive set of risk factors and fully understand their effect on norovirus transmission. Second, we were not able to include socioeconomic status (SES) as potential confounder in our analysis because of logistical constraints which would have been useful to understand norovirus transmission in this setting. Third, some variables, particularly water treatment methods, may have been affected by recall bias. Filtering water, boiling water and chemically treating water were associated with increased odds of norovirus infection. There is a possibility that people can over report “good” hygiene practices, instead of reporting what they actually do. Another issue of concern would be how they define these water treatment methods and the way they actually treat their drinking water. Biases associated with health facility utilization should also be considered as this is a health facility based surveillance system.

Our aim was to gain a better understanding of the norovirus epidemiology addressing the gap of knowledge in transmission type, setting and risk factors that are associated with increased transmission specifically in developing countries. The risk factors identified in this study suggest that waterborne transmission of norovirus is a possible route of transmission for endemic norovirus, although a causal relationship between each risk factor and norovirus outcome remains to be established. Data from this study also indicate that there might different set of risk factors for norovirus and rotavirus transmission. Even though in this study, water treatment methods were either shown to be risk factors or insignificant, interventions for sporadic non-bacterial gastroenteritis should focus on improving water quality and access to safe water sources. Further in depth studies of risk factors and routes of transmission for norovirus are warranted to truly understand the risk factors that influence norovirus epidemiology. Noroviruses are an emerging public health threat among children and adults, in both developed and developing countries, a better understanding of disease transmission will allows us to implement effective interventions to reduce morbidity and mortality due to norovirus infections.

PUBLIC HEALTH IMPLICATIONS

Noroviruses are the most common cause of epidemic, non-bacterial gastroenteritis in the world. They are responsible for 50% of gastroenteritis outbreaks globally and cause 21 million cases and 300 deaths annually in United States [3, 4]. Norovirus infections result in 900,000 clinic visits among children and cause 64,000 episodes of diarrhea resulting in hospitalizations in developed countries every year [5]. In developing countries it is estimated that norovirus infections cause over 1 million hospitalizations and up to 200,000 deaths among children under the age of 5 in developing countries [5]. Previous studies have helped us understand the transmission of norovirus in developed countries but the epidemiology of noroviruses in the developing world remains largely unknown.

Little is known about the true disease burden and risk factors that influence transmission of the norovirus in resource poor settings [5, 6]. This study aimed at addressing that gap by identifying risk factors for waterborne transmission for endemic norovirus in rural communities in Guatemala and has important implications for norovirus control and prevention activities. First, population based surveillance systems are important for detecting endemic norovirus in developing countries and should be an integral part of any control program. Secondly, this study highlights the importance of using robust laboratory techniques such as rRT-PCR in accurately detecting and characterizing strains of norovirus in the study population. This will help better our understanding of the epidemiology of norovirus and design specific interventions based on the strain characteristics. Third, water sources and water treatment methods are likely associated with transmission of norovirus. Even though some relationships in this study were not found to be significant, any intervention to reduce waterborne transmission should include access to safe water source and provide resources to adequately treat drinking water. Finally, this study reinforces the fact that children under the age of five are disproportionately affected as they experience majority of the disease burden. All control and prevention programs should include this population to effectively curb norovirus morbidity and mortality.

Further in depth studies of risk factors for all routes of transmission for norovirus are warranted to truly understand the risk factors that influence norovirus epidemiology in developing countries.

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Figures:

Figure 1: Study site: Department of Santa Rosa

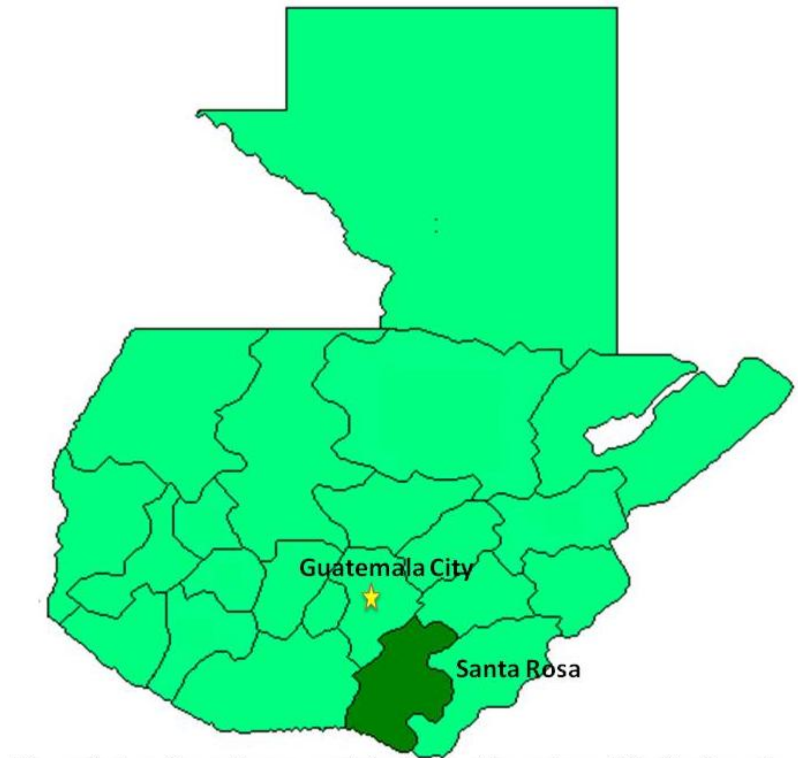
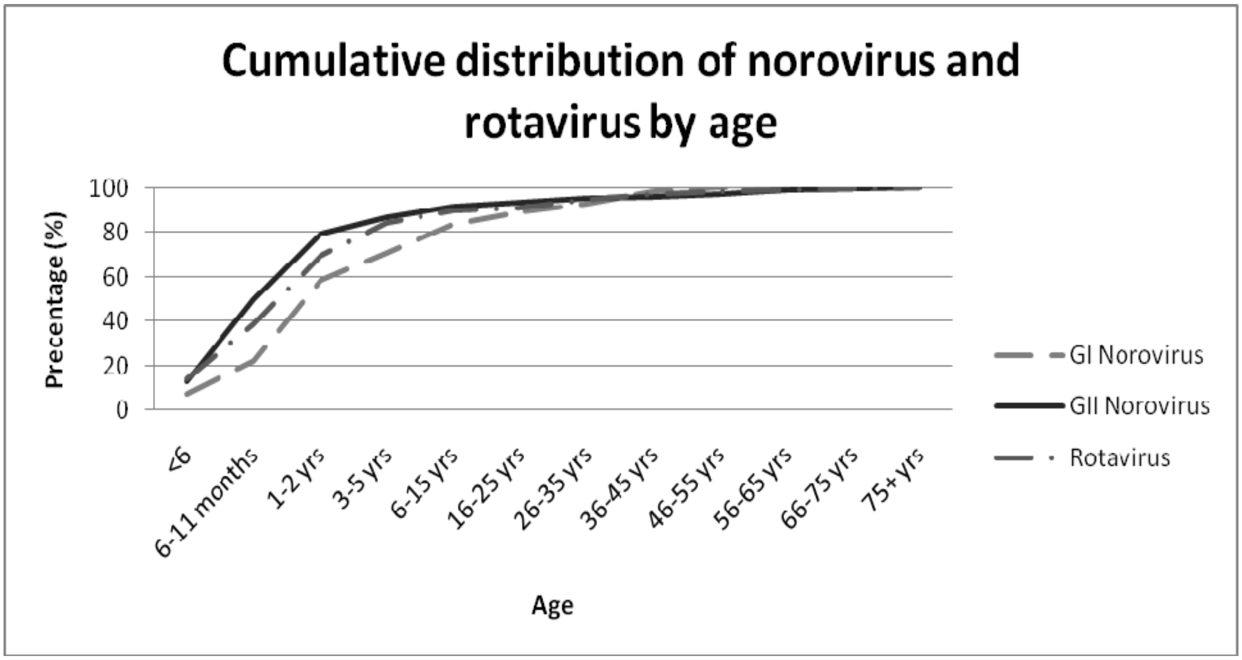
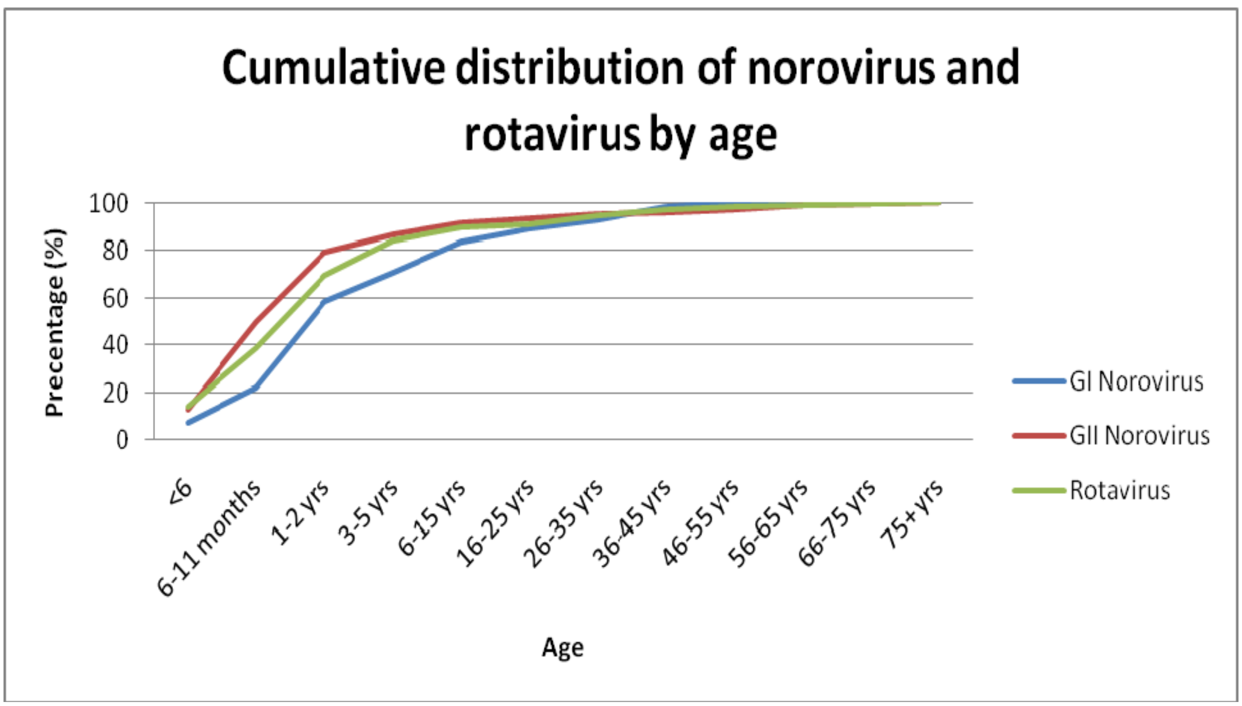


Figure 2: Cumulative distribution of norovirus and rotavirus cases by age in study population in the Department of Santa Rosa





Tables:

Table 1: Distribution of norovirus by strain and age among the study population in the Department of Santa Rosa

Norovirus Strain	<5 yrs	>5 yrs	N
GI	32 (70.9%)	16 (27.3%)	48
GII	217 (86.8%)	34 (13.2%)	251
Mixed	7	0	7
Total	256	50	306

Table 2: Distribution of rotavirus by age among the study population in the Department of Santa Rosa

	<5 yrs	>5 yrs	N (%)
Rotavirus	259 (84.4%)	48 (15.6%)	307

Table 3: Distribution of hospitalized norovirus and rotavirus cases by municipalities within the Department of Santa Rosa

Municipal district	Norovirus (N (%)) N= 108	Rotavirus (N (%)) N = 157
Barberena	35 (32.4%)	45 (28.7%)
Cassillas	5 (4.6%)	5 (3.2%)
Chiquimulilla	6 (5.6%)	3 (1.9%)
Cuilapa	21 (19.4%)	30 (19.1%)
Guzacapan	1 (0.9%)	1 (0.6%)
Nueva Santa Rosa	2 (1.9%)	14 (8.9%)
Oratorio	13 (12.0%)	13 (8.3%)
Pueblo Nuevo Vinas	7 (6.5%)	9 (5.7)
San Juan Tecuaco	1 (0.9%)	0
San Rafael las flores	1 (0.9%)	5 (3.2%)
Santa Cruz Naranjo	2 (1.9%)	6 (3.8%)
Santa Maria Ixhuatan	6 (5.6%)	16 (10.2%)
Santa Rosa de Lima	7 (6.5%)	10 (6.4%)
Taxisco	1 (0.9%)	0

Table 4: Patient characteristics of norovirus and rotavirus cases stratified by hospitalized and ambulatory cases with the Department of Santa Rosa

Patient Characteristics	Norovirus (N (%))		Rotavirus	
	Hospital N = 108	Ambulatory N = 198	Hospital N = 157	Ambulatory N = 150
Age				
<6 months	17 (15.7%)	19 (9.6%)	32 (20.4%)	11 (7.3%)
6 – 11 months	49 (45.4%)	52 (26.3%)	42 (26.8%)	34 (22.7%)
1-2 years	35 (32.4%)	59 (29.8%)	63 (40.1%)	32 (21.3%)
3-4 years	2 (1.9%)	23 (11.6%)	17 (10.8%)	28 (18.7%)
5+ years	5 (4.6%)	45 (22.7%)	3 (1.9%)	45 (30.0%)
Sex:				
Female	41 (38.0%)	97 (49.0%)	51 (32.5%)	77 (51.3%)
Ethnicity:				
Ladino	105 (97.2%)	178 (89.9%)	155 (98.7%)	132 (88%)
Indigenous	2 (1.9%)	20 (10.1%)	2 (1.3%)	18 (12.0%)
Vomiting	68 (63.0%)	92 (46.6%)	108 (68.8%)	83 (55.3%)
Stool with blood	6 (5.6%)	11 (5.6%)	2 (1.3%)	2 (1.3%)
Stool with mucous	57 (52.8%)	132 (66.7%)	90 (57.3%)	88 (58.7%)
Watery stools	27 (25.0%)	37 (18.7%)	66 (42%)	76 (50.7%)
Fever	34 (31.5%)	28 (14.1%)	42 (26.8%)	50 (33.3%)
Dry oral mucosa	82 (75.9%)	104 (52.5%)	123 (78.3%)	81 (54.0%)
Sunken eyes	39 (36.1%)	75 (37.9%)	60 (38.2%)	66 (44.0%)
Time to feel pinch (>2secs)	5 (4.6%)	8 (4.0%)	6 (3.8%)	11 (7.3%)

Table 5: Potential risk factors for waterborne transmission of norovirus within the Department of Santa Rosa

Risk Factor	Norovirus cases	Non norovirus total	Unadjusted OR (95% CI)
Sex (male)	168 (54.9%)	803 (45.0%)	1.49 (1.16-1.90)
Treat drinking water	111 (48.1%)	646 (43.4%)	1.21 (0.91-1.60)
Filter water	91 (60.7%)	302 (41.1%)	2.21 (1.54-3.16)
Chemically treat water	110 (73.8%)	466 (63.3%)	1.63 (1.10-2.43)
Boil water	129 (84.3%)	544 (73.5%)	1.94 (1.22-3.08)
Store drinking water	252 (82.6%)	1383 (77.6%)	1.37 (1.00-1.88)
Piped water - Private	202 (66.5%)	1050 (58.9%)	1.38 (1.07-1.78)
Piped water - Public	31 (10.2%)	187 (10.5%)	0.97 (0.65-1.44)
Bottled water	57 (18.8%)	330 (18.5%)	1.02 (0.75-1.39)
Public Laundry	14 (4.6%)	49 (2.8%)	1.71 (0.93-3.13)
Well Water	61 (20.1%)	356 (20.0%)	1.00 (0.74-1.36)
Surface water	3 (1.0%)	23 (1.3%)	0.76 (0.23-2.56)

Reference = non norovirus cases

Table 6: Potential risk factors for waterborne transmission of rotavirus within the Department of Santa Rosa

Risk Factor	Rotavirus cases	Non rotavirus total	Unadjusted OR (95% CI)
Sex (male)	170 (59.7%)	783 (44.8%)	1.82 (1.41-2.35)
Treat drinking water	104 (48.6%)	636 (43.5)	1.23 (0.92-1.64)
Filter water	40 (28.8%)	353 (48.62%)	0.43 (0.29-0.63)
Chemically treat water	74 (52.9%)	493 (67.9%)	0.53 (0.37-0.76)
Boil water	96 (69.1%)	565 (76.9%)	0.67 (0.46-1.00)
Store drinking water	205 (71.9%)	1392 (79.7%)	0.65 (0.49-0.87)
Piped water - Private	197 (69.1%)	1020 (58.5%)	1.59 (1.22-2.08)
Piped water - Public	22 (7.7%)	192 (11.03%)	0.67 (0.43-1.07)
Bottled water	53 (18.6%)	319 (18.3%)	1.02 (0.74-1.41)
Public Laundry	3 (1.1%)	58 (3.3%)	0.31 (0.10-0.99
Well Water	41 (14.4%)	370 (21.3%)	0.62 (0.44-0.88)
Surface water	1 (0.4%)	24 (1.4%)	0.25 (0.03-1.87)

Reference = non rotavirus cases

Table 7: Adjusted odds ratio of risk factors for norovirus and rotavirus among the study population in the Department of Santa Rosa

Norovirus	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Filter water[a]	2.21 (1.54 - 3.16)	2.44 (1.68-3.56)
Rotavirus		
Filter water[a]	0.43 (0.29 – 0.63)	0.48 (0.33-0.70)
Piped private water[b]	1.59 (1.22 – 2.08)	2.17 (1.33-3.54)

[a] Not filtering water was used as the reference

[b] Water sources that were not piped water were used as the reference

APPENDICES

APPENDIX A – Flow chart of the study