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Changes in etiology of acute gastroenteritis following introduction of the Rotavirus vaccine, Rotavac, in Tirupati, India

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Abstract

Changes in etiology of acute gastroenteritis following introduction of the Rotavirus vaccine, Rotavac, in Tirupati, India

By Alexia Eve Rodriguez

India introduced Rotavac, a locally produced, oral Rotavirus vaccine, to the national immunization program beginning in 2016. Understanding the change in the etiological mix of diarrheal disease in India following the introduction of this vaccine is necessary to inform treatment and prevention efforts surrounding diarrheal diseases. The effects of the rotavirus vaccine introduction on the etiology and severity of diarrheal diseases were evaluated using data collected from children < 5 years of age hospitalized for acute gastroenteritis (AGE) in Tirupati, India between September 2012 and April 2019. Stool samples underwent laboratory analysis to detect the presence of norovirus, sapovirus and astrovirus. For each pathogen, we used age adjusted negative binomial regression to model the counts of pathogen positive stool samples prior to and following the introduction of the rotavirus vaccine. Ordinal logistic regression was used to compare the severity of AGE between these two periods. Over the study period, 1,581 children presented to hospital for AGE. At least one pathogen (norovirus, sapovirus or astrovirus) was detected in 47% of all cases. We found a relationship between the vaccine period and astrovirus infection (IRR = 2.4; $CI_{95\%}$: 1.2, 5.1; p = 0.02) and no relationship between the vaccine period and norovirus or sapovirus infection. The odds of a child presenting with a moderate or severe episode in the post-vaccination period is 2.4 times that of the pre-vaccination period ($CI_{95\%}$: 1.9, 3.1). These findings suggest changes in the etiology of pediatric viral AGE in Tirupati, India following the introduction of the rotavirus vaccine where astrovirus, norovirus, and sapovirus contribute highly to detected pathogens of AGE in children.

Changes in etiology of acute gastroenteritis following introduction of the Rotavirus vaccine, Rotavac, in Tirupati, India

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by

Alexia Eve Rodriguez

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Chapter I. Background

Diarrheal Diseases and Rotavirus

Although diarrheal diseases have decreases substantially in the past 25 years, they remain a leading cause of death among all age groups and the fourth leading cause of death among children less than 5 years of age globally(1-3). It is estimated that diarrhea is responsible for over one million deaths worldwide and is a leading cause of disability-adjusted life-years (DALYs) as it disproportionately impacts young people (2). Diarrhea is a major cause of death in children less than 5 years worldwide, falling only behind lower respiratory infection, complications of preterm birth and neonatal encephalopathy (4). Among children in low-income countries, severe diarrhea is a major cause of death as a result of several factors including limited access to health care, malnutrition and delays in referral to health facilities (5). The greatest burden of these deaths befalls Africa and South Asia which account for over half of all diarrheal cases and lead in mortality for children under 5 years (2, 6). While estimates of annual mortality due to diarrhea in this age group ranges from nearly 1.5 million deaths per year in 2004 to 500,000 deaths annually in 2015 it is agreed that rotavirus is a leading cause of diarrheal mortality among children in worldwide (2, 7).

Over the ten-year period of 2005 to 2015, it is estimated that rotavirus-associated death among children this age group have decreased by 44% (2). However, severe diarrheal disease in young children caused by rotavirus still accounts for about 3.4% of all child

deaths globally (8). Additionally, rotavirus is an important cause of death at older ages as roughly 23% of rotavirus deaths occur in those over the age of 5. Pitzer et al. report that rotavirus accounts for nearly 610,000 deaths annually worldwide and Burnett et al. report that rotavirus accounts for about 40% of hospitalization in children less than 5 years due to acute gastroenteritis (AGE) globally (7, 9-10). While rotavirus may account for the highest burden in diarrheal disease, there are a number of other pathogens that cause acute gastroenteritis.

Viral Acute Gastroenteritis Agents

In addition to rotavirus, norovirus, sapovirus and astrovirus are among the viral agents largely responsible for AGE in children under 5 years of age (11). Noroviruses and sapoviruses are human caliciviruses that are recognized as a major cause of AGE worldwide (12).

Norovirus

Noroviruses are an enteric pathogen of the *Caliciviridae* family (13, 14). These viruses are known to cause disease in animals and humans of all ages and are responsible for sporadic cases and outbreaks. Noroviruses are composed of a single-stranded, positive-sense RNA genome roughly 7.7 kilobase in size, enclosed in a non-enveloped capsid with a cup-shaped depression (15). The genome contains three open reading frames encoding

for a capsid proteins and other non-structural proteins. Norovirus strains can be divided into five genogroups (GI – GV) three of which, GI, GII, and GIV, are known to cause infection in humans (13, 12). Each of these genogroups are further divided into genetic clusters which consists of approximately 25 genotypes. The genomes of norovirus vary due to an accumulation of point mutations and errors in replication resulting in the evolution of new strains causing repeated infections of norovirus in a single host (15). Despite the wide diversity of norovirus, GII norovirus infections have been the most commonly identified in outbreaks worldwide (13).

Norovirus infections are typically associated with vomiting and abdominal cramps followed by diarrhea and other symptoms such as headache, myalgia, nausea and chills (15). Fevers associated with norovirus infection are rare (15). Additionally, noroviruses can circulate as asymptomatic infections. Studies have indicated that norovirus-associated diarrhea are considered less severe than rotavirus-associated diarrhea (13).

However, noroviruses are a leading cause of AGE in all age groups and, increasingly, are becoming recognized as the second most common cause of AGE in children (13, 16). It is estimated that one-fifth of all AGE cases and over 200,000 annual death worldwide are associated with norovirus (17). Despite being a leading cause of AGE in all ages, fatalities associated with norovirus outbreaks are typically among the elderly in long-term care facilities (15). A previous study in Ethiopia reported that among children less than 5 years of age, children aged < 2 years had a higher rate of presence of norovirus in stool than children age 3 to 5 years (17).

While norovirus is primarily spread via the fecal-oral route, it can also be spread through droplets, fomites, person-to-person, and contamination of the environment. Worldwide, norovirus infections occur year around however, cases appear to peak during cold weather months. Currently, a number of norovirus vaccines are under development however, rapid evolution of the virus and incomplete understanding of immunity make this challenging (18).

Sapovirus

Like norovirus, sapovirus is part of the *Caliciviridae* family (19-20). These viruses are also known cause disease in animals and humans of all ages and are responsible for sporadic cases and outbreaks. Sapoviruses has a single-stranded, positive-sense RNA genome between 7.1 to 7.7 kilobase in size enclosed in a non-enveloped capsid. The genome contains two to three open reading frames encoding for a capsid protein and other proteins of unknown function and, based on the capsid, sapovirus strains can be divided into five genogroups (GI – GV) (12, 14). Of the five genogroups, GI, GII, GIV, and GV are known to cause infection in humans.

Sapovirus infections are typically associated with diarrhea and vomiting (20). Other symptoms such as headache, myalgia, nausea, abdominal cramps and chills are also reported with some frequency. Similar to norovirus, fevers are rare. Sapoviruses can also circulate as asymptomatic infections, predominantly in infants and young children,

providing a reservoir of the disease for the community (19, 21, 22). Among the literature there are conflicting reports regarding the severity of sapovirus-associated diarrhea. Many studies suggest that sapovirus associated diarrhea are generally mild but that severe cases can occur (12, 23). Oka et al. report that the severity of AGE associated with sapovirus is milder than cases of AGE associated with rotavirus and norovirus However, a study by Monica et al. reports no difference in severity of diarrhea among those infected with norovirus or sapovirus alone (21).

A study by Haranda et al. shows that sapovirus infections are not as rare as previously considered however, detection of sapovirus has ranged widely from 0.9% to 12.7% in various studies worldwide (19, 24). In an assessment of sapovirus-associated infections, Svraka et al. concluded that prevalence of sapoviurses are increasing in Europe and emerging across the globe. While it is children under 5 years of age who are primarily affected by sapovirus-associated diarrhea, infection in young adults and the elderly have been reported. Of children under 5 years of age, some studies suggest that sapoviruses are more prevalent among those over the age of 2 however, a report from Ethiopia suggest that frequency in detection of sapovirus does not vary between children under the age of 2 years and those age 3-5 years (17, 19, 25). Seasonal peaks of sapovirus infection have been primarily detected during the cold months overlapping with norovirus, rotavirus and astrovirus seasonality however, this likely varies with geographic region (20, 23). In fact, sapoviruses have been detected with higher frequency in environmental water samples during the colder season (26, 20).

Astrovirus

Astroviruses are a common cause of viral AGE worldwide across various developmental regions and are particularly important agents of diarrheal disease in children under 5 (27-29). Despite being noted as important drivers of viral AGE, astroviruses remain understudied in low- and middle-income countries.

Also known to infected animals, human astroviruses belong to the *Mamastrovirus* genus of the *Astroviridae* family, are characterized as nonenveloped, single-stranded positive sense RNA viruses and are related to other viruses such as picornavirus and caliciviruses. Of the eight serotypes of astrovirus, HAstV1-HAstV8, currently identified, HAstV-1 are the most prevalent.

Astrovirus infections are typically associated with acute watery diarrhea for two to three days, vomiting, abdominal pain, anorexia, headache, mild dehydration and fever. Severity of astrovirus-associated diarrhea or illness remains at odds with some studies indicating that severity of astrovirus infection are among the lowest of enteropathogens studied and others indicating that astrovirus is strongly associated with diarrhea as or more severe than well-recognized viral enteropathogens, including norovirus and rotavirus (28-29). It is possible that this range in severity of astrovirus infections is influenced by the high frequency of co-infections between the astrovirus with rotavirus or caliciviruses. Regardless, astrovirus remains an important cause of outbreaks and hospitalizations (28).

It is estimated that astroviruses are responsible for 0.6% to 12.4% of acute viral diarrhea with higher rates of infection in children under 5 years old and Olortegui et al. suggest that astrovirus may account for up to 5.96 million cases each year (30). Astroviruses, transmitted by the fecal-oral route, are associated with outbreaks in schools, child-care and aged-care facilities and hospitals resulting in nosocomial infections (27, 30). Astroviruses are known to cause diarrhea in children, the elderly, and immunocompromised persons and one study indicates that the frequency of diarrhea caused by astrovirus appears to the similar between children and adults (25, 27). The literature suggests that children < 2 years of age are most commonly affected however, studies in France and Spain have shown astrovirus infection to more commonly occur in slightly older children. Seasonality of astrovirus infection varies by geographical region where most astrovirus infections have been shown to occur during the winter months in temperate regions and during the rainy season in tropical regions (27, 30). The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study reported undernutrition as a risk factor for astrovirus and suggests evidence of protective immunity from previous infection noting that a vaccine for this virus might be appropriate.

Severity of Acute Gastroenteritis

In 1990, Ruusak and Vesikari proposed a 20-point scoring system, the Vesikari Scoring System, to measure the severity of rotavirus infections and evaluate the effectiveness of a rotavirus vaccine among Finnish children (31). This system gives scores based on duration of diarrhea and vomiting, maximum number of stools and vomiting episode over 24 hours, fever, dehydration and treatment (Table 1). In their initial document, Ruusak and Vesikari do not indicate how the points should be used to determine mild, moderate and severe cases and a number of articles, including one or both of these authors, have indicated differing cutoffs. Xiao-Li et al. used a score of 7 or less to indicate a mild case and anything greater than 7 to indicate a moderately severe case while Monica et al used a score of 5 or less as a cutoff point between mild and moderate cases and a score of 11 or greater to indicate a severe case (32, 21). Vesikari et al. use a score of 6 or less to indicate a mild case, and a score of 11 and greater as a cutoff between moderate and severe cases (33).

Despite being initially developed for rotavirus, the Vesikari scoring system has since been adopted to measure severity for other diarrheal diseases including norovirus, sapovirus and astrovirus (13).

Viral Acute Gastroenteritis in India

As previous studies have indicated, norovirus, sapovirus and astrovirus are an important cause of AGE among young children however, the roles of these diseases in sporadic AGE in children have been understudied in low- and middle-income countries (14, 19).

Norovirus

The role that human caliciviruses play in low- and middle-income countries, including in India, is not well described (14). Norovirus is a significant cause of AGE and an early report from Vellore, south India reported norovirus to be present in 8% of sporadic cases of AGE among children and adults (34). A more recent study following birth cohort in southern India found that 15% of all hospitalized diarrheal episodes and while another reported that 6.6% of all community cases among children age <5 years to be associated with the virus (13, 21). The study also reports seasonality of norovirus in this more tropical region suggesting a mild increase of norovirus during the summer months (13). Monica et al. report detection of norovirus in asymptomatic children indicating that the virus can circulate in the population in the absence of disease where children may act as a reservoir (21). The investigators go on to report GII as the most frequently detected genogroup of norovirus in Vellore. Detection rates of norovirus vary greatly between study sites. A study from New Delhi in northern India showed that 16% of children presenting with AGE tested positive for norovirus while a study form Lucknow, north India reported a 1.2% detection rate of norovirus (14, 22). A study in Chennai City in

southern India found that 44% of diarrheal patients tested positive for norovirus and that the seasonal peak occurred in the summer months (April) (16).

Sapovirus

A 5-year study in Maharashtra, western India detected sapovirus in 2.7% of children hospitalized with AGE over the study period. Positivity rates of sapovirus among children in India do vary slightly. Five percent in children hospitalized for AGE in southern India, 3.4% in the community of Vellore, 2.9 to 10.2% in in children hospitalized for AGE in northern India, and 2.3% in children hospitalized for AGE in eastern India (14, 19, 21-22). The former most study reports that only children 3 years of age or younger were found to test positive for the virus and that cases peaked in the summer months. During the study period of 2001-2004, investigators in southern India reported GII.1 as the most frequently identified sapovirus genotype. In years following, investigators in northern India found GII.1 to be the most predominant sapovirus genotype in 2007 and 2009, GIV.1 in 2008 and GV.1 in 2011.

Astrovirus

The MAL-ED study determined that India was among the countries with high incidence of astrovirus infection in children under the age of 5 years (27). Studies have shown that positivity rates of astrovirus in hospitalized children ranging from 2% in southern India, to 5.8% in eastern India, 1.8% in Lucknow, north India and 3.1% in western India (22,

29). The latter study reported higher rates of infection among children ≤ 1 year of age with a majority of infections among children < 2 years of age and no infections among adults hospitalized for acute diarrhea. This study also reports that the majority of astrovirus cases were detected during the winter with lower detection during the summer months however, a study from Kolkata reports that astrovirus infections were not more prevalence during the winter months (30). Bhatt et al. go on to argue that, in addition to rotavirus, astrovirus plays an important role in viral AGE among children.

Rotavirus

An assessment by the Expanded National Rotavirus Surveillance Network highlights the high disease burden that rotavirus poses for India with a prevalence near 40% nation-wide (35). Prior to the inclusion of Rotavac in the national vaccine schedule, India led the world in rotavirus burden with 22% of all deaths due to rotavirus globally (36). Badur et al. report that rotavirus was responsible for nearly 26% of all children < 5 presenting to a government hospital for AGE in Tirupati, India, from 2012 to 2013 (37).

Implications of Rotavirus Vaccines

Commercial rotavirus vaccines have been available since 2006 and, in 2009, WHO recommended that all countries add rotavirus to their national immunization schedules (38). Rotavirus vaccines have been shown to be efficacious against severe rotavirus

gastroenteritis in developed countries however, in developing countries or low-income settings rotavirus vaccine is less effective (9, 10, 39-41). Studies from Ghana and Finland report that yearly hospitalization for all-cause diarrhea declined following introduction of the rotavirus vaccine (5, 18). The latter also reported an upward shift in the age of children presenting to hospital with rotavirus-associated AGE. A study from Nicaragua reported an association between rotavirus vaccination and a lower risk of severe rotavirus diarrhea in young children however, they also note that this association was lower than what had previously been reported in high-income countries (9).

Recent studies have suggested changes in etiology of AGE, including norovirus- and sapovirus-associated AGE, following rotavirus vaccination (5, 17, 23, 39, 42). A study conducted in Nicaragua showed a change in the predominant causes of AGE from rotavirus to human caliciviruses norovirus and sapovirus following the introduction of a rotavirus vaccine (39). A study from the United States also reported norovirus as a leading cause of AGE in medically attended cases following its introduction (11). Rotavirus vaccination was found to be associated with a reduction in severity of sapovirus associated diarrhea in Finnish children (23). Hemming et al. report an increase in norovirus and sapovirus following the introduction of rotavirus vaccines and suggest that, while this was not the case at the study site, that other viruses could potentially replace rotavirus (18).

Despite WHO's 2009 recommendation regarding the rotavirus vaccine, it was not until 2016 that India introduced Rotavac, an indigenous, oral rotavirus vaccine to the nation

immunization program (43). The recent introduction of a rotavirus vaccine in India provides an opportunity to assess how such a vaccine may contribute to changes in the etiology and severity of AGE in India. Prior to the introduction of a rotavirus vaccine, India accounted for 22% of all rotavirus related deaths globally (8, 36). Likewise, norovirus, sapovirus and astrovirus appear to play a significant role in viral AGE throughout the world and in India. The recent addition of Rotavac to the national immunization program allows for the opportunity to assess the early effects of the vaccine on norovirus, sapovirus and astrovirus. If and how the reduction in rotavirus gastroenteritis may influence these viral pathogens and AGE severity in India has not yet been assessed.

Chapter II. Manuscript

Changes in etiology of acute gastroenteritis following introduction of the Rotavirus vaccine, Rotavac, in Tirupati, India

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Abstract

India introduced Rotavac, a locally produced, oral Rotavirus vaccine, to the national immunization program beginning in 2016. Understanding the change in the etiological mix of diarrheal disease in India following the introduction of this vaccine is necessary to inform treatment and prevention efforts surrounding diarrheal diseases. The effects of the rotavirus vaccine introduction on the etiology and severity of diarrheal diseases were evaluated using data collected from children < 5 years of age hospitalized for acute gastroenteritis (AGE) in Tirupati, India between September 2012 and April 2019. Stool samples underwent laboratory analysis to detect the presence of norovirus, sapovirus and astrovirus. For each pathogen, we used age adjusted negative binomial regression to model the counts of pathogen positive stool samples prior to and following the introduction of the rotavirus vaccine. Ordinal logistic regression was used to compare the

severity of AGE between these two periods. Over the study period, 1,581 children presented to hospital for AGE. At least one pathogen (norovirus, sapovirus or astrovirus) was detected in 47% of all cases. We found a relationship between the vaccine period and astrovirus infection (IRR = 2.4; $CI_{95\%}$: 1.2, 5.1; p = 0.02) and no relationship between the vaccine period and norovirus or sapovirus infection. The odds of a child presenting with a moderate or severe episode in the post-vaccination period is 2.4 times that of the pre-vaccination period ($CI_{95\%}$: 1.9, 3.1). These findings suggest changes in the etiology of pediatric viral AGE in Tirupati, India following the introduction of the rotavirus vaccine where astrovirus, norovirus, and sapovirus contribute highly to detected pathogens of AGE in children.

Introduction

Diarrheal diseases are a leading cause of death among all age groups and the fourth leading cause of death among children less than 5 years of age (1-3). Rotavirus has been a primary contributor to the severe diarrheal disease burden globally and accounts for about 3.4% of all child deaths. Commercial rotavirus vaccines have been available since 2006 and, in 2009, WHO recommended that all countries add rotavirus to their national immunization schedules (38).

In addition to rotavirus, norovirus, sapovirus and astrovirus are among the viral agents largely responsible for acute gastroenteritis (AGE) in children under 5 years of age (11).

However, these diseases have been understudied in low- and middle-income countries. Norovirus is a significant cause of AGE and a recent study in southern India found that 11.2% of all diarrheal episodes to be associated with the virus (13). Sapoviruses, which are responsible for sporadic outbreaks of gastroenteritis, often circulate as asymptomatic infections providing a reservoir of the disease for the community (19, 21). Moreover, India is among the countries with high incidence of astrovirus infection in children under the age of 5 years (27). While astrovirus is typically less severe than norovirus or rotavirus, it remains an important cause of outbreaks and hospitalizations (28).

Recent studies have suggested changes in etiology of gastroenteritis (GE), including GE due to norovirus and sapovirus, following rotavirus vaccination (4, 23 39, 42). There was a change in the predominant causes of GE from rotavirus to norovirus, sapovirus and other pathogens, following the introduction of a rotavirus vaccine in Nicaragua while rotavirus vaccination was found to be associated with a reduction in severity of sapovirus associated diarrhea in Finnish children.

The recent introduction of a rotavirus vaccine in India provides an opportunity to assess how such a vaccine may contribute to changes in the etiology and changes in severity of GE in India. In 2016, India introduced the oral rotavirus vaccine, Rotavac, to the national immunization program (43). Prior to the introduction of a rotavirus vaccine, India led the world in rotavirus burden with 22% of all rotavirus related deaths globally (8, 36). An assessment by the Expanded National Rotavirus Surveillance Network highlights the high disease burden that rotavirus poses for India with a prevalence near 40% nation-wide

(35). If and how the reduction in rotavirus gastroenteritis may influence the AGE burden in India has not yet been assessed. Additionally, rotavirus vaccines have been shown to be efficacious against severe rotavirus in developed countries and, to varying degrees, in developing countries or low-income settings where rotavirus vaccine is less effective (9, 40-41). However, the influence of the rotavirus vaccine on severity of all-cause AGE is unknown in this setting.

This study aims to provide an assessment of the early effects of nation-wide rotavirus vaccine introduction on the etiology of norovirus-, sapovirus- and astrovirus-associated diarrhea in Tirupati, India. We explore the prevalence of these diseases in hospitalized children prior to and following the introduction of the Rotavac vaccine; a time frame of nearly seven years from 2012 to 2019. As a secondary aim, we explore the trends in AGE severity over time.

Methods

Study Population

This study took place in the municipality of Tirupati, Andhra Pradesh in southern India. Hospitalization data from the Sri Venkateswara Medical College in Tirupati over the period of September 2012 through April 2019 were obtained from the Indian National Rotavirus Surveillance Network (NRSN) and the Rotavirus Impact Surveillance Programme (RVIS).

The NRSN, from which data prior to rotavirus vaccine introduction was obtained, was established by the Indian Council of Medial Research in December of 2005 in an effort to estimate and monitor diarrheal disease in children under the age of 5 (35, 44). Following recommendations from advisory groups, the program was expanded in 2012 to include 28 recruitment sites including in Tirupati. Patients, children age 0 to 59 months, were considered for enrollment if they were admitted to one of the sites for rehydration due to acute diarrhea with a duration of no longer than five days.

Data for the years of and following the introduction of the rotavirus vaccine were obtained from the RVIS. The goal of this multisite surveillance system is to monitor and evaluate the impact of the rotavirus vaccine following its introduction into the national immunization program (43). Patients, children under 5 years of age, were considered for enrollment if they were admitted to one of the sites for rehydration due to acute diarrhea and produced a stool sample during the first two days of presenting at hospital.

From September 2012 to April 2019, 1,581 children under 5 years of age were included in the two surveillance programs from the Tirupati site. Data on patient age, sex, date of onset, clinical features of the diarrheal episode and Vesikari scores were collected and recorded. Vesikari scores are a measure of severity of diarrheal disease originally developed for rotavirus which use clinical features of diarrheal episodes such as duration of diarrhea and duration of vomiting to categorize severity (13, 31). On this 20-point scoring system, we define mild cases as those with a score of 6 or less, moderate cases

with a score from 7 to 10 and severe cases as those with a score greater than 10. Stool samples were tested for rotavirus, norovirus, sapovirus, astrovirus and adenoviruses using real-time (semi-quantitative) polymerase chain reaction.

Statistical Analysis

Relative frequencies of viral pathogens over various age groups, severity scores, and over time were analyzed to investigate trends. The impact of rotavirus vaccine introduction on diarrhea etiology was estimated by comparing norovirus, sapovirus and astrovirus prevalence prior to and following vaccine introduction using Chi-square tests. Similarly, impact of rotavirus vaccine introduction on AGE severity was estimated by comparing average severity in the years following vaccine introduction to the average severity prior to vaccine introduction using Student's t-test and an ordinal logistic regression model treating the numeric scores, 0 to 20, as categories.

Data were tested for overdispersion to determine the appropriate use of the regression model. Negative binomial regression was used to model counts of AGE prior to and following the introduction of the rotavirus vaccine with the introduction of the virus (or the post-vaccine period) as the predictor. Models were adjusted for time using a linear variable to take into account secular trends such as changes in surveillance and adjusted for age in categories of 0-5 months, 6-11 month, 12-17 months, 18-23 months, 24-35 months, 36-47 months and 48-59 months. The year of vaccine introduction (2016), was considered the transition year and was excluded from the analysis. The pre-vaccine period was defined from 2012 through 2015 and the post-vaccine period was defined

from 2017 through 2019. Data were analyzed using R 3.5.1 software and p-values of < 0.05 were considered statistically significant.

Ethical Approval

This research was not subject to institutional review board approval at Emory University as the data were deidentified.

Results

Participants

From September 2012 to April 2019, 1,581 children under 5 years of age (0-59 months) were enrolled in the study and their stool was tested for various viral pathogens (Fig. 1). Of those children, 689 (43%) were females (Table 2). The average age of enrolled children was 13 months (13 ±10.2) and a majority (55%) were under 1 year of age. The average severity as measured by the Vesikari Score was 10.09 (±3.3).

Norovirus

Of those evaluated, norovirus was the most common pathogen detected (Fig. 2). Norovirus was observed in 265 (17%) of the 1,581 children included in the study and the majority of children infected with norovirus were under the age of 24 months. Norovirus was observed in 79 (17%) of 458 children with severe diarrhea. The majority of cases

belonged to GII (91%) followed by GI (9%) and one case was co-infected with both GI and GII.

Sapovirus

Sapovirus was detected in 234 (15%) of the 1,581 participants. Similar to our observations with norovirus, the majority of children infected with sapovirus were under the age of 24 months with a mean age of 12.7 months (median = 11). Of the sapovirus infections observed, the majority constituted moderate to severe cases of AGE. Two hundred and eight of the 234 (89%) detected sapovirus infections were successfully genotyped. The most common genogroup was GI at 55% (115/208) followed by GII at 41% (84/208), GIV at 2% (5/208), and GV at 2% (4/208).

Astrovirus

A total of 247 (16%) astrovirus cases were detected among participating children. The majority of children infected with astrovirus were under the age of 24 months with a mean age of 12.3 months (median = 10). Of the 234 detected astrovirus infections, 208 were successfully serotyped. The most common serotype was found to be AstV 1 at 51% (106/208) followed by AstV 3 at 16% (33/208) and AstV 4 at 15% (32/208).

Pre and Post Vaccination Comparison

In comparison of viral AGE in the pre and post rotavirus vaccine eras, the results indicate that there is no statistically significant relationship between the vaccination periods and the proportion of samples that tested positive for at least one of the viral pathogens,

norovirus, sapovirus, astrovirus or rotavirus, of AGE ($X^2_{df=1}$ = 0.5; p = 0.49) (Table 3). Comparisons between the pre-vaccination period and each individual year following vaccine introduction indicates that there is no difference in the proportion of samples positive for at least one of the viral pathogens between these time periods.

Vesikari scores were lower in the pre-vaccine (9.1 $\pm$ 2.7) compared to the post-vaccine (10.8 $\pm$ 3.4) period ($t_{494.61}$= -7.9; p < 0.001) (Fig. 3). For children who presented in hospital for AGE in the post-vaccination period, the odds of higher (i.e. >6 versus <=6) severity score is 2.4 times that of children presenting in hospital in the pre-vaccination period ($CI_{95\%}$: 1.9, 3.1)

Tests for dispersion indicated that the data were over-dispersed and that negative binomial regressions should be utilized in place of the Poisson regressions. A negative binomial regression indicated no evidence of change in counts of overall viral AGE cases post-vaccine introduction compared to pre-vaccine introduction (IRR: 1.1; $CI_{95\%}$: 0.8, 1.5; p = 0.69) (Table 4). We found no evidence for a relationship between the vaccine period and norovirus cases (IRR = 0.7; $CI_{95\%}$: 0.4, 1.3; p = 0.25) nor between the vaccine period and sapovirus infection (IRR = 0.9; $CI_{95\%}$: 0.4, 1.8; p = 0.75) indicating that the incident rate of samples positive for norovirus or sapovirus did not change between the pre- and post-vaccination periods. However, we found a relationship between the vaccine period and astrovirus infection (IRR = 2.4; $CI_{95\%}$: 1.2, 5.1; p = 0.02). There is no relationship between the vaccine period and coinfected cases (IRR = 1.6; $CI_{95\%}$: 0.8, 3.5; p = 0.2).

Discussion

We investigated the early effects of nation-wide rotavirus vaccine introduction on the etiology of norovirus, sapovirus and astrovirus in Tirupati, India. We examined the prevalence of these diseases in pediatric gastroenteritis cases in a hospital-based setting over a seven-year period. The rates of these viruses present in diarrhea remains high following rotavirus vaccine introduction as the proportion of AGE caused by norovirus, sapovirus, astrovirus and rotavirus combined remained the same between the pre- and post-vaccination eras. Overall, there were higher rates of astrovirus in post-vaccine period than in the pre-vaccine period however the rates of norovirus, sapovirus, and coinfections did not change substantially. Additionally, we see higher average severity in the post-vaccine period than the pre-vaccine period.

The proportion of AGE caused by norovirus, sapovirus, astrovirus and rotavirus, as measured by a positive test for any one of these pathogens, combined remained the same between the pre- and post-vaccination periods. A study in Finland found an overall decrease in hospital/clinic visits for GE (18) however, that is ultimately challenging to assess with a short time frame following vaccine implementation. Despite the relative stability in the proportion of these pathogens combined, we do see a higher rate of astrovirus infection in the post-vaccine period compared to the pre-vaccine period and no relative change in rates of norovirus or sapovirus infection. A few studies indicate that

norovirus or caliciviruses in general become the leading cause of GE following implementation of the rotavirus vaccine. This contrasts with our finding that, in the post-vaccine period, astrovirus became the leading cause of viral GE in the early years following vaccine implementation (11, 14, 18). In agreement with a study from Nicaragua, rotavirus vaccination does not reduce incidence of norovirus and sapovirus (39).

We further investigated severity as measured by Vesikari scores between the pre- and post-vaccination periods and found higher average severity in the post-vaccine period than the pre-vaccine period. Due to the nature of the hospitalized setting, we would presume that the cases being identified are already those that are severe and, following, that severity would not change because we would continue to identify severe cases. Studies of Finnish children indicated a reduction of severity in sapovirus-associated diarrhea following the introduction of the rotavirus vaccine and suggested that, overall, severe AGE is expected to decrease following rotavirus vaccination (18, 23). A study in Ghana noted the possibility of a significant reduction of severe diarrhea hospitalization resulting from the implementation of rotavirus vaccination program (5). While we did not look at changes in severity for individual pathogen across time, it appears that severity for all-cause AGE increased.

We note a number of limitations in this study. Firstly, these data were only collected from one hospital in southern India and, therefore, the results may not reflect changes in viral pathogens of AGE in all of India. Second, these data are solely hospital based and

provide no information regarding community-based AGE which limits calculations of prevalence and incidence. Third, the data include little information on the patient and so it is unknown which children may have presented in hospital multiple times for AGE or which had received the rotavirus vaccine. Another important limitation is that the sensitivity of surveillance may have changed over time which would confound the trends seen here though we attempted to account for this using our linear time variable. Although previous studies have indicated that rotavirus vaccination has no effect on incidence of infection with other pathogens among individuals there is no way to measure this from the data (45). Lastly, AGE is influenced by seasonality which was not assessed in this study (16, 18, 46). Seasonality may also have implications on generalizability of this study between different areas of India (19).

The findings from these data have a number of implications for treatment and prevention of AGE. The high detection of viral AGE highlights the importance of pathogen detection prior to prescription of antibiotics for AGE for children under 5 years of age (39). Despite little change in the pathogen specific rate of viral AGE between the pre- and post-vaccination periods, norovirus remains a large contributor of viral AGE. This suggests that a potential norovirus vaccine could be supported in this area (15, 18). Additionally, sapovirus, astrovirus, and norovirus have generally been described as mild diseases however the data suggest that these pathogens are also causing moderate and severe disease (12, 20, 26, 42). As these pathogens are understudied in low- and middle-income countries, further research of these pathogens is critical to addressing GE in Tirupati (13-14, 19).

To conclude, this study shows that the etiology of pediatric viral AGE in India has been altered following the introduction of the rotavirus vaccine and that astrovirus has become a leading cause of viral gastroenteritis at the hospital studied. Together with norovirus and sapovirus, astrovirus contribute highly to detected pathogens of moderate to severe AGE among children in Tirupati, India.

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Tables and Figures

Table 1. Vesikari Scoring System

Symptom	Score		
	1	**2**	**3**
Diarrhea			
Duration (days)	1-4	5	≥ 6
Maximum number of stools / 24 hours	1-3	4-5	≥ 6
Vomiting			
Duration (days)	1	2	≥ 3
Maximum number of episodes / 24 hours	1	2-4	≥ 5
Fever (°C)	37.1 – 38.4	38.5 – 38.9	≥ 39
Dehydration (%)	N/A	1-5	≥ 6
Treatment	Rehydration	Hospitalization	N/A

Table 2. Characteristics of study population

Characteristic	**All AGE (n = 1,581)**	**Norovirus (n = 265)**	**Sapovirus (n = 234)**	**Astrovirus (n = 247)**	**Coinfections (n = 177)**
Female, n (%)	689 (42.6%)	123 (46.4%)	102 (43.6%)	114 (46.2%)	82 (46.3%)
Age (mo), n (%)					
0-5	358 (22.6%)	43 (16.2%)	41 (17.5%)	68 (27.5%)	33 (18.6%)
6-11	495 (31.3%)	94 (35.5%)	81 (34.6%)	79 (32.0%)	58 (32.8%)
12-17	336 (21.3%)	75 (28.3%)	58 (24.8%)	45 (18.2%)	48 (27.1%)
18-23	173 (10.9%)	28 (10.6%)	28 (12.0%)	20 (8.1%)	19 (10.7%)
24-35	144 (9.1%)	17 (6.4%)	20 (8.6%)	27 (10.9%)	15 (8.5%)
36-47	50 (3.2%)	6 (2.3%)	3 (1.3%)	4 (1.6%)	2 (1.1%)
48-59	23 (1.5%)	2 (0.8%)	3 (1.3%)	4 (1.6%)	2 (1.1%)
Vesikari Scores, n (%)					
Mild (< 7)	120 (7.6%)	17 (6.4%)	14 (6.0%)	14 (5.7%)	10 (5.7%)
Moderate (7 - 10)	655 (41.4%)	101 (38.1%)	91 (38.9%)	105 (42.5%)	75 (42.3%)
Severe (> 10)	458 (29.0%)	79 (29.8%)	72 (30.9%)	64 (25.9%)	56 (31.6%)
Missing	348 (22.0%)				
Year , n (%)					
2012*	55 (3.5%)	2 (0.8%)	0 (0.0%)	5 (2.0%)	1 (0.6%)
2013	191 (12.1%)	22 (8.3%)	12 (5.1%)	11 (4.5%)	11 (6.2%)
2014	217 (13.7%)	31 (11.7%)	46 (19.7%)	38 (15.4%)	30 (17.0%)
2015	222 (14.0%)	45 (17.0%)	31 (13.3%)	16 (6.5%)	17 (9.6%)
2016	278 (17.6%)	50 (18.9%)	36 (15.4%)	54 (21.9%)	40 (22.6%)
2017	254 (16.1%)	46 (17.4%)	56 (23.9%)	57 (23.1%)	43 (24.3%)
2018	272 (17.2%)	48 (18.1%)	42 (18.0%)	51 (20.7%)	26 (14.7%)
2019*	92 (5.8%)	21 (7.9%)	11 (4.7%)	15 (6.1%)	9 (5.1%)
Pre-vaccine Introduction, n (%)	685 (43.3%)	100 (37.7%)	89 (38.0%)	70 (28.3%)	59 (33.3%)
Post-vaccine Introduction, n (%)	618 (39.1%)	115 (43.4%)	109 (46.6%)	123 (49.8%)	79 (44.6%)

*2012 and 2019 are not full calendar years; sampling began in September of 2012 and ended in April of 2019

Table 3. Results of Chi-square comparing proportions of samples testing positive for at least one viral pathogen (norovirus, sapovirus, astrovirus, or rotavirus) between the pre- and post-vaccination periods

Comparison	χ^2 Statistic	95% CI	P-value
Pre- vs. Post-Vaccination periods	0.5	-0.1, 0.0	0.49
Pre-Vaccination period vs. 2017	3.3	-0.5, 0.6	0.07
Pre-Vaccination period vs. 2018	0.3	-0.5, 0.5	0.56
Pre-Vaccination period vs. 2019	0.0	-0.1, 0.1	0.85

Table 4. Results of age and date adjusted negative binomial regression used to model counts of AGE prior to and following the introduction of the rotavirus vaccine

Model	Incident Rate Ratio	95% CI	P-value
Viral AGE ~ Vaccine period + Age + Time	1.1	0.8, 1.5	0.69
Norovirus ~ Vaccine period + Age + Time	0.7	0.4, 1.3	0.25
Sapovirus ~ Vaccine period + Age + Time	0.9	0.4, 1.8	0.75
Astrovirus ~ Vaccine period + Age + Time	2.4	1.2, 5.1	0.02
Coinfections ~ Vaccine period + Age + Time	1.6	0.8, 3.5	0.20

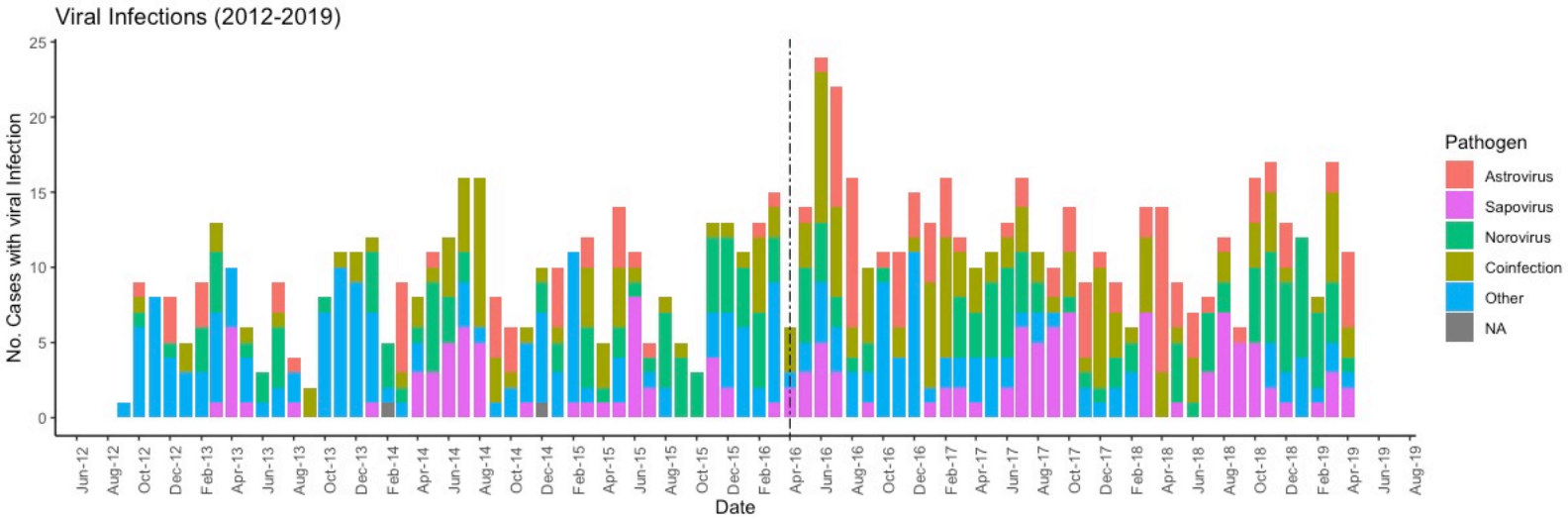

Figure 1. Time series of viral cause AGE cases presenting in hospital form September 2012 to April 2019. Agents in varying colors and vertical line at month of rotavirus vaccine introduction (April 2016).

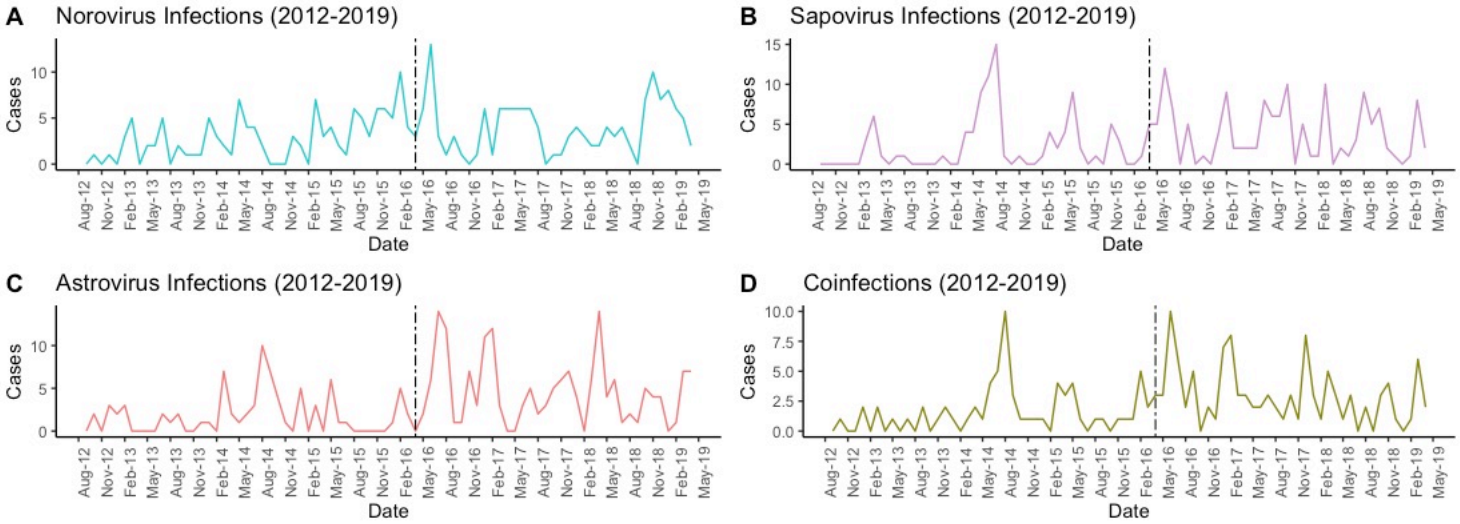

Figure 2. Time series (line graphs) of viral cause AGE cases presenting in hospital form September 2012 to April 2019 with a vertical line at month of rotavirus vaccine introduction (April 2016); Norovirus (A), Sapovirus (B), Astrovirus (C), and Coinfections (D).

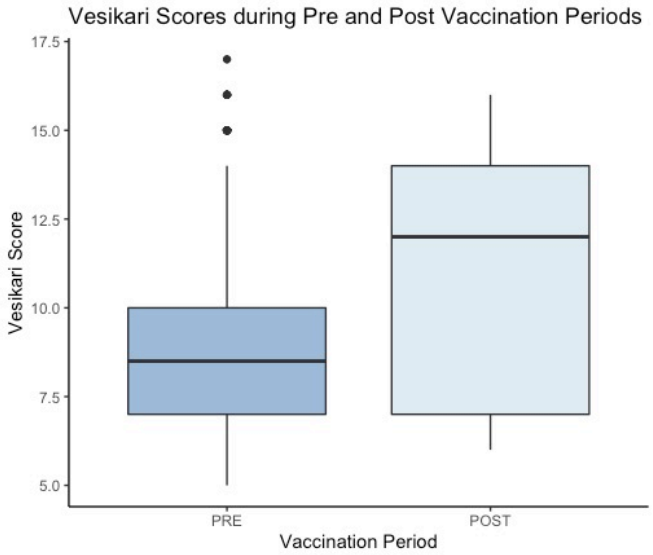

Figure 3. Boxplot of Vesikari Scores during the pre and post vaccination periods ($t_{494.6}$= −7.9, p < 0.001)

Chapter III. Summary, Implications and Future Directions

Summary

The intent of this study was to assess the early effects of the introduction of Rotavac, India's indigenous, oral Rotavirus vaccine, on the etiological mix and ecology of norovirus-, sapovirus-, and astrovirus-associated diarrhea in Tirupati, India. Data from the Sri Venkateswara Medical College in Tirupati were obtained from the Indian National Rotavirus Surveillance Network (NRSN) and the Rotavirus Impact Surveillance Programme (RVIS) and includes all children < 5 years of age hospitalized for AGE between September 2012 and April 2019. Our analysis showed that the proportion of samples that tested positive for at least one of the viral pathogens, norovirus, sapovirus, astrovirus or rotavirus, did not change following the introduction of the rotavirus vaccine. Analysis also indicated that the rate of astrovirus detected in stool sample increased in the post-vaccine period while detection of norovirus and sapovirus remained constant. Severity of all AGE cases overall increased following vaccine introduction and the odds of moderate to severe episodes presented in hospital increased in the post-vaccine period.

This was one of the first studies to evaluate the effects of the rotavirus vaccine on the etiology of norovirus-, sapovirus- and astrovirus-associated diarrhea in India. Recent studies in Nicaragua, Finland and the United States have suggested changes in etiology of GE, including GE due to norovirus and sapovirus, following rotavirus vaccination (11-

14). Rotavirus vaccination does not reduce incidence of norovirus and sapovirus in some settings (11). While the relationship between rotavirus vaccine implementation and other viral pathogens has not been well studied due to the relative novelty of the rotavirus vaccine, the results from this study help support the idea that introduction of the rotavirus vaccine influences the mix of diarrheal disease.

Public Health Implications

These findings show a change in pediatric viral acute gastroenteritis in Tirupati, India following the introduction of the rotavirus vaccine where astrovirus and sapovirus contribute highly to detected pathogens of AGE in children < 5 years old. Understanding this change in the etiological mix and ecology of diarrheal disease is necessary to inform treatment and prevention efforts surrounding diarrheal diseases, particularly in regard to pathogens for which there is limited data.

The literature has shown India to be among the countries with high incidence of astrovirus infection in children under the age of 5 years (9). Our results indicate that astrovirus becomes increasingly identified in AGE patients after the implementation of the rotavirus vaccine. While there is no relationship between the vaccine period and counts of norovirus cases, norovirus continues to be an important cause of AGE. Identifying the leading contributors to viral AGE can help focus research initiatives

critical to addressing GE in Tirupati and India as these pathogens, sapovirus and astrovirus in particular, are understudied in low- and middle-income countries (6, 8, 25).

In informing treatment and prevention efforts, the high detection of viral GE highlights the importance of pathogen detection prior to prescription of antibiotics for GE for children under 5 years of age (4). Norovirus remains a large contributor of viral AGE in this setting suggesting that a potential norovirus vaccine, of which there are some in development, could be supported in this area (24, 29).

Possible Future Directions

As mentioned previously, these pathogens are understudied in low- and middle-income countries and further research of these pathogens is critical to addressing GE in Tirupati. Future studies should be aimed at better understanding the role that sapovirus and astrovirus play in GE in children < 5 and in children < 2. Our study was limited to children who presented in hospital, however, research on asymptomatic or subclinical cases in the community could further aid in identifying the impact of these viruses on child health. Further research can be done on the seasonality of each pathogen to better understand patterns in infection and if and how these patterns might change after rotavirus vaccine introduction. Additionally, future studies should seek to address prevention of these enteric viruses including, but not limited to, reductions in childhood undernutrition, improvements in safe water, sanitation and hygiene and vaccination.