PREVALENCE OF ROTAVIRUS IN CHILDREN PRESENTING WITH ACUTE GASTROENTERITIS AT GERTRUDE'S CHILDREN'S HOSPITAL AND CLINICS

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Pediatrics by:

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DECLARATION

I declare that this dissertation is my original work and has not been submitted by any other person for a degree in any other university.

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DEDICATION

To my husband Chege who has been of tremendous support during this study; and to Sara our daughter.

ACKNOWLEDGEMENTS

I wish to thank my supervisors for all the support they have accorded me throughout this study.

I also thank Gertrude's Children's hospital management for allowing me to conduct the study there and for all their help in data collection and stool analysis.

I acknowledge the World Health Organisation (WHO) for financial support.

There are many other people who in one way or another have contributed in making this study a success. My heartfelt gratitude goes to all.

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ABBREVIATIONS

EIA Enzyme Immunoassay

ELISA Enzyme Linked Immunosorbent Assay

ETAT Emergency Triage and Treatment

GCH Gertrude's Children's Hospital

GSK GlaxoSmithKline

HRV Human Rotavirus

IV Intravenous

KNH Kenyatta National Hospital

NSP Non- structural protein

ORT Oral rehydration therapy

PAGE Polyacrylmide Gel Electrophoresis

PFC Pediatric Filter Clinic

RNA Reverse transcriptase – Polymerase Chain Reaction

VP Viral Protein

WHO World Health Organization

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ABSTRACT

Background

Rotavirus is the leading cause of severe gastroenteritis among young children worldwide. An estimated 600,000 children die from rotavirus each year with 80% being from developing countries. Rotavirus vaccination is considered the most effective public health strategy to prevent infection and reduce the severity of gastroenteritis.

Objectives

The primary objective of the study was to determine the prevalence of rotavirus among children aged 6 to 24 months presenting with acute gastroenteritis. The secondary objectives were to determine rotavirus vaccine coverage and to compare vaccination status, sociodemographic characteristics, breastfeeding practices and z scores in children with severe and non-severe gastroenteritis and also those with rotavirus positive and rotavirus negative stools respectively.

Study site

The study was carried out at Gertrude's Children's Hospital.

Study population

The study population comprised 195 children aged 6-24 months presenting with acute diarrhea at Gertrude's Children's Hospital (GCH) and its satellite clinics.

Study design

This study was a hospital based cross-sectional study.

Methods

A standard clinical assessment was carried out and stool samples collected from all children who met the inclusion criteria using standard tools. Rotavirus antigen testing was done and data analyzed using the SPSS program and presented in figures and tables as applicable.

Results

One hundred and ninety five children aged 6-24 months presenting with acute diarrhea were recruited. Overall, 77 stool samples tested positive for rotavirus giving a prevalence of 39.5%. Seventy one (36.4%) children had received the rotavirus vaccine. Vaccination was associated with a 70% reduction in the risk of severe gastroenteritis OR 0.3(95% CI (0.1-0.6); P=0.002 and a similar lower risk of having rotavirus recovered in stool OR 0.3(95% CI 0.2-0.7); P=0.001. Children aged 6-12 months were twice as likely to have severe gastroenteritis OR 2.2 (95% CI 1.1-4.6), P=0.033. Children whose parents had education level below secondary were twice as likely to have severe gastroenteritis and to have rotavirus recovered in stool OR 2.1(95% CI 1.0-4.4), P=0.042 and OR 2.3(95% CI 1.2-4.1), P=0.007. Multivariate analysis showed that vaccination status was the only variable independently associated with severity of gastroenteritis and rotavirus recovery from stool.

Conclusion

The prevalence of rotavirus in stool was 39.5%. The vaccine coverage against rotavirus was 36.4%. Rotavirus vaccination was associated with a 70% reduction in the risk of severe gastroenteritis and 60% reduction in the rate of rotavirus recovery from the stool.

LITERATURE REVIEW

Background

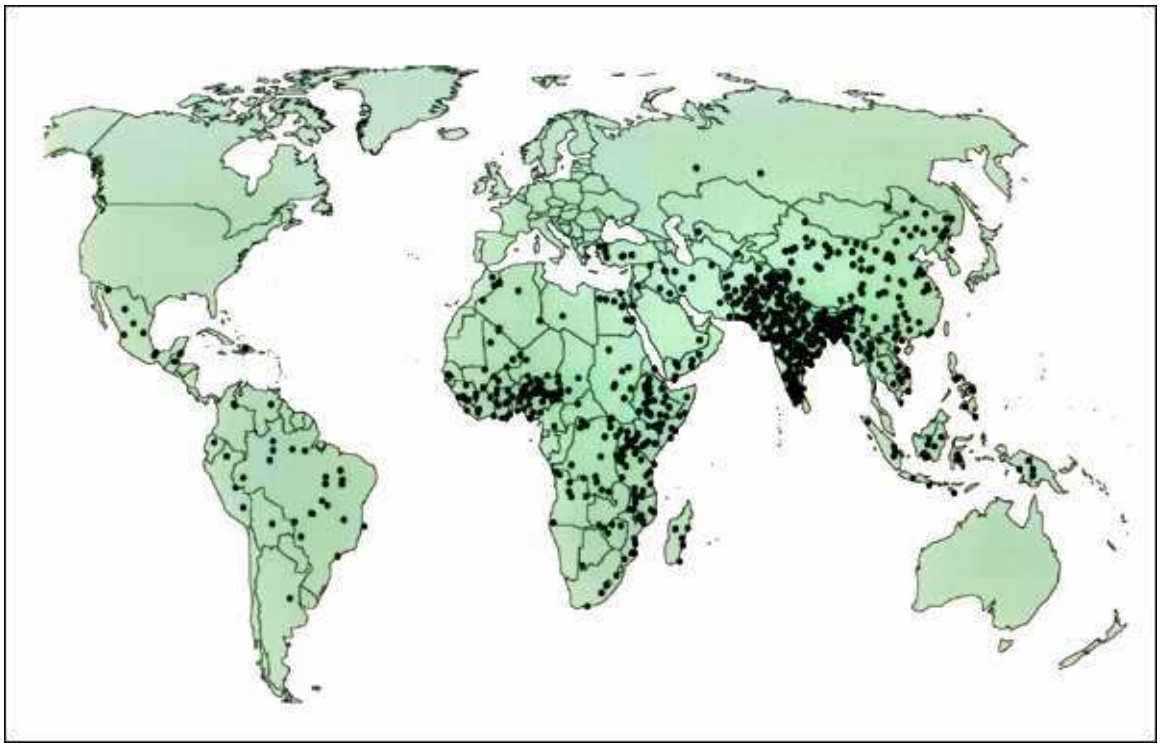
Rotaviruses are the most common cause of severe diarrheal disease in infants and young children world wide (1). Rotavirus infection is responsible for about 600,000 deaths per year (2). The mortality of rotavirus falls disproportionately on children in developing countries, where adequate and timely medical care is often inaccessible and unaffordable. More than 80% of rotavirus deaths occur in South Asia and sub-Saharan Africa (3). The observation that naturally acquired rotavirus infections provide protection against disease stimulated research into the development of a vaccine (4). In 1999 a highly efficacious rotavirus vaccine Rotashield licensed in the United States, was withdrawn after less than one year in the market because of its association with intussusception (4). In 2006, two new oral live-attenuated rotavirus vaccines were licensed: the monovalent human rotavirus vaccine (Rotarix®) and the pentavalent bovine-human reassortant vaccine (Rotateq™) (5). Both vaccines have been shown to have a reasonable safety profile in clinical trial settings and have been recommended by WHO for use in developing countries.

Epidemiology of diarrheal disease

Diarrheal disease is responsible for 4 – 6 million deaths per year according to WHO and is especially dangerous for infants and young children aged between 3-35 months (6). Rotavirus accounts for approximately 22% of hospitalizations for childhood diarrhea (7). Limited data suggest that children from disadvantaged socioeconomic backgrounds and premature infants have an increased risk of hospitalisation from gastroenteritis.

Rotavirus infection tends to occur year round in the tropics, whereas seasonal winter epidemics occur in temperate climates (8).

Figure 1. Estimated distribution of deaths from rotavirus diarrhea annually (1)

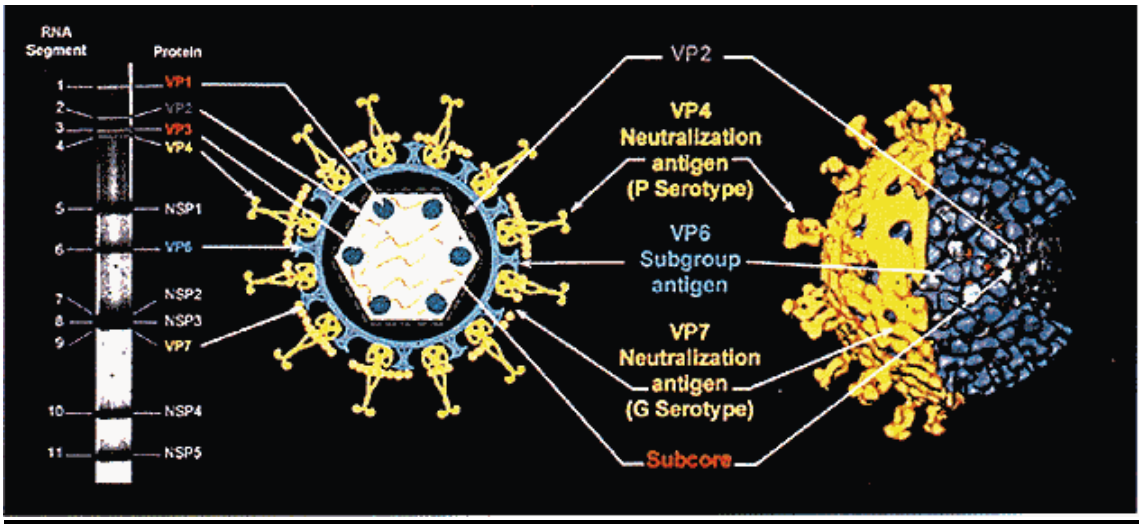


The annual incidence of diarrhea in Kenya is 3.4 – 4.6 episodes /child/year making it the 3rd leading cause of morbidity and mortality among children (9). At least half of the children presenting to the outpatient department with diarrhea have rotavirus infection. A study done in KNH showed a prevalence of 53% in children presenting with acute gastroenteritis at the pediatric filter clinic (PFC) (10). Preliminary findings from ongoing rotavirus surveillance in KNH from August 2006 to May 2007 indicated a prevalence of 47% among hospitalized children under 5 years of age (11).

Introduction and promotion of an effective rotavirus vaccine would therefore have significant impact on childhood diarrheal morbidity and mortality. Since diarrhea frequently precipitates malnutrition, rotavirus vaccine would be of benefit in the reduction of diarrhea related malnutrition.

Virology

Figure 2. Structure of rotavirus (14)



Rotavirus is a non enveloped virus of the family Reoviridae with an eicosahedral capsid 70nm across. It derives its name from the wheel – like appearance it has when viewed under an electron microscope (rota is Latin for wheel). Its genome is made up of 11 segments of double stranded RNA held in the inner core of the three layered virus.

The genome codes for 6 virus proteins (Vp1, 2, 3, 4, 6 and 7) and 6 non – structural proteins (NSP 1 – 6). Rotaviruses are classified in groups A-E and subgroups on the basis of the major virus protein VP6. Group A rotaviruses are the most common cause of human disease. Rotaviruses are further classified into serotypes on the basis of the neutralizing epitopes of VP7 (a glycoprotein), called G serotypes and VP4 (protease

sensitive), called P serotypes. There are 15 G and 14 P serotypes. Once in the small intestine, the virus undergoes a change and becomes infective to the villi. Proteins then mediate the invasion of the host cells and replication of the virus genome (1)

Transmission

Rotaviruses are transmitted by the faecal – oral route. Only 10 – 100 infectious particles are required to cause infection. This amount can readily be acquired through contact with contaminated hands and objects. Notably standard sanitary measures that kill most bacteria and parasites are ineffective in controlling rotavirus as demonstrated by the fact that rotavirus incidence is similar in countries with both low and high sanitation standards (13, 14).

Pathology

After ingestion, the rotavirus particles are carried to the small intestine where they infect mature enterocytes in the mid and upper part of the villi, leading to diarrhea. Invasion of target cells is thought to be by two ways; by direct entry or fusion with the enterocytes and through calcium dependent endocytosis (12). Within 24 hours of infection, the villus epithelium changes from columnar to cuboidal and the villi become shortened. Changes are most severe in the upper portions of the small intestine and there is little or no inflammation. The severity of these changes is correlated with the severity of the resulting illness.

Clinical presentation

In all the age groups the classical presentation of rotaviral infection is fever and vomiting for 2 – 3 days, followed by non – bloody diarrhea. The diarrhea can be profuse, with patients commonly having 10 – 20 bowel movements per day. Especially when associated with vomiting, the diarrhea caused by rotavirus can lead to severe and potentially life threatening dehydration and electrolyte imbalance. Gastrointestinal symptoms generally resolve in 3 - 7 days. Re- infections with rotavirus are generally less severe (15).

Diagnosis

Because the clinical features of rotavirus gastroenteritis do not differ from those of gastroenteritis caused by other pathogens it is necessary to confirm infection for reliable rotavirus surveillance and in making decisions about the use of antimicrobials.

The diagnosis of rotavirus can be done by identifying the virus in the patients stool.

The most popular technique is enzyme immunoassay (EIA), other techniques include electron microscopy (EM) polyacramide gel electrophoresis (PAGE) and reverse transcription – polymerase chain reaction (RT - PCR) (12).

Treatment

There is no cure for rotavirus. Most people develop immune response that is eventually adequate to clear the virus from the body (16). The treatment is therefore supportive, aimed at rehydration to prevent severe dehydration. Antidiarrheal medicines are not recommended because they may prolong the infection (17). In developing nations, the

primary treatment for dehydration is oral rehydration therapy (ORT). In a diarrheal state the normal mechanism for sodium absorption is impaired but glucose absorption is not affected. If sodium is given with glucose, absorption occurs via a co-transport mechanism. This discovery led to the development of ORT which was adopted by the World Health Organization (WHO) in 1978 as its principal strategy for preventing deaths from diarrheal disease.

Children with rotavirus often suffer frequent bouts of vomiting which lessens the effectiveness of ORT. Intravenous fluids (IVF) are often used to treat severe dehydration caused by rotavirus. Several studies comparing the effectiveness of ORT and IV administration have found no discrepancy in the effectiveness of the two methods (18). Enhancing diarrheal disease control through a combined strategy of prevention and treatment – incorporating the rotavirus vaccine, ORT and Zinc supplementation during diarrhoeal episodes can significantly reduce child mortality (20). Zinc is a micronutrient that is important for the growth of intestinal mucosa. It is essential for growth, protein synthesis and epithelial repair. It improves transport of water and electrolytes across the intestinal mucosa. Acute diarrhea can cause significant Zinc loss and supplementation is effective and affordable.

Prevention

Studies of natural rotavirus infection indicate that initial infection protects against subsequent severe gastroenteritis. Therefore vaccination early in life, which mimics a

child's first natural infection will not prevent all subsequent disease but should prevent most of severe rotavirus disease including hospitalization and dehydration (21).

Table 1. Characteristics of the rotavirus vaccines (25)

Rotavirus Vaccines		
	Rotarix® (GSK)	**RotaTeq® (Merck)**
Origin	**Human monovalent**	**Bovine pentavalent**
Strain	**G1, P**	**G1, G2, G3, G4, P**
Dosage	**2 doses (with DTP1, DTP2)**	**3 doses (with DTP1, DTP2, DTP3)**
Presentation	**Lyophilized; reconstituted**	**Liquid**
Administration	**Oral; applicator**	**Oral; squeeze tube**
Co-administration	**OPV, IPV, DTaP, DTwP HepB, Hib, PCV-7**	**IPV, DTaP, DTwP HepB, Hib, PCV-7**
Phase II & III safety & efficacy trials	**N=63,225 healthy infants USA, Canada, Latin America, Taiwan, Singapore, Hong Kong, Belgium, Germany, Finland, South Africa, Bangladesh, Sweden**	**n=70,301 healthy infants USA, Mexico, Costa Rica, Jamaica, Guatemala, Puerto Rico, Taiwan, Belgium, Finland, Germany, Italy**
Efficacy vs. rotavirus gastroenteritis	**85% vs. severe rotavirus gastroenteritis and 100% vs. more severe episodes**	**98% vs. severe G1-G4 rotavirus gastroenteritis**

Rotavirus vaccines

In 1998, the world's first rotavirus vaccine, a rhesus-based tetravalent rotavirus vaccine (RRV-TV) Rotashield™ was licensed for use in the United States. It was found to be 80 – 100% effective in preventing severe rotavirus diarrhea (22). It was however withdrawn from the market in 1999, after it was associated with an increased risk for intussusception in 1 out of every 12000 vaccinated infants (23).

Two oral live –attenuated vaccines against rotavirus infection (Rotarix®) manufactured by Glaxo Smithkline, and Rota Teq® manufactured by Merck & Co., Inc.) were licensed by the European medicines Agency and the US Food and Drug Administration respectively, in 2006(24). Their characteristics are outlined in table 1.

Clinical trials have demonstrated that these vaccines are safe and highly efficacious at preventing rotavirus associated gastroenteritis (25)

The immunogenicity of rotavirus vaccines is generally measured by detecting rotavirus group specific serum IgA seroconversion or by detecting serum neutralizing antibodies to vaccine strains and to the prevalent human strains (26). In all studies, vaccinated children developed significantly higher neutralizing antibodies to rotavirus than children who received placebo.

In recent trials, the efficacy of RotaTeq against rotavirus gastroenteritis of any severity was 74%; and for severe rotavirus gastroenteritis was 98%(27). A phase III trial conducted in 6 European countries showed that Rotarix and Rota Teq reduced the

incidence of all gastroenteritis hospitalizations of any etiology by 75% and 59% respectively during the first two years of life (28). Live oral vaccines are less efficacious in the developing world. Differences in nutritional status, breastfeeding patterns, bacterial and parasitic infections, HIV prevalence as well as different rotavirus serotypes pose a challenge to vaccine introduction in the developing world (31). Host related factors include malnutrition and the microbial load in African infants. Differences in the epidemiology of the rotavirus strains in these countries are also another factor (31). The reasons for these apparent differences in efficacy need to be investigated and elucidated, since they may hold important clues to completing development of a vaccine suitable for African children.

Breastfeeding or concurrent administration of other childhood vaccines does not appear to diminish either the immune response to or the efficacy of the rotavirus vaccines (29). Among 204 vaccinated premature infants, the point estimate of vaccine efficacy against rotavirus gastroenteritis of any severity was comparable to that among term infants (70%) (29). The vaccine is not recommended for children with known or suspected immunodeficiency. These are at risk of prolonged rotavirus gastroenteritis and can shed the virus for prolonged periods of time (30).

In clinical studies the most common adverse events were irritability, cough, runny nose, anorexia and vomiting and loose stool. The vaccine carries no significant increased risk of intussusception in either vaccine dose or placebo dose (25).

STUDY JUSTIFICATION

Rotavirus diarrhea contributes to about a quarter of all deaths related to diarrhoeal disease in children under five years of age. The bulk of these deaths are in the developing countries where severe dehydration and electrolyte imbalance are often not managed adequately due to unavailability of timely and optimal medical care. Public health measures such as good hygiene, proper sanitation and environmental cleanliness have not been shown to reduce the disease prevalence. Vaccination therefore holds the key to combating morbidity and mortality from rotavirus gastroenteritis worldwide. Following the introduction of the rotavirus vaccine into the private sector in mid- 2006, the number of admissions from rotavirus gastroenteritis has gone down. It is hoped that data generated on the prevalence of rotavirus gastroenteritis and rotavirus vaccine coverage from this study will be useful in evaluating the usefulness of the vaccine in the Kenyan community.

OBJECTIVES

Primary objective

To determine the prevalence of rotavirus in children presenting with acute gastroenteritis at Gertrude's Children's Hospital (GCH)

Secondary objectives

1. To determine rotavirus vaccine coverage among the children presenting with acute gastroenteritis.

2. To compare vaccination status, sociodemographic characteristics, breastfeeding practices and z-scores in children with severe and non-severe gastroenteritis.

3. To compare vaccination status, sociodemographic characteristics, breastfeeding practices and z-scores in children with rotavirus positive and rotavirus negative stools.

Methodology

Study area

The study was carried out at Gertrude's children's Hospital (GCH) in the out patient department and the satellite clinics.

Gertrude's Children's Hospital is one the leading children's hospitals in East and Central Africa. It is a 75 bed hospital with 5 satellite clinics within Nairobi. The average rate of admission for gastroenteritis was 360 cases per month in 2008. About 250 children per month received the rotavirus vaccine at the well baby clinics both in the main hospital and at the satellite clinics. The vaccine is also given in other private health care facilities.

Study population

Infants aged 6 – 24 months presenting with acute diarrhea at GCH.

Study design

Cross-sectional hospital based study

Inclusion criteria

1. Clinical diagnosis of acute diarrhea
2. Acceptance by guardian to participate in study by signing informed consent
3. Age 6-24 months

Exclusion criteria

1. Infants with diarrhea more than 14 day's duration

2. Those who did not give consent

Sample size calculation

Sample size was calculated using the formula for the calculation of a sample size in a cross-sectional study. The prevalence of acute gastroenteritis was estimated at 53% from a study done in KNH (10). Design effect of 2 was used to control for the clustering effects of the targeted population since the study was carried out both in the main Hospital and the satellite clinics.

$$n = \frac{\frac{1}{2}Z\alpha^2 P(1-P)\times DEFF}{d^2}$$

where

n = minimal number of infants aged 6 - 24 months presenting with Acute diarrhea

$\frac{1}{2}Z\alpha^2$ = the cut off point along the x-axis of the standard normal probability distribution that represents probabilities matching the 95% confidence interval (1.96).

P = prevalence of acute diarrhea among infants aged between 6-24 months estimated at 53%

d = level of precision 5% (0.05)

Design effect (DEFF) = 2

Using the above formula, the targeted sample size for the study was 191.

Case definition

1. An episode of diarrhea was defined as passage of three or more liquid stools per day

2. Acute diarrhea was defined as duration of diarrhea less than 14 days

3. Acute gastroenteritis was defined as diarrheal disease of rapid onset, with or without accompanying symptoms such as nausea, vomiting, fever or abdominal pain.

4. Severe gastroenteritis was defined as a score $\geq$ 11 using the **Vesikari 20 point scale** (Appendix C). This was modified because fever was not assessed during data collection as the decision to use the scoring system was made later.

Sampling procedure

The investigator identified children aged 6-24 months who presented at the emergency department at the main hospital or at the satellite clinics with acute diarrhea as per the case definition. After initial clinical assessment and commencement of treatment, recruitment into the study followed of those who met the inclusion criteria and from whom informed consent was obtained. (Appendix A)

Clinical methods

Clinical history and physical examination were carried out by the principal investigator and the details entered in the questionnaire (Appendix B). The degree of dehydration was assessed using clinical signs such as skin turgor, sunken eyeballs, capillary refill, peripheral pulse character and level of consciousness. The level of dehydration was classified using the World Health Organization (WHO)/Emergency Triage and Treatment

(ETAT) guidelines as shock, severe dehydration, some dehydration or no dehydration. The Vesikari 20 point scoring system (Appendix C) was used to classify severity of acute gastroenteritis. Severe gastroenteritis was defined by a score $\geq$ 11 points.

Samples collection

The investigator collected one stool sample approximately 3mls, in a plastic polypot from every child recruited into the study.

Laboratory methods

The stool sample was submitted to the GCH laboratory within one hour of collection. Those from the satellite clinics were stored in a refrigerator at 2-8°C and transported to GCH laboratory within one week where rotavirus antigen tests were carried out by trained laboratory personnel using a rapid immunochromatographic test for detecting rotavirus antigen in stool. The rotavirus antigen tests were performed as per the manufacturer's protocol. The test had 96.4% sensitivity and 100% specificity.

Statistical analysis

Data was collected using a well structured questionnaire (Appendix B).

It was entered into a data entry sheet, cleaned and verified to ensure that quality was maintained. Statistical analysis was performed using Statistical Package for Social Sciences version 11.2 software for windows and Epi Info 3.2.2. Atlanta Georgia.

Descriptive statistics such as mean, median and standard deviation were determined for continuous variables like age, weight and length and duration of symptoms.

Frequency distribution was used for categorical variables like sex, rotavirus test results, vaccination status and severity of gastroenteritis. Proportions were calculated for each of the outcome variables. Associations between the two groups were assessed using Chi-square test and Mann-Whitney U test for medians. Odds ratios and P values were determined. P-value of less than 5% was considered statistically significant and the data was presented in tables and figures as applicable.

ETHICAL CONSIDERATIONS

The study was conducted after approval by the Department of Pediatrics (University of Nairobi), Kenyatta National Hospital Scientific and Ethical Review Committee and Gertrude's Children's Hospital. Informed consent was obtained from the parent or guardian for every child recruited into the study. Emergency treatment took precedence over the interview and no treatment was delayed due to interview. Patients suffered no loss if they declined to participate in the study and confidentiality was safeguarded.

RESULTS

Recruitment

The study was carried out between March and June 2009.

One hundred and ninety five children were recruited by consecutive sampling of all the patients aged 6-24 months who presented to the outpatient department at GCH and the satellite clinics with acute diarrhea.

Demographic data and Anthropometric measurements

The mean age of all the patients included in the study was 13.96 months (± 5.37SD) with a range of 6 - 24 months and a median age of 13 months. Of the 195 patients, 115 (59%) were male and 80 (41%) were female. The mean weight of the children recruited was 10.40 kg (± 2.06 SD) and the range was 4.7-16kg. The mean length was 77.37cm (±3.44SD) with a range of 62-100cm. This is shown on table 2

Table 2. Anthropometric measurements

Anthropometric measure	N	Mean	median	mode	SD	Range
Age(months)	195	13.96	13	13	5.37	6-24
Weight(kg)	195	10.40	10	10	2.06	4.7-16
Length(cm)	195	77.37	77	75	3.44	62-100

Using the National Center for Health Statistics as the reference, the weight for age, weight for height and height for age z-scores of the study population were determined. Weight for age z-scores greater than -2SD were 192(98.5%) while 3(1.5%) had z-scores less than or equal to-2SD. In the weight for height z-scores, 192(98.5%) children had z-scores greater than -2SD and 3(1.5%) less than or equal to -2SD. Height for age z-scores for these children were also computed. Those that had z-scores greater than -2SD were 180(92.3%) while those with z-scores less than or equal to -2SD were 15(7.7%). This was a population of well nourished children. This is shown on table 3.

Table 3. Nutritional status

Z Score	**Frequency (%)**
W/A >-2SD	192 (98.5%)
≤-2SD	3 (1.5%)
W/H >-2SD	192 (98.5%)
≤-2SD	3 (1.5%)
H/A >-2SD	180 (92.3%)
≤-2SD	15 (7.7%)

Parent/guardian and breastfeeding characteristics

Most of the mothers were educated with 126 (64.6%) tertiary, 57 (29.2%) secondary and 11(5.6%) primary while 1(0.5%) parent reported no formal education. One hundred and twenty (61.5%) mothers were employed, 50 (25.6%) unemployed and 25 (12.8%) self employed. Of the 173 children who had been exclusively breastfed, 88(50.9%) had been exclusively breastfed for a period of 4 or more months and 85(49.1%) for less than 4 months. These characteristics are outlined on table 4.

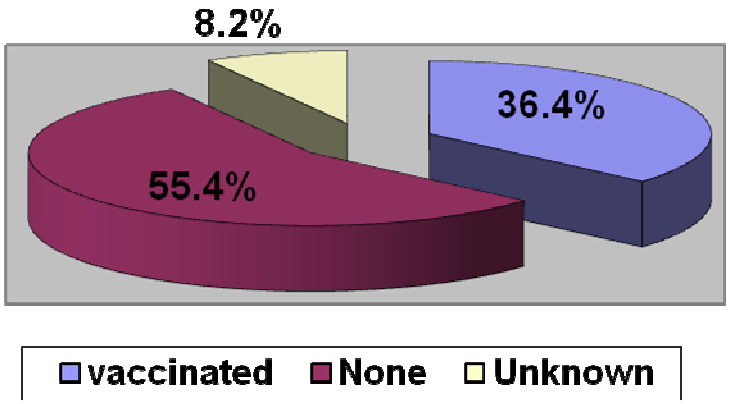
Table 4. Parent/guardian and breastfeeding characteristics

characteristic	Frequency (%)
Education level	
None	1(0.5%)
Primary	11(5.6%)
Secondary	57(29.2%)
Tertiary	126(64.6%)
Employment status	
Employed	120(61.5%)
Unemployed	50(25.6%)
Self-employed	25(12.8%)
Exclusively breastfed	
<4months	85(49.1%)
≥ 4 months	88(50.9%

Vaccination

Of the 195 children enrolled into the study, 108 (55.4%) children had not received the rotavirus vaccine, 71 (36.4%) had been vaccinated and 16 (8.2%) had unknown vaccination status. This is illustrated in figure 3.

Figure 3. Proportion of children vaccinated



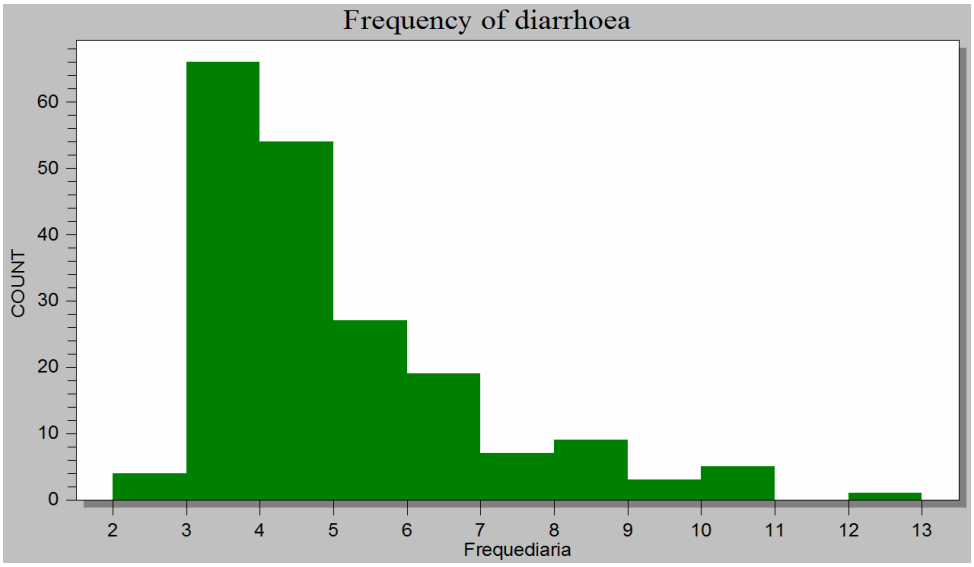
Cli

nical variables of the diarrhoeal disease

Diarrhea

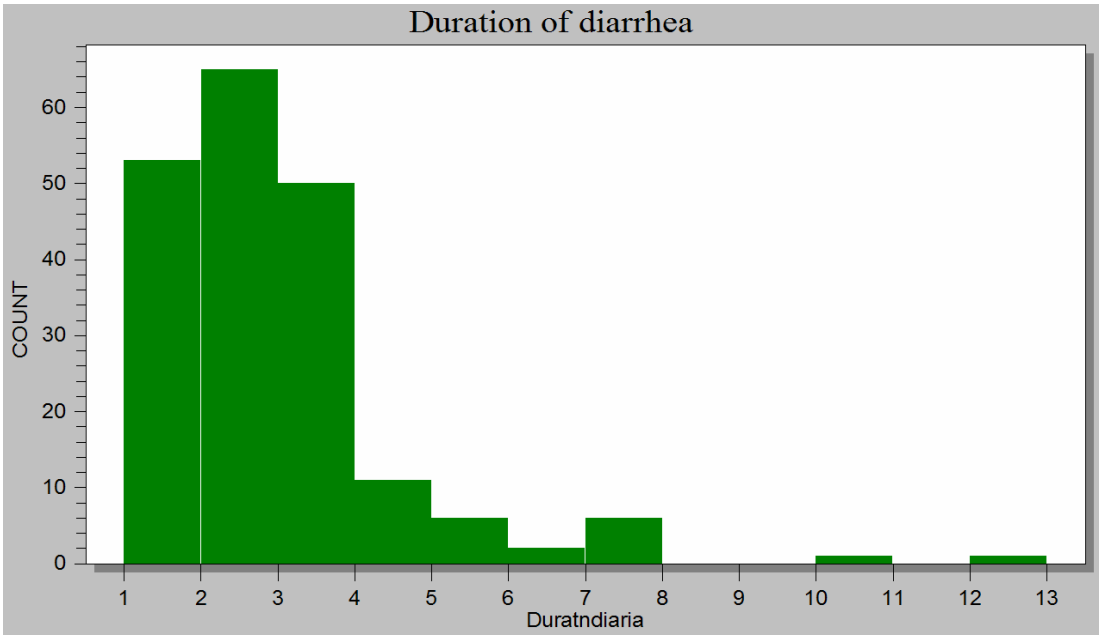
The frequency of diarrhea per day ranged from 2 to 12 episodes with a median of 4.0 and an interquartile range of 3.0-5.0 episodes as shown in figure 4.

Figure 4. Median frequency of diarrhea



The median duration of the diarrhea at the time of first clinical contact was 2.0 with an interquartile range of 1.0-3.0 days as shown in figure 5.

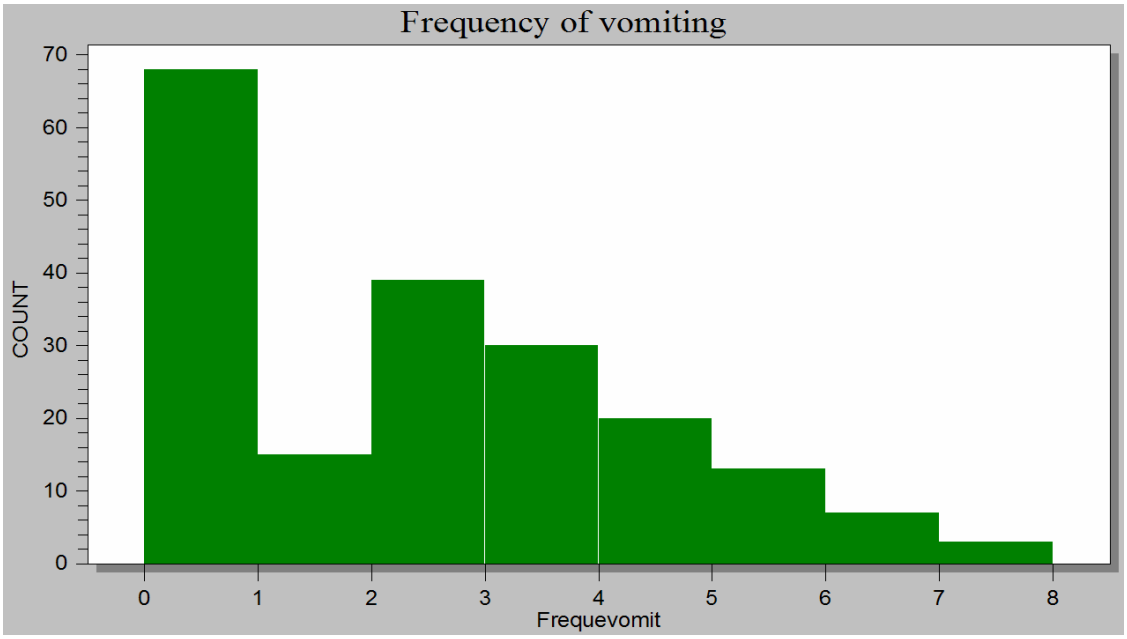
Figure 5. Median duration of diarrhea



Vomiting

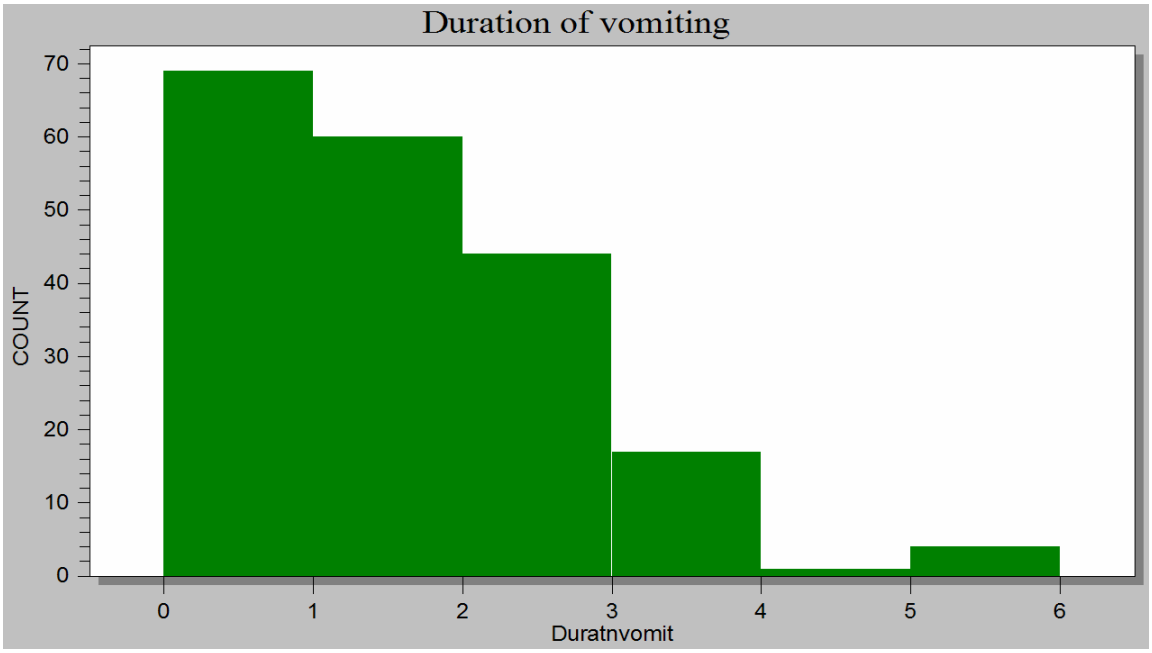
The frequency of vomiting ranged 0 - 7 episodes in 24 hours with a median of 1.0 and an interquartile range of 0.0-3.0 episodes. This is illustrated in figure 6.

Figure 6. Median frequency of vomiting



The duration of vomiting was 0 - 5 days with a median of 2.0 and an interquartile range of 0.0-3.0 days as shown in figure 7.

Figure 7. Median duration of vomiting



Blood in stool

Only 6(3.1%) of the 195 children recruited had blood in stool.

Comparison of diarrhoeal disease symptoms between the vaccinated and unvaccinated

The symptoms of the diarrheal disease were compared between the vaccinated and unvaccinated children as shown in table 5. There was no difference noted in the median frequency and duration of diarrhea and vomiting between the two groups. The median frequency of diarrhea was 4.0 with an interquartile range of 3.0-6.0 episodes in 24 hours in both the vaccinated and unvaccinated children. Unvaccinated compared to vaccinated

children had a similar median duration of diarrhea of 2.0 with an interquartile range of 1.0-3.0 days. The median duration of vomiting was similar among the unvaccinated and unvaccinated children at 1.0 with an interquartile range of 0.0-2.0 days. The median frequency of vomiting in the vaccinated compared to the unvaccinated children was also found to be similar at 2.0 with an interquartile range of 0.0-3.0 days. One (1.4%) of the vaccinated children had detectable blood in stool compared to 5(4.6%) of the unvaccinated children. This difference was not statistically significant, P= 0.235.

Table 5. Diarrhoeal disease symptoms in vaccinated compared to unvaccinated children

Diarrheal disease symptom	**Vaccinated N=71**	**Unvaccinated N=108**	**Odds ratio (95% CI)**	**P-value**
Duration of diarrhea(days) Median (IQR)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	N/A	0.753
Duration of vomiting (days) Median (IQR)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	N/A	0.070
Blood in stool				
Present	1(1.4%)	5(4.6%)	0.3 (0.0-2.6)	0.234
Absent	70(98.6%)	103(95.4%)		
Frequency of diarrhea Median (IQR)	4.0 (3.0-6.0)	4.0 (3.0-5.0)	N/A	0.653
Frequency of vomiting Median (IQR)	2.0 (0.0-3.0)	2.0 (0.0-3.0)	N/A	0.249

Of the 195 children recruited, 89 (45.6%) reported previous episodes of acute gastroenteritis while 106 (54.4%) had not had gastroenteritis before. Among them, 13

(6.7%) had been admitted previously for acute diarrhea and 182 (93.3%) reported no prior admission.

Table 6. Comparison of vaccination status with previous admission for acute gastroenteritis

characteristic	**Previous admission for acute gastroenteritis**		**Odds ratio (95% CI)**	**P value**
Vaccination status(n=179)	Yes (n=12)	No(n=167)		
none	10(83.3%)	98(58.7%)	0.5(0.1-1.8)	0.284
vaccinated	2(16.7%)	69(41.3%)		

Two (2.8%) of the 71 vaccinated children compared to 10(9.3%) of the 108 unvaccinated children reported previous admissions for acute gastroenteritis. The difference was not significant OR 0.5(95%CI 0.1-1.8); P=0.284). This is shown on table 6.

Table 7. Comparison of vaccination status with previous episodes of acute gastroenteritis

Characteristic	**Previous episodes of acute gastroenteritis**		**Odds ratio (95% CI)**	**P value**
Vaccination status(n=179)	Yes(n=88)	No(n=91)		
None	58(65.9%)	50(54.9%)	0.8(0.5-1.5)	0.579
vaccinated	30(34.1%)	41(45.1%)		

Thirty (42.3%) of the 71 vaccinated children compared to 58(53.7%) of the 108 unvaccinated children reported previous episodes of acute gastroenteritis. The difference was not significant OR 0.8((95%CI 0.5-1.5); P=0.579. This is shown on table 7.

Table 8. Distribution of cases within the clinical parameters of the Vesikari scoring system for severe gastroenteritis (modified)

Clinical parameter	score	% distribution of cases within parameter
Duration of diarrhea (days)		
1-4	1	77%
5	2	21%
≥ 6	3	2%
Frequency of diarrhea/24 hours		
1-3	1	23%
4-5	2	46%
≥6	3	31%
Duration of vomiting (days)		
0	0	41%
1	1	46%
2	2	10%
≥3	3	3%
Frequency of vomiting/24 hours		
0	0	49%
1	1	20%
2-4	2	26%
≥5	3	5%
Fever (this parameter was not assessed in the data collection tool used)	1 2 3	Not assessed
37.1-38.4		
38.5-38.9		
≥39		
Dehydration		
None	1	83%
1-5%(some)	2	10%
≥6%(severe or shock)	3	7%
Treatment		
None	0	26%
Rehydration	1	69%
Admission	2	5%
Total score	20	

Severe gastroenteritis = score ≥11 out of a maximum score of 20 points

The 20 point Vesikari numerical score gives the full clinical picture of acute gastroenteritis in a more balanced way as opposed to just assessing for dehydration. It is recommended for use in studies on rotavirus vaccine use. The percentage distribution of cases within the clinical parameters of the Vesikari scoring system were computed and outlined in table 8. Seventy seven percent of the children had duration of diarrhea less than 5 days prior to presentation to hospital. Almost half (46%) of the children had 4-5 episodes of diarrhea in 24 hours. Eighty seven percent of children had duration of vomiting of less than 1 day before presenting to hospital. Close to half (49%) of children had no vomiting at all and majority (83%) had no dehydration. Only 5% of the children had severe disease requiring admission. Fever was not assessed because the decision to use the scoring system was made after data collection had taken place. When the individual scores were determined for each patient, 36(18.5%) out of the 195 children recruited had scores $\geq$ 11 and so were classified as having severe gastroenteritis. One hundred and fifty nine (81.5%) children had non- severe gastroenteritis.

Table 9. Comparison of rotavirus vaccination status, socio-demographic characteristics, breastfeeding practices and z -scores in children with severe and non-severe gastroenteritis

characteristic	N	Severe gastroenteritis	Non severe gastroenteritis	Odds ratio (95% CI)	P value
Vaccination status*					
Vaccinated	71	6 (8.5%)	65 (91.5%)	0.3 (0.1-0.6)	0.002
None	108	29 (26.9%)	79 (73.1%)		
Age (months)					
6-12	88	22 (25%)	66 (75%)	2.2 (1.1-4.6)	0.033
13-24	107	14 (13.1%)	93 (86.9)		
Guardian education	69	18 (26.1%)	51 (73.9%)	2.1 (1.0-4.4)	0.042
≤Secondary	126	18 (14.3%)	108 (85.7%)		
> Secondary					
Exclusive breastfeeding	85	18 (21.2%)	67 (78.8%)	1.9 (0.8-4.3)	0.127
<4 months	88	11 (12.5%)	77 (87.5%)		
≥4 months					
W/A z-score					
>-2SD	192	35 (18.2%)	157 (81.8%)	0.4 (0.0-5.1)	0.460
≤-2SD	3	1 (33.3%)	2 (66.7%)		
W/H z-score					
>-2SD	192	34 (17.7%)	158 (82.3%)	0.1 (0.0-1.2)	0.088
≤-2SD	3	2 (66.7%)	1 (33.3%)		
H/A z-score					
>-2SD	180	31 (17.2%)	149 (82.8%)	0.4 (0.1-1.3)	0.118
≤-2SD	15	5 (33.3%)	10 (66.7%		

*n=179 in this group because 16 children with unknown vaccination status were excluded.

Six (8.5%) of the 71 vaccinated children compared to 29(26.9%) of the 108 unvaccinated had severe gastroenteritis. The difference was significant. Vaccination was associated with a 70% reduced risk of severe gastroenteritis compared to the unvaccinated children OR 0.3 (95%CI 0.1-0.6); P=0.002. Children aged 6-12 months had a significantly higher risk of severe gastroenteritis. Twenty two (25%) of the 88 children aged 6-12 months compared to14 (13.1%) of the 107 children aged 13-24 months had severe gastroenteritis

OR 2.2(95%CI (1.1-4.6); P-value 0.033). The risk of severe gastroenteritis was twice as likely in the children aged 6-12 compared to the 13-24 months. The parent's educational level was also significantly associated with severity of gastroenteritis. Eighteen (26.1%) of the 69 parents with secondary level education and below compared with 18(14.2%) of parents educated beyond secondary level had a two fold increased risk of severe gastroenteritis OR2.1 (1.0-4.4); P=0.042. The breastfeeding and nutritional status of the children was not significantly associated with severity of gastroenteritis. This is outlined in table 9.

Table 10. Predictors of severity of gastroenteritis

Variables	**OR (95% CI)**	**P value**
Age	2.1 (1.0-4.6)	0.059
Education	1.7 (0.7-3.7)	0.215
Vaccination	0.3 (0.1-0.8)	0.012

Multivariate analysis was then done to determine which variables independently influenced the severity of gastroenteritis. Vaccination status was found to be the only variable significantly associated with severity of gastroenteritis OR 2.1 (1.0-4.6), P= 0.012. This is shown on table 10

Stool analysis

118(60.5%) stool samples tested negative for rotavirus and 77 (39.5%) were positive for rotavirus.

Figure 8. Prevalence of rotavirus in stool

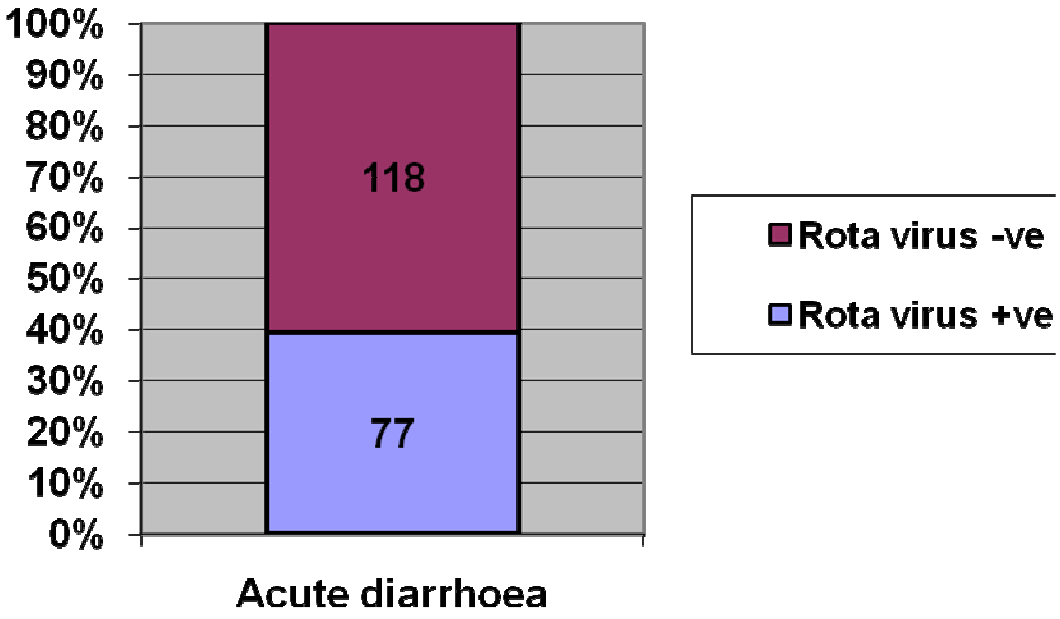


Table 11. Comparison of rotavirus vaccination status, socio-demographic characteristics, breastfeeding practices and z scores in children with rotavirus positive and negative acute gastroenteritis

Characteristic	N	Rotavirus		Odds ratio (95% CI)	P value
		Positive	Negative		
Vaccination status*					
Vaccinated	71	17 (23.9%)	54 (76.1%)	0.3 (0.2-0.7)	0.001
None	108	52 (48.1%)	56 (51.9%)		
Age (months)					
6-12	88	39 (44.3%)	49 (55.7%)	1.4 (0.8-2.7)	0.211
13-24	107	38 (35.5)	69 (64.5%)		
Guardian education					
≤Secondary	69	36 (52.2%)	33 (47.8%)	2.3 (1.2-4.1)	0.007
> Secondary	126	41 (32.5%)	85 (64.5%)		
Exclusive breastfeeding	85	30 (35.3%)	55 (64.7%)	0.8 (0.4-1.5)	0.543
<4 months	88	35 (53.8%)	53 (49.1%)		
≥4 months					
W/A z-score					
>-2SD	192	75 (39.1%)	117 (60.9%)	0.3 (0.0-3.5)	0.344
≤-2SD	3	2 (66.7%)	1 (33.3%)		
W/H z-score					
>-2SD	192	75 (39.1%)	117 (60.9)	0.3 (0.0-3.5)	0.344
≤-2SD	3	2 (66.7%)	1 (33.3)		
H/A z-score					
>-2SD	180	73 (40.6%)	107 (59.4)	1.9 (0.6-6.1)	0.220
≤-2SD	15	4 (26.7%)	11 (73.3)		

*n=179 because the 16 children with unknown vaccination status were excluded from this group.

Seventeen (23.9%) of the 71 children who had received the rotavirus vaccine had detectable rotavirus in the stool compared to 52 (48.1%) of the 108 unvaccinated children. This difference was significant with vaccination being associated with a 70% reduced risk of having rotavirus in stool OR=0.3 (95% CI 0.2-0.7); P =0.001. Thirty six (52.2%) of the 69 parents educated below secondary level compared to 41(32.2%) of the

126 parents with secondary level education and above had a two fold increased risk of rotavirus recovery in stool OR 2.3 (95%CI 1.2-4.1); P= 0.007. The age group (whether below or above 12 months), breastfeeding and nutritional status of the children did not significantly increase the risk of having detectable rotavirus in stool. This is shown on table 11.

Table12. Predictors of rotavirus positivity in stool

Variables	**OR (95% CI)**	**P value**
Education	1.5 (0.8-3.1)	0.186
Vaccination	0.4 (0.2-0.8)	0.009

Following multivariate analysis to determine which variables were independently associated with the rate of rotavirus recovery in stool, vaccination was found to be significantly associated with the risk of rotavirus recovery in stool OR 0.4(95%CI 0.2-0.8), P= 0.009. This is shown on table 12

DISCUSSION

Rotavirus is the leading cause of acute gastroenteritis in children under 5 years of age. Rotavirus vaccine has been shown to be effective in reducing the frequency, duration and severity of rotavirus diarrhea. Vaccination against rotavirus has been available in the private healthcare facilities in Kenya since mid-2006.

The prevalence of rotavirus was found to be 39.5% among the children recruited into the study. This is lower than that found in previous studies carried out before the

introduction of the rotavirus vaccine. In the year 2000, a prevalence of 43.3% among children aged 5 years and below was found by Abbas et al in a hospital based study in Iraq (40). Mwenda et al in 2003 found a prevalence of 11% among children under five years of age seen at out patient clinics in Nairobi and its suburbs (32). This prevalence was much lower because the study was on the prevalence of Group C rotaviruses which are not as common as Group A rotaviruses and the fact that the study was based in out patient clinics was likely to have excluded children requiring hospitalization. Gatinu et al in 2007 observed a prevalence of 53.3% in KNH (10). This higher frequency could be explained by the fact that these patients being from a lower socioeconomic background than those seen at GCH were unlikely to have received the vaccine against rotavirus.

Findings from this study showed an association between rotavirus vaccination and a 70% risk reduction of rotavirus recovery from stool OR 0.3(95%CI0.2-0.7); P=0.001. Other studies have shown similar findings. In a large clinical trial in Europe, Chandran et al demonstrated that rotavirus vaccination prevented 74% of rotavirus gastroenteritis (35).

According to this study, there was an association between rotavirus vaccination and a 70% reduction in the risk of severe gastroenteritis OR 0.3(CI 0.1-0.6); P=0.002. Chandran et al in Europe found the risk reduction of severe rotavirus gastroenteritis to be 98% (35). The study design in that trial was powered to assess vaccine efficacy which this study was not and assessed severity among children with rotavirus etiology whereas this study assessed severity regardless of etiology. A case control study done in Nicaragua, a developing country showed risk reduction of severe gastroenteritis to be lower at 46.6% (39). Live oral vaccines are less efficacious in the developing countries

due to the differences in nutritional status, breastfeeding patterns and different rotavirus serotypes (31). The children at GCH are from relatively affluent backgrounds and are well nourished. This may explain why the vaccine performance appeared better than that in Nicaragua where most of the children came from low socio-economic families. The study done in Nicaragua was a case control study whereas this was a cross-sectional study. The difference in study designs could have contributed to the different findings. A modified form of the Vesikari scoring system for severe gastroenteritis was used in this study where fever was not assessed. This means that children with more severe disease were likely to have been selected than would have been had the whole score been used and could also have accounted for the different findings.

The prevalence of severe dehydration in this study was 8%. This is lower than that found by Gatinu of 47.8% (10). The median duration of diarrhea was 2.0 days with an interquartile range of 1.0-3.0 days. Gatinu found a longer duration of diarrhea of 4.9 days (10). These differnces can be explained by the fact that children seen at GCH were from a higher socioeconomic class as compared to those seen at KNH and so presented earlier to hospital. In this study, the risk of severe gastroenteritis was found to be increased two fold in children aged 6-12 months compared to those aged 13-24 months OR 2.2(95%CI 1.1-4.6), P=0.033. This is comparable to a study done in India that showed a similar increase in the prevalence of severe gastroenteritis in children between 7-12 months (37). Younger children have an increased risk of developing severe gastroenteritis. Given their small body size, they appear to lose a greater portion of their total fluid volume during diarrhea. The lower prevalence of severe disease in this study

can be explained by the fact that most of the children seen at GCH presented early to hospital and rehydration was therefore started early.

In this study, rotavirus vaccine coverage was found to be 36.4%. The vaccine is relatively new in the market having been introduced into the private sector mid-2006. At GCH, vaccination against rotavirus was started in June 2006. Since then, awareness about the vaccine has increased following health talks and pamphlets given to parents both in the outpatient department and at the well baby clinics. In 2008, about 250 children per month received the vaccine both at GCH and at the satellite clinics (unpublished hospital reports). The children enrolled into this study do not all attend the well baby clinics for immunization at GCH. Of the children assessed during the study, 9.7% attended public health care facilities for immunization. This may also have contributed to the relatively low vaccine coverage found in this study.

Rotavirus vaccine was incorporated into the National Immunization Program in Brazil in 2006. Gurgel et al in 2008 found the found the vaccine coverage to be 90.3% and the prevalence of rotavirus to be 11% (34). This high vaccine coverage can explain the marked decline in rotavirus infection in Brazil. Vaccine coverage in the United States in 2008 stood at 58% there was 74% reduction in rotavirus cases of any severity (27). This decline was greater than would have been expected suggesting that vaccination of a proportion of the population could be conferring herd immunity due to decreased viral transmission in the community.

Rotavirus diarrhea is usually non-bloody (15).Only 6(3.1%) of the children had blood in stool. Of these, one (13.7%) was positive for rotavirus. This number was too small to draw any valid conclusion about association of rotavirus with bloody diarrhea.

In this study, nutritional status did not appear to be a contributing factor in rotavirus positivity in stool or severity of gastroenteritis. The majority of children were relatively well nourished with 98.5% having weight for height Z scores greater than or equal to -2SD. Binka et al reported increased prevalence of severe gastroenteritis among the malnourished children in Northern Ghana (41). The Ghanaian study was carried out among hospitalized children. It is possible that a significant proportion of children were malnourished since they are more likely to suffer severe gastroenteritis necessitating admission. The children assessed in the KNH study by Gatinu et al with Z- scores greater or equal to -2SD were 82% and prevalence of rotavirus was not significantly increased in those with severe malnutrition (10). Malnourished children given their small body size lose a greater proportion of their total fluid volume in diarrheal stool. Black et al showed that children with lower weights for height had rates of stool loss 14-61% more than higher weight children. Duration of diarrhea was also shown to be prolonged by 30-70 %(38).

Most of the parents whose children were recruited into the study had received formal education. The education level of the parents was shown to contribute significantly to both rotavirus recovery in stool and severity of gastroenteritis. The finding of an increased risk of rotavirus positivity in stool among those children whose parents had

been educated below secondary level could be attributed to the fact that these parents were less likely to know about availability of a rotavirus vaccine and hence their children were more likely to be unvaccinated. Educational status of secondary and above was found to be significantly protective against the risk of severe gastroenteritis. This is comparable with findings from a Jamaican study on caregiver knowledge, attitudes and practices regarding childhood diarrhea indicating that low literacy levels were associated with inadequate home management of diarrhea and dehydration, poor household hygiene and delay in seeking health care with consequently higher morbidity and mortality (36).

In this study exclusive breastfeeding was not significantly protective against severe gastroenteritis. This contrasts with other studies. A case control study in Bangladesh, among children aged 24 months and below, showed that exclusive breastfeeding was associated with significant protection against severe rotavirus disease RR 0.1(95%CI 0.03-0.04)(42).

STUDY LIMITATIONS

This study being cross-sectional in nature was not able to address adequately the causal relationship between rotavirus vaccination and rotavirus disease correlates. Some children may have been excluded from the study because they presented in the early stages with vomiting only.

CONCLUSIONS

Findings from this study indicate that:

1. The prevalence of rotavirus diarrhea is 39.5%.

2. Vaccination against rotavirus is associated with a 70% lower risk of severe gastroenteritis and a 60% reduction in the rate of recovery of rotavirus from stool.

3. Rotavirus vaccine coverage in GCH is 36.4%.

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APPENDIX A – CONSENT FORM

Dear parent/ guardian,

We are conducting a research study investigating the **prevalence of rotavirus among children presenting with acute gastroenteritis at GCH.** We would like to include your child as a participant. This will require that we administer to you a questionnaire, examine your child and also take stool samples from them. Participation in this study is voluntary and the decision on whether to participate or not, will not prejudice your child's care in any way. There is no risk your child will be exposed to by taking part in this study and strict confidentiality will be observed at all times. In all the instances, the child's primary care giver will be informed of all the results in view of treatment.

We hope that you accept for your child to participate in this study, as its outcome will impact on the future of the disease prevention strategy in our country.

Thank you for your co-operation.

Consent

Having understood the above and in the knowledge that it is voluntary

I_____of_____

Do hereby accept to participate in the study

_____ _____

Parent's/ guardian's signature Investigator's signature

Date _____ Date_____

Incase of any ethical concern regarding the study and the procedures, please contact Prof. A GUANTAI, secretary KNH-ERC on tel. 020725272 ext 44355.

APPENDIX B- QUESTIONNAIRE

Date……. OP No…… Study No……

SECTION A: SOCIO-DEMOGRAPHIC INFORMATION OF THE CHILD

1. Age (months)….. 2. Sex ☐ male ☐ female

3. Weight (grams …… 4.length (cm) ……

SECTION B: CAREGIVER SOCIODEMOGRAPHIC INFORMATION

5. Age of primary caregiver (years) ……

6. Relationship of primary caregiver to child

☐ mother ☐ father ☐ other specify ……

7. Level of education of caregiver

☐ none ☐ primary ☐ secondary ☐ tertiary

8. Occupation

☐ employed ☐ unemployed ☐ self employed

SECTION C: VACCINATION AND FEEDING HISTORY

9. Vaccine type used ☐ rotateq ☐ rotarix ☐ none ☐ unknown

10. If vaccinated, has the child completed vaccination against rotavirus

☐ Yes ☐ no

11. Date vaccination completed …/ …/…..

12. Are the other EPI vaccines up to date ☐ yes ☐ no

13. Where do they normally attend well baby clinic

☐ private clinic ☐ public facility ☐ both

14. Breastfeeding status of child

☐ Never breastfed ☐ Still breastfeeding ☐ Stopped breastfeeding

15. exclusively breastfed ☐ yes ☐ no

16. duration of exclusive breastfeeding (months) ……

17. If not breast fed, which mode of feeding ☐ bottle ☐ Other

18. Total duration of breastfeeding (months) ……

SECTION D: CLINICAL HISTORY

19. **Assessment of diarrhea**

- Frequency of diarrhea/day ……. -Frequency of vomiting/day …….
- duration of diarrhea (days) ……. -duration of vomiting (days) ……
- blood in stool ☐ yes ☐ no

20. Previous episodes of acute diarrhea ☐ yes ☐ no

21. If yes, how many ……

21. Previous admission for acute diarrhea ☐ yes ☐ no

22. **Presence of severe dehydration**

- sunken eyeballs ☐ yes ☐ no
- skin pinch delayed ☐ yes ☐ no
- level of consciousness ☐ alert ☐ verbal ☐ pain ☐ unresponsive
- cold extremities ☐ yes ☐ no
- pulses ☐ normal volume ☐ weak ☐ impalpable
- capillary refill time (sec) ……

23. **hydration status** ☐ no dehydration ☐ some dehydration ☐ severe dehydration ☐ shock

24. **nutrition status**

☐ Normal ☐ visible severe wasting ☐ bilateral pedal oedema

25. **Outcome**

☐ None ☐ rehydrated ☐ Admitted

Duration of admission (days) …….

Outcome of hospitalization ☐ Discharged ☐ Dead

26. Stool analysis for rotavirus ☐ positive ☐ negative

APPENDIX C- Vesikari Scoring System for severe gastroenteritis (33)

Clinical parameter	score
Duration of diarrhea (days)	
1-4	1
5	2
≥ 6	3
Frequency of diarrhea/24 hours	
1-3	1
4-5	2
≥6	3
Duration of vomiting (days)	
0	0
1	1
2	2
≥3	3
Frequency of vomiting/24 hours	
0	0
1	1
2-4	2
≥5	3
Fever	
37.1-38.4	1
38.5-38.9	2
≥39	3
Dehydration	
None	1
1-5%(some)	2
≥6%(severe or shock**)**	3
Treatment	
None	0
Rehydration	1
Admission	2

Maximum score = 20 points

Severe gastroenteritis = ≥ 11points