ROTAVIRUS INFECTION AMONG CHILDREN UNDER TWO YEARS OF AGE WITH ACUTE DIARRHOEAL ILLNESS SEEN AT MOI TEACHING AND REFERRAL HOSPITAL

BY: DR. CECILIA KATUU KIILU

SM/PGCHP/06/14

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE, CHILD HEALTH AND PAEDIATRICS, MOI UNIVERSITY

DECLARATION

This thesis is my original work and has not been presented before for another degree in any other University/Institution.

……. ……..

DR. CECILIA KATUU KIILU **DATE**

SM/PGCHP/06/14

This thesis has been submitted to Moi University with our approval as University supervisors.

……. ……..

DR. IRENE MARETE **DATE**

Senior lecturer, Department of Child Health and Paediatrics, Moi University.

……. ……..

DR. EDITH APONDI **DATE**

Consultant Paediatrician, Moi Teaching and Referral Hospital

DEDICATION

This study is dedicated to my entire family for their material, intellectual and moral support towards its success.

ACKNOWLEDGEMENT

I am indebted to a large number of individuals for their priceless input without which I would not have been able to write this thesis. First to God for His never ending grace. To my supervisors Dr. Marete and Dr. Apondi for their continuous input and guidance throughout the development process. My colleagues in the department of Child Health and Paediatrics for their objective and constructive criticism. To Dr. Ann Mwangi for biostatistics consultation. Last but not least, to my entire family and my life partner for each and every effort put in during this process.

Thank you all and God bless.

ABSTRACT

Background: Diarrhoea carries an unacceptably high morbidity and mortality rate in Sub-Saharan Africa. Rotavirus infection in the pre-vaccine era had been the leading cause of diarrhoea in Kenya. There is limited data on the etiology and characteristics of diarrhoea following the inclusion of Rotavirus vaccination, in the regular vaccination schedule in Kenya in July 2014. The rotavirus vaccine is a monovalent vaccine targeting Group A rotavirus, the commonest strain in this region.

Objective: To determine the proportion of children with rotavirus associated diarrhoea among children, presenting with Acute Diarrhoeal Illness seen at Moi Teaching and Referral Hospital's (MTRH) Paediatric emergency department.

Methods: This was a cross-sectional study carried out at MTRH Paediatric Emergency Department between November 2015 and June 2016. Children aged two years and below with Acute Diarrhoeal Illness were recruited. The participants' parents/guardians were interviewed and a rapid Certest® rotavirus stool antigen test carried out. Data was collected on the socio-demographic and clinical characteristics of the participants.

Results: A total of 311 participants were recruited. The median age was 12 months (IQR 8,19). The largest proportion of participants were between 6-12 months of age 35%(109/311). The prevalence of rotavirus infection was, 55.6% (173/311), with the peak during the months of February and March. Rotavirus vaccine coverage was at 85.2%(265/311). Age appropriate completion of the standard Ministry of Health Kenya vaccines was 83.3%(259/311). Majority of the participants were undernourished (WHZ <-1) at 38.9%(121/311). Incomplete rotavirus vaccination (p=0.005), not having age appropriate completion of routine vaccination (p=0.030), and under-nutrition (p=0.009) were positively associated with rotavirus infection. On logistic regression, mild wasting (WLZ >-2 TO -1) (OR 2.581; 95% CI [1.068-6.236]; p=0.035) and moderate wasting(WLZ -3 TO -2) (OR 3.424; 95% CI [1.221-9.604]; p=0.019) were associated with rotavirus infection. Severe malnutrition was not statistically significant (OR 0.795; 95% CI [0.373-1.692]; p=0.552). Having Received two rotavirus vaccines (OR 0.151; 95% CI [0.032-0.709];p=0.017) and age appropriate completion of routine vaccination (OR 0.478; 95% CI [0.256-0.892];p=0.003) decreased the odds of rotavirus infection. Receiving a single dose of rotavirus vaccine was not protective against rotavirus infection. Socio-demographic characteristics such as age (p=0.244), gender (p=0.901)and the child's primary caregiver (p=0.783)were not associated with rotavirus infection. Although majority of the children with rotavirus positive diarrhoea had non-severe dehydration 63% (109/173) this was also not significant (OR 1.066; 95% CI 0.6695-1.699;p=0.786). Bristol grade 6 and 7 stool consistency was positively associated with rotavirus infection (OR1.941; 95% CI [0.999-3.77]; p=0.05).

Conclusions: Prevalence of rotavirus diarrhoea is still high among children aged two years and below in our setup, with those undernourished being more likely to have rotavirus infection. Completion of rotavirus vaccination and the other routine childhood vaccinations is protective against rotavirus associated diarrhoea.

Recommendations: Rotavirus vaccination and completion of the other routine childhood vaccination should be intensified. Studies looking into the effect of nutritional status on rotavirus diarrhoea should be carried out.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADI Acute Diarrhoeal Illness

CDC Center for Disease Control

DALYs Disability Adjusted Life Years

DNA Deoxy-Ribonucleic Acid

GAPPD Global Action Plan for the Prevention and Control of Pneumonia and Diarrhoea.

GAVI Global Alliance for Vaccine Initiative

GE Gastroenteritis

GEMS Global Enteric Multicenter Study

HIV Human Immunodeficiency Virus

IgA Immunoglobulin A

IREC Institutional Research and Ethics Committee

IVAC International Vaccine Access Center

KDHS Kenya Demographic Health Survey

KDVI Kenya Division of Vaccine and Immunization

KEMRI Kenya Medical Research Institute

MTRH Moi Teaching and Referral Hospital

NEC	Necrotizing enterocolitis
OPV	Oral Polio Vaccine
PAGE	Polyacrylamide gel electrophoresis
PATH	Programme for Appropriate Technology in Health
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
RT -PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction.
RTI	Respiratory Tract Infection
SAGE	Strategic Advisory Group of Experts
UNICEF	United Nations Children's Fund
URTI	Upper Respiratory Tract Infection
USA	United States of America
UVIS	Unit of vaccine and Immunization Services
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

1. **Diarrhoea** – history of three or more stools per day of decreased form from the normal, or abnormally frequent discharge of semisolid or fluid fecal matter from the bowel excluding passing of loose, "pasty" stools by breastfed babies. Stool was visually assessed and graded on the Bristol stool chart. See figure 1.

2. **Vaccinated participant** – was defined as any participant with written evidence of having received the specified vaccinations (clinic card or booklet) or self-reported description of the vaccine administration at the appropriate age.

3. **Rotavirus infection** – was defined as a positive stool antigen test for rotavirus.

4. **Acute Diarrhoeal Illness** - history of having diarrhoea for a duration less than or equal to seven days.

5. **Appropriate duration of exclusive breastfeeding** – a child who has received exclusive breastfeeding from birth to six months of age or till the time of the study if younger than six months of age.

CHAPTER ONE

INTRODUCTION

1.1 Global Burden of Diarrhoea

Diarrhoea morbidity in the world affects up to 1.9 million children aged five years and below. This is out of the 2 billion persons who suffer diarrhoea in the general population worldwide every year (Farthing M, 2012). The mortality rate is however at 18% for children under five years of age. This translates to 5,000 deaths daily. Up to 90% of these deaths occur in Africa and Asia (Farthing M, 2012). On average, a five year old will experience at least three episodes of acute diarrhoea annually (WHO,2013). In children, diarrhoea is the second most prevalent cause of mortality after pneumonia (Farthing M, 2012). Children under five years are at the highest level of risk of mortality due to diarrhoea. In this group, most deaths occur among those under two years of age, especially in Sub-Saharan Africa and South Asia.(UNICEF, 2014). Statistically, rotavirus causes up to 47% of worldwide hospitalization among children with gastroenteritis (CDC, 2011). In Kenya, there is a 27% hospitalization rate, out of all children under five years of age, admitted with acute diarrhoeal illness (PATH,2014).

1.2 Causative Agent

There are numerous causative agents for infective diarrhoea; bacterial, viral and parasitic in classification. Viral causative agents are however the most predominant cause of acute diarrhoea in children. The prevalence is almost equal across the socio- economic classes(Farthing, 2012). Studies over the last 30 years have consistently shown that rotavirus is the most important cause of infantile gastroenteritis worldwide (WHO, 2011a). Rotavirus

diarrhoea accounts for a third of diarrhoea hospitalizations and up to 500,000 mortalities worldwide annually. The severity of rotavirus diarrhoea is greater than other causes of acute diarrhoea. Neonatal infections do occur but are frequently asymptomatic. Rotavirus diarrhoea has its peak of occurrence at 4-23 months of age (Farthing, 2012). By the age of 3 years, every child has been infected by rotavirus (PATH, 2015).

In Africa, diarrhoea is one of the leading causes of death among children under five years of age. Rotavirus accounted for 30% of hospitalizations among all the children under five years of age admitted with diarrhoeal illness, in 2014(PATH, 2014) . In the most recent African Rotavirus Surveillance Network publication, in 2009 reported a rotavirus prevalence rate of 30% to 41% in East Africa. Central and western parts of Africa reported range of 21% to 59%. These did not include all the countries in Africa, since only fifteen countries were involved in the surveillance (Mwenda et al., 2010). Africa also accounts for half of all deaths globally from Rotavirus associated diarrhoea. Kenya has documented prevalence of Group A Rotavirus ranging from 14% to 39 % of rotavirus infection among those with acute diarrhoeal illnesses in hospitals (Nokes et al., 2008).

There are currently only 20 countries in Africa that have included rotavirus vaccine in their national immunization programs, Kenya being one of them (PATH, 2014). It is postulated that, with the rotavirus vaccine in place, over 2.4 million deaths shall have been prevented worldwide by the year 2030(GAVI, 2013).

1.3 Status of rotavirus vaccination in Kenya

Since the introduction of rotavirus vaccination in the Kenyan public hospitals, there has been an increase in the coverage of the vaccination. the second dose however has a lower coverage compared to the first dose (Apondi et al, 2017). A decline in rotavirus associated diarrhoea

has reduced by 49.8% with a concurrent decrease in all cause diarrhoea by 40.2 % (Apondi et al, 2017) .

1.4 Problem Statement

Diarrhoeal diseases in children are 1.7 billion per year, with a childhood mortality of 11% of globally(WHO, 2017). Of the top four leading causes of diarrhoea in children, rotavirus is leading, followed by *Shigella, Cryptosporidium, E. Coli(*Kotloff KL, 2013). Rotavirus diarrhoea has had a high morbidity and mortality burden in Africa and Kenya. The vaccine is created to cause a decrease in severity of disease. However studies done in the postvaccine period have also shown a decline in hospitalizations(WHO, 2013). Rotavirus has great strain diversity with presence of numerous reassortants which pose a challenge in the eradication of infection (WHO, 2013. Out of the top fifteen countries with highest rotavirus diarrhoea mortality rates globally, fourteen are in Africa. Kenya is among the top ten countries with the highest mortality rates for rotavirus diarrhoea in Africa and is ranked among the top in East Africa (Jason M Mwenda, 2015; Kotloff KL, 2013). Rotavirus causes moderate to severe dehydration worse in those less than two years of age(Lee, 2013; UNICEF, 2015). There has been a varying prevalence of up to 53.4% (Gatinu et al, 2016) in Kenya, a value higher than the average worldwide prevalence. Rotarix®, a monovalent vaccine against rotavirus vaccine was introduced in Kenya in July 2014. This vaccine is hoped to decrease the number of children with rotavirus diarrhoea and disease severity in this region. However, there is still a large number of children with diarrhoea in the country and the region. In Moi Teaching and Referral Hospital (MTRH), diarrhoea accounts for over 2000 hospital visits in the paediatric outpatient department annually and 19% of all inpatients under the age of 12 years with all cause diarrhoea(MTRH, 2014). The etiology of diarrhoea in the paediatric

population at MTRH is unknown. Since the introduction of rotavirus vaccination, there has not been any published assessment of the prevalence, clinical presentation and factors associated with of all cause diarrhoea in the region and countrywide. The overall vaccine coverage in Kenya and Uasin Gishu is still low and hence a poses a significant challenge in vaccination as a strategy of reducing rotavirus diarrhoea (KDHS, 2014).

1.5 Justification

One and a half years after introduction of the rotavirus vaccine in Kenya, the burden of diarrhoea in children is still high. The children's vaccination status is unknown and the etiological factors of diarrhoea in this population are still unknown. It is therefore necessary to assess whether the large number of patients presenting with ADI are still due to rotavirus infection and further describe the pattern of clinical presentation. Studies in low income countries have demonstrated a comparatively lower vaccine efficacy compared to their high to middle income country counterparts (Barzeev et al,2016). Regional surveillance of rotavirus in the postvaccine period has not been published hence this data is unavailable. The data generated from this project adds vital information on the proportion of rotavirus diarrhoea and the pattern of presentation. This is useful in not only assessing tentative results arising from introduction of the vaccine in our set up, but also give a comparative assessment of the burden of rotavirus associated diarrhoea in this region. This study has also described the socio-demographic and clinical factors associated with rotavirus diarrhoea. The concurrent assessment of the prevalence and the corresponding associated factors, will guide a multifaceted clinical approach to rotavirus diarrhoea prevention and management. This study's findings can guide interested stakeholders in identification of ways to reduce diarrhoeal illnesses and rotavirus infection by highlighting the problem areas.

1.6 Research Question

What is the proportion of children with rotavirus associated diarrhoea among children under two years of age, presenting with acute diarrhoeal illness (ADI) at the Moi Teaching and Referral Hospital Paediatric Emergency Department?

1.7 Objectives

1.7.1 Main Objective

1. To determine the prevalence of rotavirus infection among children with acute diarrhoeal illness seen at Moi Teaching and Referral Hospital and their characteristics.

1.7.2 Specific Objective

1. To determine the proportion of children with rotavirus infection, among children presenting with Acute Diarrhoeal Illness at MTRH Paediatric Emergency Department.
2. To describe the socio-demographic and clinical characteristics of children presenting with Acute Diarrhoeal Illness at MTRH Paediatric emergency department.
3. To determine the socio-demographic and clinical factors associated with rotavirus infection among children presenting with Acute Diarrhoea Illness MTRH Paediatric emergency department.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diarrhoea in Kenya

Diarrhoea is among the top 10 causes of death and DALYs (Disability Adjusted Life Years) in Kenya at 6% each. In the GAPPD (Global Action Plan for the Prevention and Control of Pneumonia and Diarrhoea) released by WHO in conjunction with UNICEF, several interventional measures have been proposed. These have been universally applied in an effort to end preventable diarrhoea. They are; vaccination, appropriate diarrhoea treatment and exclusive breastfeeding for six months. Unfortunately, during their assessment Kenya was found to be one of the fifteen countries worldwide that contribute up to 75% of the mortalities related to diarrhoea. Kenya however has adopted the recommendations and received a GAPPD score of 53% – 57 %. This is interpreted with the goals of the GAPPD as the benchmark. This means that there is at least 80% coverage countrywide for the diarrhoeal interventions (IVAC, 2013; KDVI, 2011 - 2015).

2.2 Rotavirus Diarrhoea

Sentinel surveillance data in Africa for children under 5 years over the past 7 years has shown a range of prevalence of rotavirus infection in ADI children to be between 35.6 – 49.1% with an average of 40% (Mwenda et al., 2014).

2.2.1 Rotavirus as a Causative Agent

Rotavirus was discovered 42 years ago by Ruth Bishop and has since been found to be a major contributor to childhood diarrhea(Bishop,2009). There has been evidence that the Global Rotavirus emissions are about $2*10^8$ viral particles per grid per annum. Most of these

have been found in the urban population and especially in regions that are densely populated. As a result, the Sustainability Development Goals, Goal number six, seeks to ensure availability and sustainable management of water and sanitation for all, is hoped to ensure a reduction in diarrhoeal diseases. Between 2002 and 2014, the mean rotavirus detection rate among all children with acute diarrhoeal illness was documented at 33.4%. This was found as an average among several countries in Africa, Asia, Europe, Middle East and South America. The range was between 6% and 56% (Kulia, 2015). In every five children under two years of age, one suffers from moderate to severe diarrhoea at least once. They also have an 8.5 times higher chance of death and this occurs mostly after leaving hospital. These children also end up stunted in later years of life as they grow. Worldwide, the top four etiological causes of diarrhoea are *Shigella, Cryptosporidium*, ST-ETEC and Rotavirus. Of these Rotavirus is the most frequent(Kotloff KL, 2013).

2.2.2 Structure

The rotavirus is a double stranded RNA reoviridae that is non-enveloped. Its neocapsid has 3 concentric shells that enclose eleven RNA segments that are double stranded. There are two main structural viral proteins VP4 and VP7 (Rachel M Lee, 2013; Roush, 2012). These proteins are also called P and G proteins respectively. P is protease cleaved whereas G is a glycoprotein. These two proteins are key in identification of the various serotypes. Due to the independent segregation of these proteins during division, the naming system currently in use identifies the various serotypes using both the P and G protein types. There are over 40 known strains worldwide with the most common being G1-4 and G9 number of serotypes (WHO, 2009,2011).

Rotavirus affects both human beings and animals. Despite having distinct number of serotypes that affect humans, animal rotavirus can infect humans hence having unusual rotaviruses arising through interspecies transmission. There are also animal-human reassortants that can cause disease (WHO, 2009,2011). These reassortants are due to the genetic changes occurring in the viral structure.

2.2.3 Strains

There are over 70 combinations of the G and P proteins that lead to great variations in the strains. The commonest combinations being between G1, 3, 49 with P1A[8] and G2 with P1B[4] at a total of 75% of the rotavirus serotypes. These combinations in Africa are only a third of the strains found. In Africa, the reassortants are believed to be responsible for rotavirus and zoonotic co-infection. While there are seven groups of alphabetically named rotavirus genus, only A, B and C are found in humans. Of these, group A is of the highest public health importance. It is divided into various sub-groups (Apondi, 2012).

G1P[8] which has had a prevalence of up to 31% in Africa is the most prevalent strain. There were also mixed G and P strains detected in the African rotavirus surveillance network of participating countries, Kenya being one of them. Other strains found in the Africa region include: G1P[6], G2P[6], G3P[6], G1P[4], G9P[4], G12P[6] and G12P[8]. These strains accounted for up to 40% of circulating strains. Genotypes P[8] was most prevalent. Rotavirus vaccine studies have however found that the vaccine was effective against these strains (Mwenda et al., 2014).

The eleven gene segments of the rotavirus particles can be rearranged to form reassortants. These reassortants are responsible for the wide number of available strains in Africa. The viral proteins on the outer layer VP7 (G types) and VP4 (P types) are responsible for the

antigen-antibody triggered reaction once in a host. There are 12 G types and 15 P types commonly known among the human strains. Due to the variability in strains based on the G and P proteins, a binomial typing system is used to name them. There are currently 5 G-P combinations accounting for approximately 90% of infections in majority of the countries worldwide. Type G1P[8] being most common. The prevalent types vary from one season to the next and Africa has greater strain diversity. There has also been evidence found in Africa of several rotavirus types occurring simultaneously in the same geographical region. Rotavirus virulence can however not be tested in the laboratory due to lack of markers of virulence(WHO, 2013c).

2.3 Disease Process and Manifestations

Neonates experience asymptomatic infection (WHO, 2011a) . Neonatal protection against rotavirus is due to maternal factors such as transplacental antigen supply and breast milk IgA(Apondi et al, 2012).

Rotavirus disease has age-dependent susceptibility. Severe disease is mostly between 6 and 24 months of age. Infants younger than 6 months are believed to be protected by circulating maternal antibodies. In older children and adults, the decline in disease severity is believed to be due to prior infections(WHO, 2011a).

However, rotavirus is known to cause more severe infection than other viral causes of gastroenteritis. It is even more severe in immunosuppressed persons. Apart from causing diarrhoea, patients may also present with encephalitis and meningitis. Other illnesses that have been linked to rotavirus include: Upper and lower respiratory tract infection, otitis media, laryngitis, pharyngitis and pneumonia. There have also been reports of accompanying Kawasaki syndrome, Sudden Infant Death Syndrome (SIDS), hepatic abscesses and

pancreatitis over the years. Patients with rotavirus infection have also been noted to have elevated liver function tests and neonates having necrotizing enterocolitis (NEC). The role of circulating rotavirus in extra-intestinal manifestations is however poorly understood. Mouse models have demonstrated rotavirus causes biliary atresia (Hertel & Estes, 2012).

Rotavirus disease manifestation is unique to it. Its primary site of infection is the small intestine where it affects the mature enterocytes of the villi. Its transmission being faeco-oral implies initial passage through the stomach. Here the viral particles stay for approximately 9 days without causing disease (Lee, 2013). Once the viral particles move further down, the virus causes alteration of the villous physiology by altered phosphorylation of p7056K, mitogen activated kinase and myosin light chains. These are vital in maintenance of the integrity of the villous wall. Their alteration leads to release of inflammatory agents. These changes in combination lead to malabsorption, abnormal gastrointestinal motility and eventually the characteristic diarrhoea (Surendran et al, 2008).

Clinical manifestations are after an incubation period of 1-4 days (Lee, 2013). The symptoms begin suddenly with hotness of the body accompanied by emesis and typical explosive and watery diarrhoea.(WHO, 2011a).

In approximately 50% of patients, vomiting precedes diarrhoea. Up to one fifth of children will complain of mucoid diarrhoea. Often the stools will not be blood-stained. Vomiting may also occur that causes mild to severe dehydration and electrolyte imbalance. The onset is abrupt. Fever is a major presentation with varying frequency of between 45-84% of the children. The clinical presentation spectrum is wide ranging from frequent loose stools with progression to severe diarrhoea. Chronologically the events start with fever, then vomiting that eventually leads to explosive and watery diarrhoea. These symptoms on average

disappear within 3-7 days. However, they may last up to 3 weeks. Microscopically, the stool from rotavirus diarrhoea is normally lacking in leucocytes.(WHO, 2013b).

The disease being viral, is self-limiting and hence the overall duration of the symptoms is 2-22 days. On average, most patients are asymptomatic after 8 days. Hospitalization is normally brief with an average 4 day in-patient stay(WHO, 2013b).

2.3.1 Transmission and Incubation Period

Transmission is mainly faeco-oral. This is by ingestion of contaminated food, water or fomites. (Roush, 2012). It is highly contagious with and infectious dose of <100 virus particles(WHO, 2013b).

Rotavirus Infection: During the first episode of infection, there is a large quantity of shedding of rotavirus in stool and vomiting reaching concentrations of up to $>10^{12}$ particles/gram(Lee, 2013).

Lee et al in a systematic review, showed that on average rotavirus has an incubation period of 1.4-2.4 days. The incubation period can be up to 4 days. Group A has been claimed to be responsible for more than 600,000 deaths especially in infants and young children. Group B is mostly in adults but Group C has not been found to be of public health significance. The clinical presentation includes fever and vomiting. This is soon followed by profuse and watery diarrhoea that often leads to dehydration. Standard public health methods of cleaning do not effectively kill rotavirus as effectively as they do bacteria and parasites. Between 10-100 viral particles are adequate once ingested to cause infection. This explains high prevalence even in hygienic conditions. Rotavirus concentrations shed via faeces for both symptomatic and asymptomatic persons can be as high as 10^9 particles in every gram of stool. This is the reason behind the high infectivity and transmission rates. Transmission via

airborne and waterborne means has been reported. This is after rotavirus was found in other body fluids e.g. respiratory secretions (Apondi et al, 2012).

2.3.2 Pathogenesis and Pathophysiology

Various studies have highlighted the pathological changes in the small intestine caused by rotavirus infection. It has been shown that the villus epithelium changes from columnar to cuboidal. This results in shortening and stunting of the intestinal villi. There is accompanying claudication of the villous tips. The lamina propria develops an increased number of reticulum-like cells and also is seen to have mononuclear cell infiltration. The infection is cranio-caudal in progression(WHO, 2011a).

2.3.3 Mechanism of Diarrhoea

The mechanisms of rotavirus diarrhoea have been studied extensively with various explanatory mechanisms described. After rotavirus enters the gut (intestines), it targets the intestinal villi and the colonic enterocytes. Calcium-dependent endocytosis and fuses with enterocytes are the main modes of entry into the cells. It causes disease by interfering with disaccharide levels; affecting electrolyte pumps hence opened calcium channels and subsequent sodium and water efflux; cell death by due to calcium influx. Resolution of infection if believed to occur due to immune response generation or when the virus runs out of mature enterocytes to affect(Apondi et al, 2012).

2.4 Seasonality

Rotavirus is seasonal with prevalence increasing during the drier months in the tropics. The reason behind this phenomenon is unknown. There is also possibility that humans are reservoirs for rotavirus(WHO, 2011a).

According to WHO, most low income countries in Africa have seasonal variation in rotavirus epidemiology. There is one or more periods of intense rotavirus circulation with a background of year-round transmission(WHO, 2013b).

The peak of rotavirus infection is mostly in the cooler and drier months(Apondi et al, 2012).

In Kenya, a 3 year study done in Kiambu suggested that the reason rotavirus diarrhoea had peaks during seasonal changes, was due to the relative climatic change and not due to the rain or absolute temperature (Apondi, 2012). Seasonality with peaks in January and February, the cooler and drier months (Mwenda et al., 2010).

2.5 Protection after Infection

Immunity developed from rotavirus infection can be acquired whether the first infection was symptomatic or asymptomatic and is not sterilizing. It however confers immunity against symptomatic disease. Up to 75% of infections especially in children less than 2 years old have been found to be asymptomatic(Clarke & Desselberger, 2015).

Infection during the neonatal period of a child's life has also been demonstrated to confer immunity against future symptomatic illnesses. The titer quantities of antibodies show an increased percentage of protection with increase in levels. A titer of 1:128 of neutralizing antibody was shown to provide homotypic immunity against G3 and heterotypic immunity against G1 and 4 despite sole exposure to G3. Higher titers of >1:800 offered protection of up to 80%. However, titers as high as 1:6400 were not sterilizing. Those higher were at 50% protection level.

In Kenya, high G1, G2, G3, G4 and P1 neutralizing antibodies have been found in unvaccinated infants. Theories put forward/proposed to explain variable vaccine efficiency include varied reassortants of rotavirus and affected microbiota in the gut in low income

countries. These have significant effects on the immune reactivations employed by the humoral and cellular mediated systems. Interaction and interface with Oral Polio Vaccine (OPV) has been disproved and hence safe to give concurrently. The rotavirus antibody titers increased with subsequent vaccinations and were higher when given concurrently with OPV. With co-financing, many developing countries including Kenya have been able to introduce rotavirus vaccine (Rotarix®) to its regular vaccination schedules(WHO).

2.6 Immunity

Protection is by both humoral and cellular components of the immune system. After the first infections, the first immune response is homotypic but with subsequent infection, one has heterotypic response that is against a wider variety of strains. Once a child gets infected, they are conferred a short term protection and immunity against future infection. This is however not lifelong. There have been reports of sequential illness. It is for this reason that the vaccine is vital(WHO, 2011a). There have been concerns that breast milk neutralizes rotavirus vaccine due to high IgA titers especially in developing countries. This is due to its (IgA) inhibiting effect on RV1. However, this has not been completely evaluated extensively(Moon S, 2010). However, WHO, found no significant difference in groups breastfed and those not breastfed one hour to vaccination in South Africa(Michelle J Groome, 2014). Immunity is induced via rotavirus specific B and T cells. IgA is the primary indicator and moderator against rotavirus infection (Apondi et al, 2012).

Immunity to rotavirus is generated by infection with the virus be it via faeco - oral or via vaccination. The exact mechanism is still under study. Protection against future infections is not dependent on whether the first exposure was symptomatic or asymptomatic. Exposure is also not sterilizing (i.e. does not confer full immunity 100%)(Clarke & Desselberger, 2015).

Trials conducted in Kenya on vaccine efficacy of pentavalent rotavirus vaccine have shown 65% protection in the first year and 40% protection after 2 years post-vaccination. Mexico had 94% efficiency on RV1 evaluation. Figures of decline in clinic visits and in-patient admissions have been between 46 -76%(Clarke & Desselberger, 2015).

Contentious issues that have arisen after several clinical trials on oral live attenuated rotavirus vaccines have been addressed. Intussusception was known to occur but in rare cases and hence the benefits outweigh the risks. The magnitude of risk was excluded for the current vaccines compared to previous preparations. A concern about vaccine efficiency in low socioeconomic status and vulnerable sub-populations e.g. HIV positive and malnourished children was raised by the WHO SAGE in 2008. Subsequent studies have however proved no difference in vaccine efficacy in malnourished, premature and HIV positive infants (Groome et al, 2014; WHO).

WHO has reported that there is a moderate level of confidence that use of RV1 in high mortality countries reduces the rate of severe rotavirus diarrhoea. They however recommended further research so as to change the estimate of the effect. Trials done in Malawi and South Africa have concluded that generalization to high mortality countries is difficult. There is however prolonged shedding in HIV positive infants (WHO, 2010, 2013b). WHO strategic advisory group of experts on immunization also described no aggravation of the immunological or HIV condition of the patients after RV1 immunizations. There were similar findings in South Africa and in Kenya(WHO, 2013b).

2.7 Vaccination

There are 2 brands of Rotavirus vaccines available in the Kenyan market. Rotateq is a pentavalent vaccine that is a reassortants bovine human rotavirus derivative, and Rotarix that

is a monovalent human rotavirus vaccine derivative(IVAC, 2013). These are given orally. Under the support of GAVI and the recommendation of WHO, Kenyan health system has introduced Rotarix in the regular schedule. It should be administered at 6 and 10 weeks after birth(WHO, 2015).

During vaccine production, animal, human or human-bovine reassortants strains have been used to prepare vaccines. Human beings are known to be the natural hosts for the rotavirus. For this reason, it has been postulated that the immune responses from human-derived rotavirus is greatest and most consistent in comparison with animal-generated strains.

Rotarix ®, is a live attenuated vaccine, given orally. Its strain of origin is human strain 89-12. This is G1P[8] derivative and has been found to be the most common in the world over. The primary rotavirus was obtained from an infant in 1998 in Ohio and after isolation was attenuated. This was done by multiple passages in tissue culture. Subsequently it was evaluated as a vaccine. It has been shown to confer 89% immunity against rotavirus infection after 2 doses. This is against any rotavirus infection. It also gives 100% protection from severe forms of gastroenteritis. The efforts towards vaccine manufacture have been towards oral live vaccine preparations. According to Widdowsonn et al, Rotarix is based on human attenuated strain of G1P[8]. Rotarix vaccine is made from human rotavirus strain 89-12 type G1P1A[8]. It is in >= 10^6 median cell culture infective dose after reconstitution per dose. Shedding percentage after the first dose is 35-80% (Widdowson et al 2009;Tate et al, 2010). Though initially both the monovalent (Rotarix®) and pentavalent (Rotateq®) vaccines were incorporated by WHO in immunization schedules in America and Europe, the coverage has since been extended. The GAVI alliance in collaboration with 105 GAVI eligible countries, have been able to license and introduce Rotarix® to the vaccine schedule of 24 eligible countries (also GAVI reference). Routine use of Rotarix® in Latin America in 2006-2009,

showed great reduction not only in the number of deaths but also hospitalizations due to diarrhoea. There was a 77% heterotrophic strain protection conferred (Widdowson et al, 2009).

Rotarix® vaccine has been very efficacious in high income settings. The levels of protection that have been demonstrated in low income countries are however markedly lower. This study provides a picture of rotavirus infection in a hospital setting, to inform on the influence of rotavirus on public health and the disease burden post-vaccine in our lower-middle income country(Clarke & Desselberger, 2015).

In South Africa and Malawi, Rotarix® was found to have 61% efficiency. The prevention of severe GE was at 3.9/100 vaccines in Malawi and 2.5/100 vaccines in South Africa. This means for each 100 children vaccinated 3.9 and 2.5 episodes of the diarrhoea were prevented (WHO,2009).

Since the launch of the vaccine in July 2014, in Kenya, there has been no vaccine evaluation to assess the efficacy of monovalent rotavirus vaccination in the population. In preparation for rotavirus vaccine evaluation to be done in the future via case control study, a sham case control study was done in Siaya County, western Kenya. They assessed the coverage of pentavalent vaccine and found it to be at an average of 81-86%. Given the high coverage, there were difficulties getting unvaccinated children to compare the efficacy of the vaccine (Khagayi et al, 2014).

2.8 Rotavirus Detection

Various methods of detection have been used over the last few years that are relatively efficient in rotavirus detection. The most ideal specimen for rotavirus detection is stool. When stool is not available then one can collect rectal swab or soiled diapers. There is usually

a large amount of intact rotavirus present in stool specimens of children during an active infection (Desselberger, 2000). The method chosen is however based on the facilities available, cost, human resources and accessibility. There is no consensus on a gold standard for rotavirus detection currently recognized(The Department of Health Australia, 2011).

The methods available are enlisted below:

2.8.1 Electron Microscopy: this method, though expensive due to the microscope and expertise required, is highly specific. Its sensitivity is equivalent to that of Enzyme Immunoassay (EIA) (WHO, 2009). This method can detect small particles with sizes up to 75nm like that of Rotavirus. One may also use Immune Electron Microscopy to increase sensitivity. This method is suitable for visualization and description of the viral structure (Desselberger, 2000).

2.8.2 Enzyme Linked Immunosorbent Assay (ELISA):

Antigen detection is a popular method of rotavirus diagnosis and is based on detection of the viral proteins on the viral particles in stool. For bulk testing of large number of specimens, EIA is most appropriate. Its high specificity and sensitivity are its primary reliability property. It is adaptable to a large sample volume in the 96 well plate(Desselberger, 2000).

2.8.3 RT PCR (Real time reverse transcription PCR) has been developed and is sensitive. It is based on primers for several specific rotavirus genes. It is able to detect rotavirus even in extra-intestinal tissues. It is relatively costly and labor intensive. For this reason, it not used for routine rotavirus detection studies (WHO, 2009). This method is suitable for genomic characterization of the various types of the Rotavirus(Desselberger, 2000). After

transcription the sample is then passed through a gel to map the viral genome. An example of a gel medium is polyacrylamide Gel. When using PAGE (Polyacrylamide gel electrophoresis), Nucleic acid detection can be done by extraction of the viral nucleic acid segments. These are then visualized after extraction by electrophoresis on acrylamide gels, and subsequent ethilium bromide/ Silver Nitrate staining (WHO, 2009). In this method, viral RNA is extracted and analyzed by electrophoresis on polyacrylamide gel with subsequent silver staining. The aim of the electrophoresis is to separate the 11 dsRNA segments according to size. The pattern is visualized and analyzed. The silver stain is sensitive and aids in this visualization. This method is however employed as part of the PCR detection method (WHO, 2009).

2.8.4 Viral culture can be used but has not been widely used due to low yield. Successful cultures have in the past been achieved by use of primary or transformed monkey kidney cells. Proteolytic activation of the virus with trypsin can also be carried out before infection to enable growth. The growth capacity on various media varies from one strain to the next (Michelle Arnold, 2009).

2.8.5 Rapid Rotavirus Antigen detection:

This method uses a single step test that aims at detecting the antigen on the viral capsid of the Rotavirus. The tests are immunochromatographic; hence there is a change in color with every immune reaction that indicated whether it is positive or negative. The test is coated with antibodies specific for rotavirus (BIOTEC, 2009). Due to the ease of testing and the rapid turnaround time, these tests have gained popularity in clinical setting.

According to WHO guideline, rotavirus strain characterized flow chart, reports a series of steps in detection. Once results are obtained, carrying out subsequent tests is at the discretion of the investigator. The antigen test is done first then subsequently PAGE profile with the RT PCR being final step. This is however for surveillance laboratories(WHO, 2009).

2.8.6 Viral shedding after vaccination:

There has been evidence that viral particles can be detected after vaccination in both term and preterm babies. In the term babies this occurs mostly from day 3 to 9. The peak period for detection is day 6 to 8. In a study done on preterm babies, there was shedding up to two weeks post vaccination but none detected after four weeks(Smith Candice, 2011; Yen Catherine, 2011).

2.9 Strategies for Rotavirus Diarrhoea Control in Kenya

Rotavirus vaccine was created and identified by WHO as a key strategy in reduction of rotavirus diarrhoea burden, The use of Rotarix® for RVGE prevention is however not the sole intervention for GE prevention. It is part of a comprehensive strategy that includes: exclusive breastfeeding, adequate complementary feeding, hand-washing, reduction in household air pollution, vitamin A supplementation, safe drinking water and sanitations, increased case management and education on health seeking behaviors. Despite these strategies being well known, children in low income areas lack a good number of these interventions. Unfortunately not all children with diarrhoea are given appropriate treatment. Only one third do receive the appropriate treatment. This has been described in GAPPD (The integrated Global Action Plan for the Prevention of Diarrhoea) (WHO/UNICEF 2013).

The various interventional strategies have been classified as: protect, prevent and treat. Protective measures include exclusive breastfeeding and appropriate complementary

feeding. The preventive measures include; measles vaccination, hand washing with soap and prevention of HIV. The measures for appropriate treatment include improved care seeking behavior and referral; improved case management at community and health facility levels and continued feeding(WHO/UNICEF, 2013).

The measures specific targeted for diarrhoea are: vitamin A supplementation; Rotavirus vaccination; safe water and improved sanitation and Los-osmolarity ORS, zinc and continued feeding(WHO/UNICEF, 2013).

a) Exclusive Breastfeeding:

Breastfeeding has been shown to improve a child's health and nutrition. Exclusive breastfeeding as a strategy has been used to prevent diarrhoeal infections and deaths by building their immunity(IVAC, 2013). This has been found to be most effective if a child is exclusively breastfed for six months. A child who is not exclusively breastfed is at a risk ten times higher than the one who is breastfed exclusively for six months. Those whose breastfeeding is not continued to two years of age have a 32% increased risk of diarrhoea during infancy(WHO/UNICEF, 2013).

b) Hand washing: studies have shown a reduction of about 40% of risk diarrhoeal illnesses if soap and water are used for hand washing (Curtis Val, 2003). Unfortunately it has not been well taken up, with a recent meta-analysis showing that hand washing has been poorly taken up, especially after contact with feces. Only a paltry 19% of persons worldwide use soap and water to wash their hands after contact with fecal matter (Matthew C. Freeman, 2014).

c) Use of Zinc for treatment of Diarrhoea: this method has been widely advocated since evidence has shown a reduction in childhood diarrhoea mortality by 20% (IVAC,

2014). A meta-analysis done in Georgia demonstrated a reduction of stool volume and output in children with acute diarrhoea who received Zinc Sulphate(Lukacik Marek, 2009). A Cochrane review also showed shorter duration of diarrhoeal symptoms when zinc is supplemented. There was however no benefit for those below six months of age(Lazzerini M., 2013).

d) Vitamin A supplementation: Vitamin A has been shown to be vital in the control of diarrhoeal illness. There is an increased chance of dying from diarrhoeal illness in vitamin A deficiency with a relative risk increasing two fold. Vitamin a supplementation has led to decrease in mortality by 32% (CSDi, 2008 - 2015).

e) Safe and improved sanitation: a systematic review done showed an increase in risk of diarrhoeal illnesses by having inadequate hygiene standards(Wolf, 2014).

f) Los-osmolality ORS and Zinc: these have been proposed to be the mainstay of treatment of acute diarrhoeal illnesses. Zinc has been shown to have numerous benefits in the management of diarrhoeal illnesses. It reduces the duration of illness, and the likelihood of a recurrence of the same infection in the following two to three months (WHO, 2011b).

2.10 Treatment

There have been studies on Racecadotril®, a drug to treat acute diarrhoea both Rotavirus and non-rotavirus associated diarrhoea. The results however have shown no reduction in stool volume, duration and intravenous fluid rehydration(Gagandeep Kang, 2016). There is currently no documented cure.

2.11 Characteristics of children with diarrhoea in the prevaccine period (Nsabimana et al, 2017; Wamalwa et al, 2014)

In the prevaccine period, the children with higher prevalence of all cause diarrhoea has been more in those with malnutrition especially if they are bottle fed. There has also been a higher incidence among those living in unhygienic environments and poor hygiene practices such as lack of hand washing and drinking untreated water. Those of low socio-economic status have also been shown to have higher incidence of diarrhoea. There has not been a statistically significant increase in diarrhoea in children who were weaned early and diarrhoea. Maternal characteristics such as lack of formal education have been shown to increase the chances of diarrhoea among children. Family size has been shown to increase incidence of children with diarrhoea especially where there are three to four children all aged under five years of age.

2.12 Factors Associated with Rotavirus Infection in the Prevaccine Period.

The studies in Africa prior to introduction of rotavirus vaccination have shown that infants are most affected by diarrhoeal illnesses with increased rotavirus positivity (Nakawesi et al, 2010; Bar-zeev et al, 2015)

Breastfeeding has shown varying results in terms of protection from rotavirus infection. While some studies have suggested that breastfeeding is protective against rotavirus infection, there are studies that have shown that the breastfeeding was not associated with protection against rotavirus infection (Nakawesi et al,2010).

Dehydration has been documented to be more severe in those with rotavirus infection. Hence increasing the chances of mortality in these children. Studies done in Uganda and Malawi showed that there was no increase in rotavirus positivity among the children with HIV

infection. There was however prolonged shedding of the rotavirus in stool in these children positivity (Nakawesi et al, 2010; Bar-zeev et al, 2015).

In Kenya, studies have shown that rotavirus diarrhoea affects children of all nutritional status (Agutu M., 2016; G. B. W. e. al, 2016). There has also been evidence of concurrent mixed bacterial and viral infection in stools collected from children with rotavirus infection. The bacteria identified include *Salmonella, Shigella* and *E. col* (Shah et al, 2017*)*

2.13 Postvaccine rotavirus diarrhea prevalence

Two years after the introduction of rotavirus vaccination in the regular vaccination schedule, the burden of diarrhoea is still high and of unknown etiology. While the vaccine has largely been successful in the high income countries, it is necessary to establish whether rotavirus is still a leading cause of diarrhoea in the Low-Middle income countries and to describe its clinical pattern of presentation, to advice on targets that can further reduce the burden of diarrhoea among children. Rotavirus diarrhoea has age dependent susceptibility and the children under two years of age have been most affected in the pre-vaccination period (Bishop et al, 2009).

Rotavirus prevalence has however varied greatly for both the pre-vaccine and post-vaccine periods. In a systematic review in the pre-vaccination period, the prevalence varied from 16% to 61% in various North African (Algeria, Egypt, Morocco and Tunisia) and Asian countries (Bahrain, Qatar, Syria, United Arab Emirates and Yemen). The highest prevalence was in Syria at 61% while the lowest was in Saudi Arabia at 16% (Khoury H., 2011). A report by the rotavirus surveillance network in 2014 showed a pre-vaccine prevalence of 40.7% (35.6% to 49.1%) in 34 sentinel sites in twenty African countries (Mwenda et al., 2014). Findings in

Kenya have shown prevalence as high as 53.4% at Kenyatta National Hospital, prior to introduction of rotavirus vaccination (Gatinu et al,2016).

The post-vaccination period has been characterized by a decline in prevalence of rotavirus infection, though at varying proportions. In Ghana, there was a decline from 49.7% to 27.8% (Enweronu-Laryea, 2014). In Malawi the post-vaccine prevalence was 31% from 50% previously (Bar-Zeev, 2015). Zambia also reported a decline from 40.1% to 24.7%% rotavirus positivity (Mpabalwani et al, 2016). These three countries are among the pacesetters in prevention of acute diarrhoeal illness by rotavirus vaccination in Africa.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

This was a descriptive cross sectional study.

3.2 Study Setting

The study was carried out at the paediatric emergency department of the Moi Teaching and Referral Hospital. MTRH is Kenya's second largest referral facility, and is ranked as tier four hospital by the Ministry of Health. It has a catchment population of about thirteen million persons (a third of the Kenyan Population) from within the Western and North Rift regions of Kenya as well as the neighboring countries (MTRH, 2014). MTRH is located in Eldoret town, about 330km from Nairobi, in Uasin Gishu County, Kenya. This is a mainly agricultural region with both large scale and small scale farming and has a mixed urban, peri-urban and rural population of varying economic power (County, 2014).

The hospital is an 800 bed capacity tertiary hospital that also serves various people and learning institutions in Eldoret and its environs. The hospital provides various services ranging from primary care to specialized care and services (MTRH, 2014).

The paediatric emergency department is a separate entity from the adult emergency department. It was christened "Sick Child Clinic", and caters for all walk in children coming into the hospital. It serves to filter paediatric patients into the various categories, offer them emergency care and then provide them the appropriate management thereafter. It offers short-stay admission services for children requiring short term interventions such as nebulization, rehydration and suturing among others (MTRH, 2014).

There are approximately 3,000 patients seen per month with about 19% being hospital visits due to diarrhoeal illnesses. The department is run by a multidisciplinary team consisting of doctors, nurses, clinical officers and non – clinical staff. The department is located at the Shoe for Africa Children's Hospital, that is part of the Moi Teaching and Referral Hospital(MTRH, 2014).

The emergency department is divided into five functional areas as follows:

Triage area: this serves as the first stop for every patient who comes into the facility. At this point, a nurse carries out a quick assessment of the child to prioritize the order in which the children shall be attended to by the clinicians, based on severity of illness. The vital signs are also taken and recorded.

Records area: This is the second step after triage. At this point, the patients' particulars are recorded and a file is opened with a unique hospital number. The patients who need emergency care receive clinical care prior to this registration process.

Clinician's rooms: There are several rooms for the clinicians available to offer services. These are usually a team consisting of clinical officers and medical officers. The medical officer serves as the team leader. The patients are directed to the rooms by the nurse at the waiting area which is between the triage area and the clinician's rooms. This is based on the availability of the clinician and the severity of the condition. From here they can either go to pharmacy, laboratory or observation room.

Observation area: Once the patient has been seen, they only go to the observation room if they require any services such as rehydration, nebulization, drug administration and observation. Emergency patients however go directly to this room and are attended to by the clinician while here due to the facilities available. This room functions as the emergency department's short stay admission unit, and hence serves patients who require emergency

services and stabilization for example, rehydration and observation, for less than twelve hours. Those for rehydration can either be using oral rehydration solutions or intravenous fluids. After this short duration of stay here, they are reviewed by the clinician to determine whether they can proceed home or to the inpatient wards for admission. Those for admission also wait here while the records, social work and nursing team prepare for transfer to the wards (MTRH, 2014).

Other staff rooms: There are a few other rooms available for support services. These include; a staff room, a room for the nurse in charge and storage rooms. These are however not static and can be converted for different uses as required(MTRH, 2014).

Diagnostic capacity:

The sick child clinic has a laboratory that is able to carry out basic investigations such as haemogram, urea, creatinine and electrolytes, and liver function tests. The clinic relies on the main hospital laboratory for its microbiology investigations.

Staff in the clinic.

The clinic is run by a medical officer with several clinical officers. It runs 24 hours a day seven days a week. The children who visit this clinic are all children below 14 years of age with medical or surgical conditions. The healthcare team also comprises of nurses, social workers, nutritionists and child life specialists. The doctors, nurses and clinical officers have all received ETAT plus training and hence are able to take care of the common paediatric emergencies.

3.3 Population

The target population was the children seeking treatment at Moi Teaching and Referral Hospital, paediatric emergency department.

The study population was all the children aged two years and below, presenting with acute diarrhoeal illness at MTRH, paediatric emergency department.

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

The study included all the children aged twenty four months and below, presenting with acute diarrhoeal illness.

3.4.2 Exclusion Criteria

1. Children presenting with a history of ingestion of a drug likely to cause diarrhoea eg. Laxatives within 24 hours.
2. Children unable to provide a stool sample at the time of interaction.
3. Children who have received rotavirus vaccination two weeks or less prior to being recruited for the study.

3.5 Sampling Procedure

3.5.1 Sample Size Calculation

This study being a cross sectional study, employed the Fischer's formula (Kasiulevicius et al, 2006) to calculate a sample size. In a study carried out over a 74 month period in Ghana, two time periods were compared, before and after the introduction of the vaccine. Prevalence of rotavirus in the post vaccine period was 27.8% (Enweronu-Laryea, 2014). Due to this regions similarity in terms of disease burden and socioeconomic characteristics of the population, this value was used as *p*. It was be presumed that the proportion of infection compares.

The level of significance (α) = 5% (0.05), therefore, percentage point of normal distribution corresponding to the two sided significance level, Z, = 1.96

Margin of error = ± 5% (0.05)

Proportion p = 27.8% = 0.278

Therefore sample size: $n = \frac{Z^2 p (1- p)}{e^2}$

$$= \frac{(1.96)^2 \times 0.278 \times 0.722}{(0.05)^2} = 308.4$$

n=309

3.5.2 Sampling Technique

Systematic sampling was employed to select children to be included in the study. The first participant was selected, and every 5th (***k*th**) patient selected. *k* was calculated based on the total number of patients with diarrhoea over a six month duration of time divided by the sample size.

Calculation:

The sampling frame was the number of patients presenting with diarrhoea over a six month duration derived from the records department of Moi Teaching and Referral Hospital is = 2402. Of these, the ones who had acute diarrhoea and were aged two or below are about a two third of the total, giving 1562. (MTRH, 2014).

Sample size = 309

Hence k = 1562/ 309 = 5.05 hence was approximated to 5.

$\boldsymbol{k = 5}$

The sample size was divided into six to allow collection of data over a six month time frame. This was to prevent collection of clustered data that may be representing an outbreak and also account for seasonal variability of rotavirus prevalence. This translated to a target of fifty seven participants per month. This figure was derived by dividing the sample size into six groups giving an equal group for every month. However data collection period was extended by an extra one month to achieve the required sample size.

3.5.3 Study Period

The study was carried out for a seven month duration, beginning 28th November 2015, up to 30th June 2016. There were a varying number of patients every month ranging from eight to fifty four.

3.5.4 Research Instruments

The data was collected into an interviewer administered data collection tool (*Appendix B*). It was structured into the following sections; socio-demographic data, clinical assessment, environmental assessment and laboratory assessment. These sections allowed for collection of all the data required to address the objectives of this study.

3.6 Data Collection and Analysis

3.6.1 Data Collection

Data was collected by the principal investigator and the research assistant into the data collection tool (Appendix B). The research assistant was a Clinical Officer with ETAT plus training; he was also trained on the study procedures according to the proposal's objectives.

The training included the test procedures as per the manufacturer's instruction. Data was collected from the participant on physical examination. Thereafter the rapid rotavirus antigen test was done and results recorded into the data collection tool. A brief interview of the participant's parent/guardian was then carried out. This enabled efficient assessment of the dehydration status and rapid response to emergency situations involving the participants such as severe forms of dehydration and among others.

3.6.2 Data Storage

Data was checked for completeness and accuracy by the principal investigator on a daily basis. Data was entered onto a prepared Microsoft Access® database. The anthropometric measurements were entered into ENA for SMART 2011® from where the Z scores were derived. Confidentiality was maintained by excluding any identifiers from the keyed dataset. The database was password protected to prevent un-authorized access. Data was backed up in a remote hard disk and flash drive to safeguard against any data loss and placed in a lockable drawer. Data cleaning was then carried out.

3.6.3 Data Analysis

Data was entered using Microsoft Access® and analyzed using Stata® version 12.0 (Statacorp Texas USA ®), at 95% confidence interval. Data analysis of the demographic, clinical and laboratory parameters was done. Descriptive statistics were used for continuous variables and frequency listings for discrete/categorical data where suitable. Means and medians were calculated and used to describe these variables based on the kurtosis and skewness of the findings. Univariate, bivariate and multivariate analysis was done on the categorical variables.

Chi-square test and fisher's exact test were used to test for associations among categorical variables. These tests were used to compare the values between the two groups being studied i.e. those with rotavirus associated diarrhoea and those without. The variables included were the demographic characteristics such as age and gender. Others included the nutritional status, co morbidities and disease severity of the diarrhoeal illness, among others. P values <0.05 were considered statistically significant. Logistic regression was subsequently carried out for those found to be statistically significant, to test for independent association.

3.7 Study Procedures

3.7.1 Participant Selection and Study Execution

The team of nurses, clinical officers and doctors, at the emergency department were sensitized on the study prior to commencement of data collection. In congruence with the patient flow at the sick child clinic (see section 3.2) the patient would go through triage as is routine at the paediatric emergency department. They were then directed by the triage nurse to the clinician's room as part of the routine service delivery and patient flow of this department. The point of entry to the study for the participants was at the clinician's room. Once they had been seen by the clinician, the patient who met the inclusion criteria were assigned reference numbers. Every morning, the first patient with acute diarrhoea to arrive at the clinician's room was considered participant number one.

The research assistant would then place a sticker with the reference number on the all the files of the patients with acute diarrhoeal illness aged two years of age and below. This sticker was used to indicate a number based on time of arrival to the clinician's room. These numbers were used as a tally method to enable selection of every fifth (k^{th}) patient as a participant with a goal of recruiting three participants during that day.

Once a participant was selected, they were moved to an adjacent private area. While the nurse was preparing and administering the prescribed treatment, informed consent was obtained from the parent/guardian. The data collection was carried out in three stages; **Stage one** was clinical assessment. Here the child's dehydration characteristics were recorded according to the parameters required by the basic paediatric protocol of Kenya. This was because this is the only parameter likely to change with treatment received.

Stage two was stool testing. For the stool collection, plastic wrapper was then placed on the child's diaper after an independent physical examination focused on dehydration and the clinical parameters was carried out and recorded in the questionnaire. The participant then continued receiving treatment as is routine while the interviewer awaited a stool sample. Once the sample was available, the rapid antigen test was carried out by the interviewer at the patient's bedside. This was strictly within an hour of passing stool to preserve viability of the viral particles. The results were then disseminated to the primary clinician after disclosure and explanation to the participant's parent/guardian.

Stage three was completion of the data collection tool. The rest of the questionnaire was then administered to completion. This involved completion of the socio-demographic data and all the remaining clinical data.

After these three stages, the participant's parent/guardian were thanked for their participation and proceeded with their treatment.

The participants, who were not in a position to give a stool sample during the interaction period, were not analyzed in the study since their data entered into the questionnaire was incomplete. The next patient who met the inclusion criteria was recruited as a participant. The next patient was selected as the one who came into the clinician's room after the current

participants duration of stay has elapsed. Every fifth patient was identified as a participant till the desired number for the day was achieved.

3.7.2 Special Considerations

The following were put into consideration during the study period:

- The questionnaire was administered by either the principal investigator or the research assistant who is herein termed interviewer.
- This study process did not hinder any rehydration or other treatment modalities required/prescribed neither did it interfere with routine operations of the emergency department.
- The total interaction time was for a duration of four hours since this is the minimum time of observation required for any child requiring rehydration.
- Those who did not require observation and did not produce a stool sample at the time of interaction were not be recruited. This was because it would be exposing the child to unnecessary risk of infection by staying in hospital for a longer duration to wait for a stool sample. They were hence left out of the study. The next child to come into the observation room with acute diarrheal illness was then recruited in their place.
- Exceptions were made for those in dire emergencies such as severe forms of dehydration and shock, where the investigator would work in collaboration with the medical officer to stabilize the patient. After the patient was stable, consent was taken and the interview process commenced. These were included in the study but priority was given to their emergency treatment since their level of dehydration is life threatening.

- The study procedures were carried out discretely while in the emergency department to ensure that there was no breach of confidentiality during the entire process.
- A member of the nursing team was always available during the interaction to ensure monitoring of the participant being rehydrated, as is the routine.

3.7.3 Sample Handling

Once the sample was collected, the rapid antigen test was then carried out at the bedside. The remaining sample was disposed of immediately after the rapid antigen test. This disposal was in the highly infectious waste category of waste, hence was placed in the red color coded waste containers. They were subsequently disposed of as is routine for the hospital, with the rest of the highly infectious waste materials.

3.8 Laboratory Procedures

The participant's parent(s)/guardian(s) were then requested to keenly monitor the child's bowel movements and informed the interviewer immediately stool was passed. A stool sample was collected from the participant and a rapid rotavirus stool antigen test carried out at the participant's bedside. The details of the principles and standard operating procedures of the test have been described in Appendix I.

3.9 Ethical Considerations

Approval was sought from IREC (Institutional Research and Ethics Committee) prior to the commencement of the study. Permission was also sought from the Moi Teaching and Referral Hospital. The IREC approval was issued on 28th July 2015, IREC Formal Approval Number 1441. These letters of approval and permission were presented to the head, paediatric

outpatient department. Informed consent was sought from the parent or legal guardian of the participant prior to the data collection.

All the information regarding the study was provided in a language best understood by the participant's parent or guardian. Participation in the study was purely voluntary and data collected was de-identified using unique identification codes for each person.

Prompt and appropriate intervention for any emergent medical condition was availed in conjunction with the health staff at the paediatric emergency department.

The guidelines on handling of human biological specimens were followed in collection, use and disposal of the samples collected during this study.

Laboratory results found during the study were communicated to the clinician in charge of the participant's treatment and also documented in the participant's health records.

Confidentiality was assured and the data collected was available only to the research team for analysis purposes. Data was stored safely and shall be disposed after the statutory duration of time.

Ascent was to be sought from any mothers who were below eighteen years of age with subsequent consent signed from the mother's guardian or parent. We however did not receive any mothers below eighteen years of age. Those who did not have a guardian to countersign were to be omitted from the study. However we did not receive any mothers below the age of eighteen.

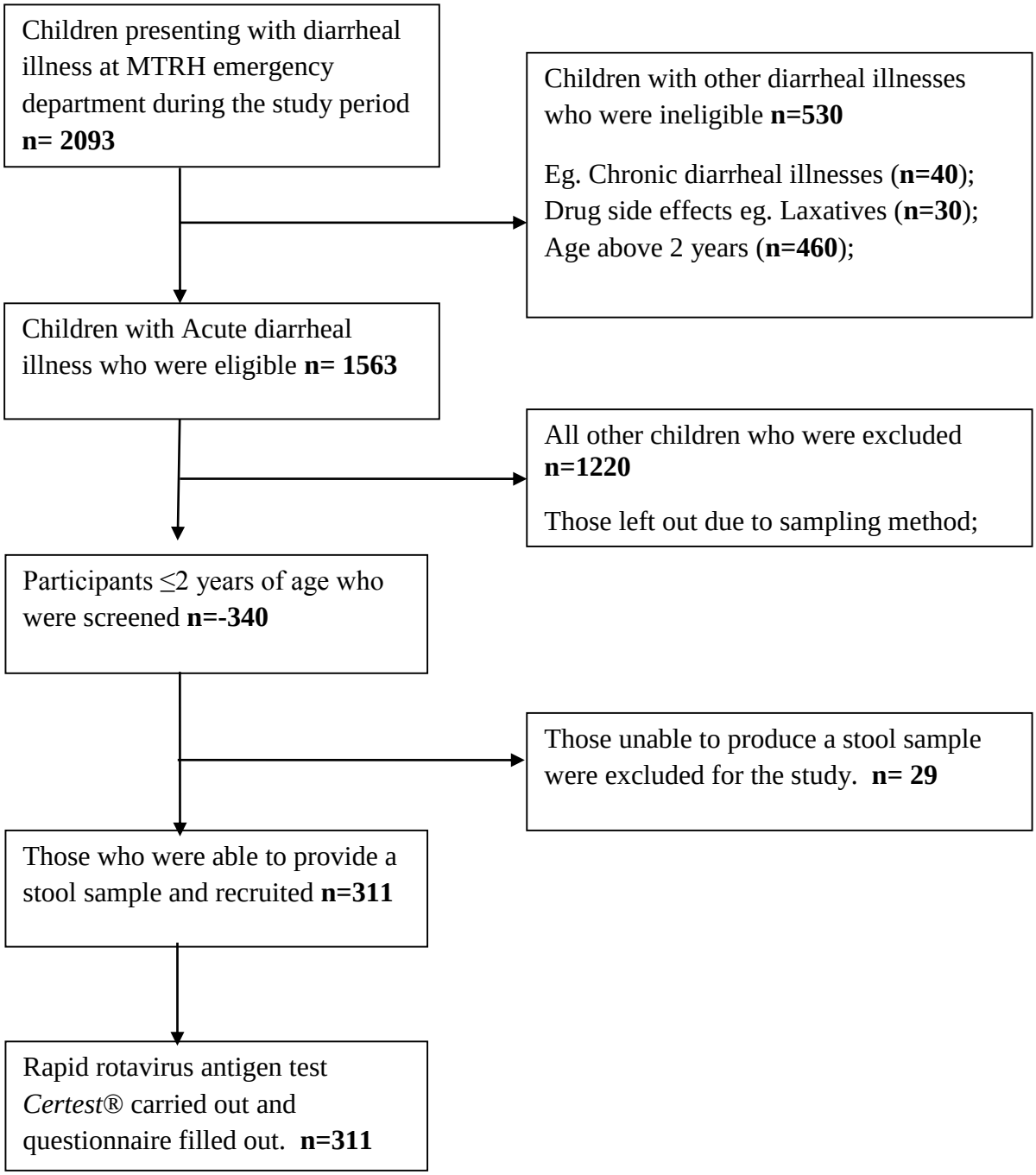
3.10 Dissemination of Results

The results of the study are being disseminated through a written thesis. An oral defense will be done in a forum that shall be convened by the School of Medicine. The results will

also be presented in national or international research meetings and published in a peer reviewed journal.

3.11 Study Flow Algorithm

A description of the participant recruitment process.



CHAPTER FOUR

RESULTS

4.1 Population Description

A total of 311 children were recruited and analyzed. Of these, 186 (60%) were males and the male female ratio was 1:0.7. Their median age of the children was 12 months (IQR 8, 19). Majority of the patients had their parents as the primary caretaker (80.7%). Majority of the participants, 89.1% were residents of Uasin Gishu County.

4.1.1 Socio-demographics of the study population

Table 1: Showing the socio-demographic characteristics of the participants recruited in the study.

Parameter:	**Number of participants: (n=311)**
Age Distribution	
< 6 months	45(14.5%)
6 – 12 months	109(35%)
12 – 18 months	69(22.2%)
>18 months	88(28.3%)
Gender	
Female	125(40.2%)
Male	186(59.8%)
Primary Caregiver	
Parent	251(80.7%)
House help	43(13.8%)
Others	17(5.5%)
County Of Residence	
Uasin Gishu	277(89.1%)
Other Counties (Bungoma, Marakwet, Nandi, Kisii, Kakamega, etc.)	34(10.9%)

4.1.2 Clinical characteristics of the study population:

Majority (83.3%) of the children were up-to-date with their vaccination and 85% had received two rotavirus vaccine doses. The children who had received age appropriate vitamin A at least once were 73.3% (228/300). The measles vaccine coverage was at 93.2% (205/220). In this study 36 % of the children had severe dehydration while 47/311 (15.1%) had WHZ score ≤-3 (wasting). Majority, 84% (261/311) of the children had Bristol stool type 6-7.

Table 2: Showing clinical features of the children in this study population

Parameter	**Number (n = 311)**
Vaccination Status	
Up to date	259(83.3%)
Not up to date	52(16.7%)
Rotavirus Vaccination	
None	30(9.6%)
Missed second dose	6(1.9%)
Received two doses	265(85.2%)
Not applicable	10(3.3%)
Dehydration Level	
Severe	113(36.3%)
Non-severe dehydration	198(63.7%)
Nutrition status (WHO WLZ score)	
Undernourished (Z score <-1)	121(38.9%)
Normal (Z score 1 to -1)	98(31.5%)
Over-nourished (Z score > 1)	92(29.6%)
Appropriate duration of exclusive breast feeding	
Yes	122(39.2%)
No	189(60.8%)
Currently breastfeeding	
Yes	210(67.5%)
No	101(32.5%)

Comorbidity at presentation	
Respiratory infection	40(12.9%)
Febrile illness	57(18.3%)
Others	25(8%)
None	189(60.8%)

The levels of dehydration were classified according to the Basic Paediatric Protocol 2015, which grades them as no dehydration, some dehydration, severe dehydration and shock. For this study, severe dehydration and shock were classified as severe forms of dehydration while the remaining two were non-severe forms of dehydration.

4.2 The Prevalence of Rotavirus Positive Diarrhoea

The prevalence of rotavirus diarrhoea based on the Rotavirus stool antigen Test result was 55.6% (173/311) with a 95% CI (49.91,61.23). Although the overall number of children with diarrhoea increased gradually from January to March, rotavirus associated diarrhoea was more prevalent during the months of February and March and then subsequently reduced as shown in the figure below. The rainfall amount recorded by the Kenya meteorological department was plotted against the monthly distribution, and showed the peak prevalence to be during the driest month i.e. February.

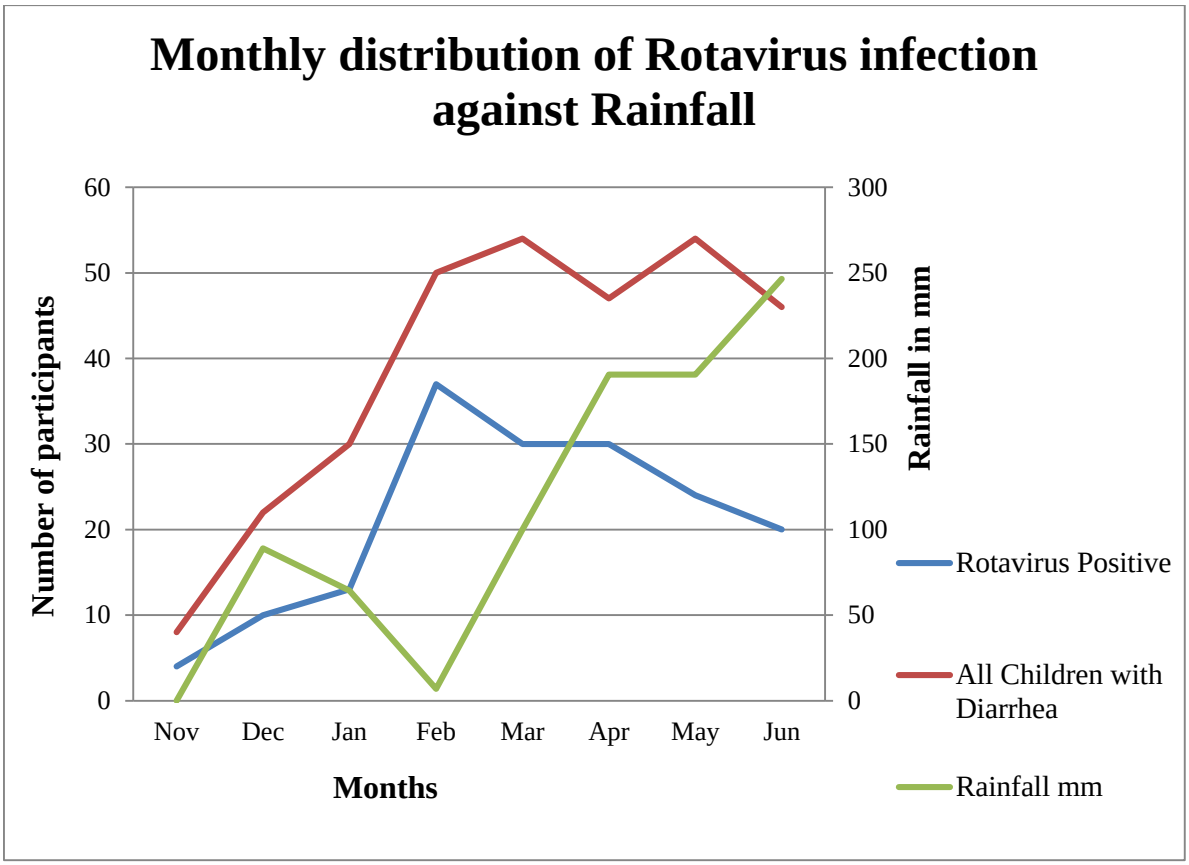


Figure 1: Line graph showing monthly distribution of participants with a positive Rotavirus antigen test and Total number of participants with Diarrhoeal illness, plotted against Rainfall in mm.

Prevalence of rotavirus diarrhoea was above 35% for all age groups except for those below 6 months. The highest proportion of rotavirus positive children, were among those aged 6 – 12 months 64/173 (37%). See figure 2 below.

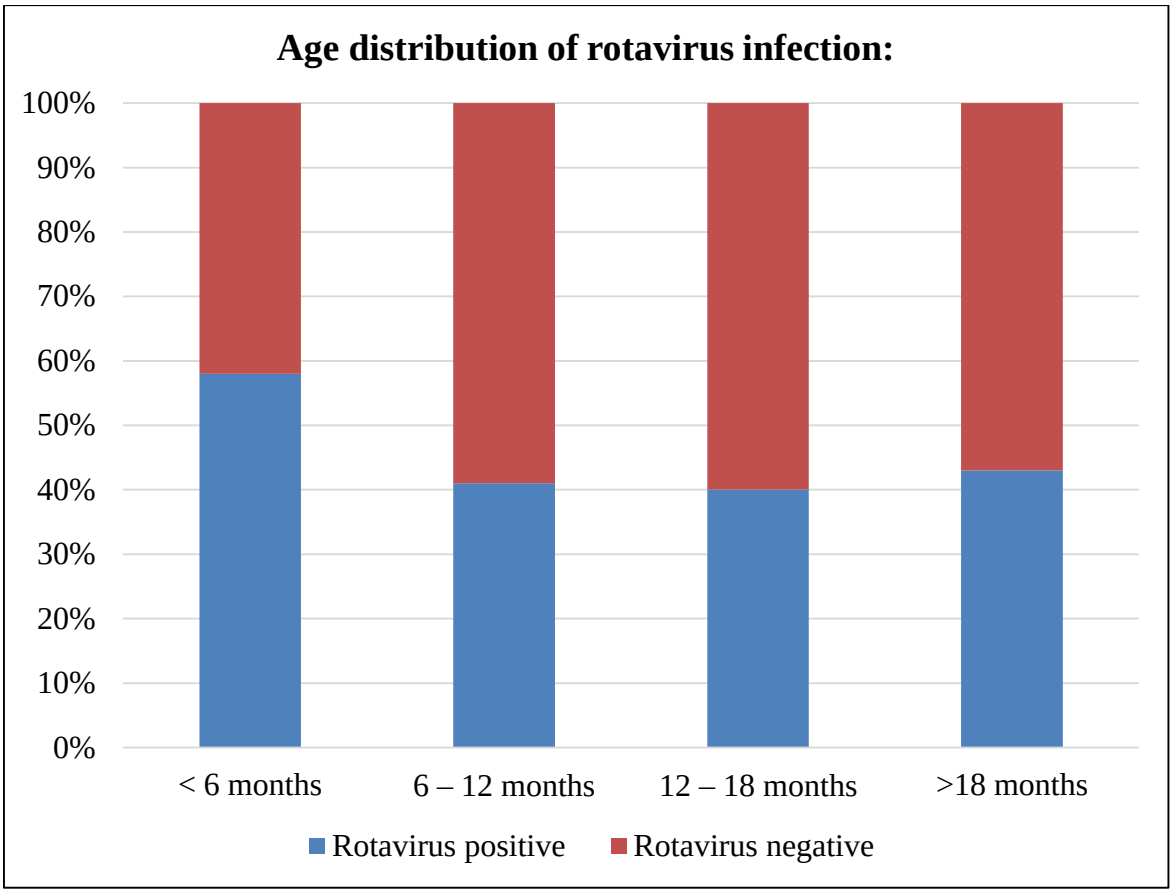


Figure 2: Bar graph showing age related prevalence of rotavirus diarrhoea among the children with diarrhoea.

4.3 Factors Associated With Rotavirus Positive Diarrhoea

4.3.1 Socio-demographic factors:

None of the socio-demographic factors assessed for were associated with Rota virus positive diarrhoea. These were as shown in table 3 below.

Table 3: Showing the socio-demographic factors associated with Rotavirus Antigen test positivity:

Parameter:	**Number of participants: (n=311)**	**Rotavirus Negative**	**Rotavirus Positive**	**p value (X^2)**
Age Distribution				
< 6 months	45	26 (57.7%)	19 (42.2%)	0.244
6 – 12 months	109	45 (41.3%)	64 (58.7%)	
12 – 18 months	69	28 (40.6%)	41 (59.4%)	
>18 months	88	38 (43.2%)	50 (56.8%)	
Gender				
Female	125	56 (44.8%)	69 (55.2%)	0.901
Male	186	82 (44.1%)	104 (55.9%)	
Primary Caregiver				
Parent	251	109 (43.4%)	142 (56.6%)	0.783
House help	43	21 (48.8%)	22 (51.2%)	
Others	17	8 (47.1%)	9 (52.9%)	
County Of Residence				
Uasin Gishu	277	124 (44.8%)	153(55.2%)	0.691
Other Counties	34	14 (41.2%)	20 (58.8%)	

4.3.2 Clinical Factors associated with Rotavirus antigen test positivity:

On univariate analysis, age appropriate completion of routine vaccination, two doses of Rotavirus vaccination and nutrition status were associated with a positive rotavirus test.

Table 4: Showing Clinical factors associated with Rotavirus antigen test positivity:

Parameter	Participants (n = 311)	Rotavirus Negative	Rotavirus Positive	p value (X^2)
Vaccination Status[1]				
Up to date	259	122 (47.1%)	137 (52.9%)	0.03
Not up to date	52	16 (30.8%)	36 (69.2%)	
Rotavirus Vaccination				
None	30	5 (16.7%)	25 (83.3%)	0.005
Missed second dose	6	2 (33.3%)	4 (66.7%)	
Received two doses	265	124 (46.8%)	141 (53.2%)	
Not applicable	10	7 (70.0%)	3 (30.0%)	
Dehydration Level				
Severe dehydration	113	49(43.4%)	64(56.6%)	0.786
Non-severe dehydration	198	89(39%)	109(61%)	
Nutrition status n=301 (WHO WLZ score) [2]				
Undernourished (Z score <-1)	109	40(36.7%)	69(63.3%)	0.009
Mild(WHO WLZ -1to >-2)	-	*10 (27.8%)*	*26 (72.2%)*	
Moderate(WHO WLZ -2 to -3)	-	*6 (22.2%)*	*21 (77.8%)*	
Severe(WHO WLZ <-3)	-	*24 (52.2%)*	*22 (47.8%)*	
Over-nourished (Z score > 1)	90	48 (53.3%)	42 (46.7%)	
Normal (Z score 1 to -1)	102	46 (45.1%)	56 (54.9%)	
Appropriate duration of exclusive breast feeding				
Yes	122	50 (41.0%)	72 (59%)	0.334
No	189	88 (44.4%)	101 (53.4%)	
Currently breastfeeding				
Yes	210	88 (41.9%)	122 (58.1%)	0.200
No	101	50 (49.5%)	51 (50.5%)	
HIV Status				
Positive	11	8 (72.7%)	3 (27.3%)	0.146[3]
Negative	291	126 (43.3%)	165 (56.7%)	
Sero-exposed	9	3 (33.3%)	6 (66.7%)	
Temperature [1]				
Low	72	31 (43.1%)	41 (56.9%)	0.089
Normal	131	67 (51.1%)	64 (48.9%)	
High	108	40 (37%)	68 (63%)	

Stool type in Consistency				
Type 4 to 5	50	28 (56.0%)	22 (44.0%)	0.071
Type 6 to 7	261	1102.2%)	151(57.8%)	

1. Age appropriate vaccine completion, according to the Ministry of Health Kenya schedule.

2.Weight for Length Z Score according to the World Health Organization(WHO, 2016).

3. Fisher's exact test

4. Normal ranges according to the Canadian Paediatric Society (36.5 to 37.5^{O}C)(Leduc D., 2015)

Vaccination status was described as up to date if a child had received all the expected vaccines for age. In those who received rotavirus vaccination, those classified as not applicable were the children below six weeks of age since the first dose of the vaccine is given at six weeks of age.

4.4 Logistic Regression Analysis

We subjected the significant socio-demographic and clinical variables to logistic regression to seek independent association with rotavirus diarrhoea. Age appropriate completion of vaccination (OR 0.478; CI 95% 0.256 – 0.892; p=0.030), Mild wasting (WHO WHZ of <-1) and Moderate wasting (<-2) increased the odds of rotavirus diarrhoea by up to 3 times (OR 2.6, p= 0.035, CI 1.068-6.23 and OR 3.4 p= 0.019 CI 1.22-9.6 respectively). Severe malnutrition (WHO WHZ of <-3) was still not associated. Two Rotavirus vaccinations reduced Rotavirus diarrhoea by 85% (OR 0.15 p=0.017 CI 0.032-0.709). One rotavirus vaccination was not statistically significant in protection.

Table 5: Showing Logistic regression analysis to test for associations

Variables:	**Odds Ratio**	**P-value**	**[95% Confidence Interval]**
Stool type (6-7 vs 4-5)	1.941	0.050	0.999, 3.771
WHZ (Overweight vs Normal)	0.787	0.440	0.428, 1.445
WHZ Mild vs Normal)	2.581	0.035	1.068, 6.236
WHZ (Moderate vs Normal)	3.424	0.019	1.221, 9.604
WHZ (Severe vs Normal)	0.795	0.552	0.373, 1.692
Rota (Missed second dose vs None)	0.445	0.456	0.053, 3.742
Rota (Two vs None)	0.151	0.017	0.032, 0.709
Rota (N/A vs None)	0.058	0.002	0.009, 0.359
Fully immunized (Yes vs No)	0.478	0.030	0.256, 0.892

CHAPTER FIVE

DISCUSSION

In the postvaccine period, the characteristics of children with acute diarrheal illnesses have had been documented in various African countries with great variation. In Malawi, the proportion of children who were breastfed and presented with diarrhea were 82%, this is significantly higher than this study where only 67.5% were breastfeeding(Bar-Zeev et al, 2015). This could be attributed to variations in the cultural practices and the health advocacy initiatives put in place in the two countries. However further studies looking into the reasons behind the low breastfeeding proportions is necessary. The age of children with diarrheal illness has however been uniformly documented with most studies showing that infants were most affected, hence an age dependent susceptibility. These include Ghana, Malawi and this study that was done in Kenya. In Malawi the children under two formed the greatest proportion of those with diarrhea at 94%(Bar-Zeev et al, 2015; Enweronu-Laryea, 2014). In Kenya there has been no statistical significance in terms of gender predilection to diarrheal illness, despite males being recorded at a higher percentage than females. This is similar to the findings in this study(Mbae et al, 2017).

Our study showed a rotavirus prevalence of 55.6%, a value higher than some of the studies carried out in the post and pre vaccination period in other hospital based studies. The high prevalence in this study, can be attributed to differences in methodology of this study compared to previous studies. These study differences include age of participants, site of the study, population studied among others.

The prevalence of Rotavirus diarrhoea is higher when only children aged two years and below are included in the study compared to when older children are also included. For instance the prevalence rose from 15% and 22.7% in Cameroon and Kenya respectively to 42.8% and 44.7% (Kotloff et al, 2013; Ndze et al,2013). This explains the relatively high prevalence in our study. Age dependent susceptibility in children below two years is due to inadequate immunity which is gained from exposure to the virus. These hospital based studies have shown a higher prevalence compared to population based studies that have showed as low prevalence as 10-15% (Kotloff et al, 2013; Njeru et al., 2016). This is due to study population biases since the hospitalized children have diarrhoeal illness at recruitment while population based studies include asymptomatic children. The prevalence also varies from hospital to hospital even in multicenter studies within one country such as that seen in Cameroon where they had a range of 33.9% to 46.5%(Ndze et al, 2013) . We can attribute this variation from one hospital to the other to the differences in catchment populations that increase their risk of rotavirus infection. Urban centers have higher prevalence of rotavirus diarrhoea compared to rural areas due to the high population density and poor sanitation(Shah et al, 2017).

High prevalence of rotavirus even after introduction of the rotavirus vaccine in other African countries, has been hypothesized to be due to various reasons. These include: lower vaccine efficacy in high burden countries as seen in Zambia and Malawi (Bar-Zeev et al, 2015; Mpabalwani et al, 2016). This has been studied further in other high burden countries and various determinants behind the lower vaccine efficacy put forward. There have been studies that show that variability in gut microbiota causes reduced vaccine efficacy; *Bacteroides* predominance in the gut is associated with poorer vaccine response compared to *Streptococcus bovis* (Harris et al, 2016). Also, variations in Rotavirus serotype and re-

emergence of strains following the introduction of the vaccine have been attributed to poorer vaccine response. In a study in Kenya there was documented re-emergence of genotypes not covered by the vaccine such as G[9] and G[12] strains (Agutu et al, 2016). Breast milk antigens have been shown lower vaccine seroconversion in high burden-low income countries where breastfeeding rates are high. The anti-rotavirus specific IgA in breast milk is high especially in high burden countries. There is also a chance that these children have had exposure to wild type rotavirus prior to vaccination, hence the response to vaccination will be poor due to preformed antibodies against rotavirus (Chilengi et al, 2015).

This study showed that mild and moderate malnutrition increased the risk of rotavirus infection (OR 2.581;CI 95% 1.068–6.236; p=0.035) and (OR 3.424; CI 95% 1.221 – 9.604; p=0.019) respectively, while severe malnutrition had no association with the infection (OR 0.795; CI95% 0.373, 1.692; p=0.552). However, the relationship between malnutrition and rotavirus infection is still unclear. Under-nutrition has been associated with increased prevalence and worsening severity of rotavirus diarrhoea and lower vaccine efficacy(Bar-zeev et al, 2015). This is thought to result from the lower immunity in under-nutrition and villous blunting. Severe malnutrition on the other hand was not associated with rotavirus infection in our study. Our findings of high rotavirus infection prevalence could further be explained by high number of mild and moderately malnourished children among the participants. There have been some contradictory studies that showed malnutrition having been protective against rotavirus infection(Hans Verkerke, 2016). The pre-vaccine studies in Kenya showed a higher prevalence among those of normal nutritional status(Agutu et al, 2016).

The rotavirus vaccine functions to decrease severity of illness in terms of dehydration level and hence a decline in hospitalization rates(WHO, 2013c). There have however been some hospital based studies showing a decline in prevalence of rotavirus after introduction of the vaccine(Enweronu-Laryea, 2014). This study did not compare the prevalence with any prior findings since there are no published studies in the facility and the region in the postvaccine period among children under two years of age. There have been no reports to the contrary. The rotavirus positivity and disease severity has been shown to decrease on an incremental proportion every year after introduction of vaccination. The total percentage improvement in the burden has increased progressively. While we did not set out to carry out a vaccine evaluation, this study found that rotavirus vaccination is protective against rotavirus infection especially when given in two doses. One dose of rotavirus vaccine conferred some protection but was not statistically significant. The vaccine coverage was however lower in this study than the World Health Organisation recommended level required for public health protection of 90% (WHO, 2013a).

This study can be of use to various policy makers. At the hospital level, it can be used to inform choice of point of care tests for diarrhoeal illnesses, to inform treatment choices for day to day patient care. The county level health policy makers can use the findings of this study to carry out evidence based interventions in reduction of rotavirus associated diarrhoea. The country should implement similar studies in other regions as a prelude to vaccine evaluation studies, and assess the prevalence and characteristics of children with rotavirus infection.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS:

6.1 Conclusion

- There is age dependent susceptibility to acute diarrhoeal illness among children, with infants being most affected.
- The rotavirus vaccine coverage has not reached the recommended level and rotavirus remains a major cause of morbidity in children under two years of age presenting at MTRH.
- Completion of rotavirus vaccination and the scheduled routine vaccinations is protective against rotavirus associated diarrhoea.
- Mild and moderate forms of malnutrition increases the odds of rotavirus infection

6.2 Recommendations

- Uptake of the 2 doses of Rotavirus vaccination and the Standard UVIS (Unit of Vaccines and Immunization) Kenya vaccines should be intensified to reach the targeted 90% coverage.
- Increased interventions towards decreasing the prevalence of acute diarrheal illness among infants should be put in place.
- Studies looking into the effect of nutritional status on rotavirus diarrhoea should be carried out.
- Further studies on the serotypes of rotavirus causing disease in the post vaccine era should be pursued.
- Vaccine evaluation studies should be carried out in Kenya to evaluate the efficacy of the rotavirus vaccine.

BIBLIOGRAPHY

Agutu, M., Ongus, J., Kombich, J.,Kamenwa, R., Nyangao, J.,…Bitek, A.,(2016) Prevalence and genetic diversity of rotavirus infection in children with acute gastroenteritis in a hospital setting, Nairobi Kenya in post vaccination era: a cross-sectional study. *Pan African Medical Journal*. *26:38.* doi:10.11604/pamj.2017.26.38.10312

Apondi, W.E.,Omondi, J. and Ichinose, Y., (2012) Detection and molecular characterization of rotavirus strains isolated from children attending selected health facilities in Kiambu district, Kenya, Masters Thesis in School of Pure and Applied Sciences of Kenyatta University, Kenya. Retrieved from: http://ir-library.ku.ac.ke

Bar-Zeev, N., Kapanda, L., Tate, J.,Jere, K., Iturriza-Gomara, M.,…VacSurv,C.,(2015) Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case-control study. *The Lancet Infectious Diseases*, 15(4):422-8. doi: 10.1016/S1473-3099(14)71060-6.

Beres, L., Tate, J., Njobvu, L., Chibwe, B., Rudd, C.,…Chilengi, R., (2016). A Preliminary Assessment of Rotavirus Vaccine Effectiveness in Zambia. *Clinical Infectious Diseases*, 62 Suppl 2:S175-82. doi: 10.1093/cid/civ1206

BIOTEC, (2009). Certest rotavirus - One step Rotavirus Card Test. 1-2. http://www.certest.es/products/rotavirus/

Bishop Ruth, (2009). Discovery of Rotavirus:Implications for child health, *Journal of gastroenterology and hepatology* 2009, 3:S81-5 doi: 10.1111/j.1440-1746.2009.06076.x.

CDC, (2011). Rotavirus Surveillance Worldwide 2009. *Morbidity and Mortality Weekly Report* (MMWR), 60(16), 514 - 516. Retrieved in August 2015 from: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6016a5.htm

Chilengi, R., Simuyandi, M., Beach, L.,Mwila, K.,Becker-Dreps,…Jiang,B.,(2015) Association of Maternal Immunity with Rotavirus Vaccine Immunogenicity in Zambian Infants. *PLOS One*. https://doi.org/10.1371/journal.pone.0150100

Clarke, E., & Desselberger, U. (2015). Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings. *Mucosal Immunology* , 8(1), 1-17. doi: 10.1038/mi.2014.114

County, U. G. (2014). General Information about Uasin Gishu County. http://uasingishu.go.ke/reports/8/about-the-county.

CSDi. (2015). Zinc and Vitamin A: Mitigating Diarrhoea in Children. The center for sustainable development. Retrieved from: http://www.csd-i.org/zinc-vitamin-a-mitigating-dia/

Curtis, V. and Cairncross S., (2003) Effect of washing hands with soap on diarrhoea risk in the community: a systematic review. *The Lancet infectious Disease Journal*, 2003. 3(5): p. 275 - 281. https://doi.org/10.1016/S1473-3099(03)00606-6

Desselberger, J. and Gray,J.(eds) (2000). Rotaviruses Methods and Protocols.Methods in molecular medicine. *Biomedical sciences*, Humana Press, Totowa, NJ.

Enweronu-Laryea, C., Boamah, I., Sifah, E., Diamenu, S., Armah, G., (2014). Decline in severe diarrhea hospitalizations after the introduction of rotavirus vaccination in Ghana: a prevalence study. *BioMedCentral Infectious Diseases*. doi: 10.1186/1471-2334-14-431.

Farthing, M., Lindberg, G., Dite, P., Khalif I, Salazar-Lindo E,… LeMair A. (2012). Acute diarrhea in adults and children: a global perspective. World Gastroenterology Organisation Global Guidelines. *Journal of Gastroenterology*. 47(1):12-20. doi: 10.1097/MCG.0b013e31826df662.

Freeman, C., Stocks, E., Cumming, O., Jeandron, A., Higgins, P.,… Curtis, V., (2014) Hygiene and health: systematic review of handwashing practices worldwide and update of health effects. *Tropical Medicine and International Health*. 19(8):906-16. doi: 10.1111/tmi.12339.

Gagandeep, K., Thuppal, V., Srinivasan, R, Sarkar, R., Subashini, B.,… Bose,A., (2016) Racecadotril in the management of rotavirus and non-rotavirus diarrhea in under-five children: Two randomized, double-blind, placebo-controlled trials. *Indian Academy of Pediatrics*. 53(7): p. 595-600. DOI: 10.1007/s13312-016-0894-0

Gatinu, B., Kiulia, N., Nyachieo, A., Macharia W., Nyangao J. and Irimu G., (2016) Clinical Features Associated with Group A Rotavirus in Children Presenting with Acute Diarrhoea at Kenyatta National Hospital, Nairobi, Kenya. *Journal of Virology and Emerging Diseases*. 2.1. DOI: 10.16966/jved.112

GAVI. (2013). Rotavirus disease - www.gavialliance.org. Retrieved August 2013 from http://www.gavi.org/support/nvs/rotavirus/.

Harris V., Armah G., Fuentes S., Weerth, C., Giaquinto, C.,…Vos, W.,(2016), The infant gut microbiome correlates significantly with rotavirus vaccine response in rural Ghana. *Journal of Infectious Disease*. 214(12) 34–41, https://doi.org/10.1093/infdis/jiw518

Hans V., Jennie Z., Petri,M., Reichman, D., Qadri, F.,…Petri, W., (2016). Malnutrition Is Associated with Protection from Rotavirus Diarrhea: Evidence from a Longitudinal

Birth Cohort Study in Bangladesh. *Journal of Clinical Microbiology*, 54(10). doi: 10.1128/JCM.00916-16

Hertel, P. M. and Estes, M. K. (2012). Rotavirus and biliary atresia: can causation be proven? *Current Opinion in Gastroenterology*, 28(1), 10-17. doi: 10.1097/MOG.0b013e32834c7ae4

IVAC. (2013). Pneumonia and Diarrhea Progress Report, 2013. International Vaccine Access Center.Retrieved in May 2015 from: https://www.jhsph.edu/research/centers-and-institutes/ivac/resources/IVAC-2013-Pneumonia-Diarrhea-Progress-Report.pdf

IVAC. (2014). Pneumonia and Diarrhea Progress Report 2014. International Vaccine Access Center. Retrieved in May 2015 from: https://www.jhsph.edu/research/centers-and-institutes/ivac/resources/IVAC-2014-Pneumonia-Diarrhea-Progress-Report.pdf

Kenya National Bureau of Statistics. (2014). Kenya Demographic Health Survey Key indicators. Retrieved in April 2015 from: https://dhsprogram.com/pubs/pdf/fr308/fr308.pdf

Kasiulevicius, V. Šapoka, R. and Filipavičiūtė, R.,(2006), Sample size calculation in epidemiological studies. *Gerontologija.* 7(4): 225–231.

Khoury H., Ogilvie I., Khoury A., Duan, Y. and Goetghenbeur (2011), Burden of rotavirus gastroenteritis in the Middle Eastern and North African pediatric population. *BioMedCentral Infectious Diseases*. 11(9): p. 1471 - 2334. https://doi.org/10.1186/1471-2334-11-9

Kotloff K., Nataro J., Blackwelder W., Nasrin D, Farag TH,… Levine MM,(2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing

countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet*, 2013. 382(9888): 209-22. doi: 10.1016/S0140-6736(13)60844-2.

Kulia N., Hofstra N., Vermulen L. C.,Obara, M., Medema, G. and Rose, J.,(2015), Global Occurrence and Emission of Rotaviruses to Surface Waters. *Pathogens*. 4: p. 229 - 255. doi: 10.3390/pathogens4020229

Lazzerini M.and Luca Ronfani (2013). Oral zinc for treating diarrhoea in children. *Cochrane Infectious Diseases* Group [cited 2016; Available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD005436.pub4/abstract.

Leduc D., S Woods. (2015). Canadian Paediatric Society, Community Paediatrics Committee, Temperature measurement in Pediatrics. [cited 2016 10th October]; Available from: http://www.cps.ca/documents/position/temperature-measurement.

Lewis SJ and Heaton KW, (1997). Stool form scale as a useful guide to intestinal transit time. *Scandinavian journal of Gastroenterology*. 32(9): p. 920 - 924. DOI:10.3109/00365529709011203

Lukacik, M., Ronfani, L., Thomas and V. Aranda,V., (2009) A Meta-analysis of the Effects of Oral Zinc in the Treatment of Acute and Persistent Diarrhea. *9med.net* [cited 2016; Available from: http://journal.9med.net/html/qikan/fckxyekx/xekyxqk/200821202/wz/20090401094054691_467801.html

Mpabalwani M.,Chibumbya J. Simwaka,Mwenda, J.,Mubanja, C.,…Tate, J., (2016) Impact of Rotavirus Vaccination on Diarrheal Hospitalizations in Children Aged <5 Years

in Lusaka, Zambia. *Clinical Infectious Diseases*. (63): p. s183 - s187. DOI: 10.1093/cid/civ1027

Michelle A., Patton, T. and McDonald S., (2009). Culturing, Storage, and Quantification of Rotaviruses. *Current Protocol for Microbiology* - doi: 10.1002/9780471729259.mc15c03s15. Retrieved May 2015 from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3403738/

Michelle J. G., Moon S., Velasquez D.,Jones, S., Koen, A.,…Madhi, S., (2014). Effect of breastfeeding on immunogenicity of oral live-attenuated human rotavirus vaccine: a randomized trial in HIV-uninfected infants in Soweto, South Africa. *Bulletin of the World Health Organisation*, 2014. 92: p. 238 - 245.

Ministry of Public Health nd Sanitation (2015). Division of vaccines and immunization Comprehensive Multi Year Plan Kenya: Government of Kenya . Retrieved in April 2015 from: http://www.nationalplanningcycles.org/sites/default/files/country_docs/Kenya/cmyp_kenya_2013-2017_1.pdf

Moon, S., Wang, Y., Shane, L.,Nguyen, T., Ray, P.,… Jiang, B., (2010) Inhibitory effect of breastmilk on infectivity of live oral rotavirus vaccines. *Pediatric Infectious Disease Journal*. 29(10): p. 919 - 23. doi: 10.1097/INF.0b013e3181e232ea

MTRH (2014). Outpatient records of children seen at Moi Teaching and Refferal Hospital. *Records Department*.

Mwenda, J. M., Ntoto, K. M., Abebe, A.,Enweronu-Laryea, C.,Amina, I.,…Steele, A., (2010). Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network.*The Journal of Infectious Diseases*, 202 Suppl, S5-S11. doi: 10.1086/653557

Mwenda, J. M., Tate, J. E., Parashar U. D., Mihigo, R, Agócs, M, Serhan, F, Nshimirimana, D., (2014). African rotavirus surveillance network: a brief overview. *The Pediatric Infectious Disease Journal,* 33 Suppl 1, S6-8. doi: 10.1097/INF.00174

Mwenda J., Mmihigo R., Benissan, C., Mumba, M., Nshimirimana, D.,(2015), Rotavirus disease burden in Africa and the need to accelerate introduction of vaccines. *WHO African Region*. Retrieved in June 2015 from: http://www.aho.afro.who.int/sites/default/files/ahm/pages/2471/AHM%2019%20-%2002%20-%20Rotavirus%20disease%20burden%20in%20Africa.pdf

Nakawesi J., Wobudeya E. and Ndeezi G., Mworonzi, E. and Tumwine, J. (2010), Prevalence and factors associated with rotavirus infection among children admitted with acute diarrhea in Uganda. *BioMedCentral Pediatrics*. 10(19). https://doi.org/10.1186/1471-2431-10-69

Ndze V., Akum A.,Kamga G.,Enjema, L., Esona, M.,…Obama, M.,(2013), Epidemiology of rotavirus diarrhea in children under 5 years in Northern Cameroon. *PanAfrican Medical Journal*. 11(73). http://www.panafrican-med-journal.com/content/article/11/73/full

NIH (2010). Rotavirus. National Institute of Allergy and Infectious Diseases. Retrieved May 2015 from http://www.niaid.nih.gov/topics/rotavirus/Pages/rotavirusIllustration.aspx

Njeru R., Mbae C.,Kariuki S., Owuor, B. and Karanja, S., (2016), Prevalence of Group a Rotavirus before and after Vaccine Introduction in Mukuru Informal Settlement in Kenya. International Knowledge Sharing Platform: *Biology Agriculture and Healthcare*. 6(14).

Njeru R., Karanja S. and Mbae C., (2017). Prevalence and Genetic Diversity of Group A Rotavirus in Children Under Five Years, before and after Vaccine Introduction at Mukuru Informal Settlements, Nairobi County, Kenya, Master in Public Health, Jomo Kenyatta University of Agriculture and Technology: JKUAT repository.Retrieved in January 2018

Nokes, D.J., Abwao J., Pamba A.,Peenze, I., Dewar, J.,…Williams, T.,(2008), Incidence and clinical characteristics of group A rotavirus infections among children admitted to hospital in Kilifi, Kenya. *PLoS Medicine*. 5(7): p. e153. doi:10.1371/journal.pmed.0050153

Nsabimana J., Mureithi C. and Habtu M., (2017), Factors Contributing to Diarrheal Diseases among Children Less than Five Years in Nyarugenge District, Rwanda. *Journal of Tropical Diseases & Public Health*. 5(3). DOI: 10.4172/2329-891X.1000238

PATH. (2014a). Rotavirus disease and vaccines in Africa. Vaccine Access and Delivery. https://www.path.org/publications/files/VAD_rotavirus_africa_fs.pdf (retrieved 2015)

PATH. (2014b). Rotavirus Disease and Vaccines in Kenya. Vaccine Access and Delivery. Retrieved in May 2015 from, https://www.path.org/publications/files/VAD_rotavirus_zambia_fs.pdf

PATH. (2015). Accelerating access to rotavirus vaccines. Retrieved in May 2015, from http://www.path.org/projects/rvp.php

Rachel Lee,Lesler J, Lee A., Rudolph, K., Reich, N.,… Cummings, D.,(2013). Incubation periods of viral gastroenteritis: a systematic review. *BMC Infectious Disease*. 13: p. 446 - 458. https://doi.org/10.1186/1471-2334-13-446

Roush S, and Baldy L., (2015). National Center for Immunization and Respiratory Diseases Centers for Disease Control and Prevention *Manual for the Surveillance of Vaccine-Preventable Diseases.* 2012, CDC: Retrieved in April 2015 from: https://www.cdc.gov/vaccines/pubs/surv-manual/index.html

Shah M., Odoyo E., Wandera E., Kathiiko, C., Bundi, M.,…Ichinose, Y., (2017), Burden of rotavirus and enteric bacterial pathogens among children under five years old hospitalized with diarrhea in suburban and rural areas in Kenya. *Japanese Journal of Infectious Diseases*, 2017. Retrieved in June 2016 from; https://www.jstage.jst.go.jp/article/yoken/advpub/0/advpub_JJID.2016.398/_pdf

Smith, C., McNeal, M., Meyer, R., Haase, S. and Dekker, C., (2011) Rotavirus shedding in premature infants following first immunization. *Vaccine*. 29(45): p. 8141-8146.

Surendran, S. (2008). Rotavirus infection: Molecular Changes and Pathophysiology. *Excle Journal*, 7, 156 - 162.

Tate, J., Patel, M., Steele, A., Gentsch, J., Payne, D.,…Parashar, U., (2010). Global impact of rotavirus vaccines. *Expert Reviews*, 9, 395 - 407. doi: 10.1586/erv.10.17.

The Department of Health. (2011). Rotavirus Laboratory Case Definition (LCD). Communicable Disease Surveillance. October 2016, Retrieved from http://www.health.gov.au/internet/main/publishing.nsf/content/cda-phlncd-rotavirus.htm

UNICEF (2014). Diarrhoea remains a leading killer of young children, despite the availability of a simple treatment solution. Retrieved May 2015, from http://www.data.unicef.org/child-health/diarrhoeal-disease

UNICEF (2015). Diarrhoea remains a leading killer of young children, despite the availability of a simple treatment solution. UNICEF Data. Retrieved September 2017, from https://data.unicef.org/topic/child-health/diarrhoeal-disease/

Wamalwa, P., Njai, D. and Laving, A., (2014). *Prevalence of and factors associated with diarrhoeal disease in moderate to severely malnourished children aged 6 to 59 months at Mbagathi District Hospital*. Master of Medicine in Pediatrics and Child Health, University of Nairobi.

Wandera, E., Ouko, S. M.,Yatitch, J.,Taniguchi, K., Ichinose, Y., (2017). Variation in rotavirus vaccine coverage by sub-counties in Kenya. *Tropical Medicine and Health*, 45(9). doi: DOI 10.1186/s41182-017-0051-z

Wandera, E., Mohammad. S., Bundi, S. M., Komoto, S.,…Ichonose, Y., (2017). Impact of rotavirus vaccination on rotavirus and all-cause gastroenteritis in peri-urban Kenyan children. *Vaccine*, 35(38), 5217 - 5223. https://doi.org/10.1016/j.vaccine.2017.07.096

WHO (2008). Rotavirus Vaccines — Atlanta, Meeting Minutes Report of the Meeting Rotavirus Vaccines: Evaluating Clinical Trial Data and Guiding Future Research Atlanta, November 2007 2015, from http://www.who.int/immunization/sage/WHOatlanta_Meeting_Minutes_Final_March2008.pdf

WHO (2009). *Manual of rotavirus detection and characterization methods - Immunization, Vaccines and Biologicals*. Retrieved in June 2015 from; http://apps.who.int/iris/bitstream/10665/70122/1/WHO_IVB_08.17_eng.pdf

WHO (2010). Grading of scientific evidence - Does RV1and RV5 induce protection against rotavirus morbidity and mortality in young children both in low and

highmortality settings. Retrieved May 2015, from http://www.who.int/immunization/position_papers/rotavirus_grad_rv1_rv5_protection.pdf?ua=1

WHO (2011a). The Immunological Basis for Immunization Series Module 21: Rotavirus. Immunization, Vaccines and Biologicals. Retrieved May 2015 from, http://www.who.int/immunization/documents/immunological_basis_series/en/

WHO (2011b). Zinc supplementation in the management of diarrhoea. *e-Library of Evidence for Nutrition Actions.* Retrieved May 2015 from, http://www.who.int/elena/titles/zinc_diarrhoea/en/

WHO (2013a). Ending preventable child deaths from pneumonia and diarrhoea by 2025. The integrated Global Action Plan for Pneumonia and Diarrhoea (GAPPD), Retrieved May 2015 from, http://www.who.int/woman_child_accountability/news/gappd_2013/en/

WHO (2013b). Rotavirus vaccines WHO position paper – January 2013. Weekly epidemiological record(5), 49 - 64. Retrieved May 2015 from, http://www.who.int/wer/2013/wer8805.pdf?ua=1

WHO (2015, 13 April 2015). Rotavirus. Retrieved 15-5-2015, 2015, from http://www.who.int/immunization/diseases/rotavirus/en/

WHO (2016). Child Growth Standards. 2016, from http://www.who.int/childgrowth/standards/en/

WHO (2017). Diarrhea. 2018, from http://www.who.int/mediacentre/factsheets/fs330/en/

WHO (17 March 2009). Detailed Review Paper on Rotavirus Vaccines. rotavirus vaccines. 2015, from

http://www.who.int/immunization/sage/3_Detailed_Review_Paper_on_Rota_Vaccines_17_3_2009.pdf

WHO/UNICEF (2013). Ending preventable Child Deaths from Pneumonia and Diarrhoea by 2025. The intergrated Global Action plan for pneumonia and Diarrhoea. Retrieved in May 2016 from, http://www.who.int/maternal_child_adolescent/documents/global_action_plan_pneumonia_diarrhoea/en/

Widdowson, A., Steele, D., Vojdani, J., Wecker, J. and Parashar, U., (2009), Global rotavirus surveillance: Determining the need and measuring the impact of rotavirus vaccines. *The Journal of Infectious Diseases*, 2009. 200 Suppl 1: p. S1-8.

Wolf, J., Prüss-Ustün, A., Cumming, O.,Bartram, J., Bonjour, S.,… Higgins, J.,(2014), Assessing the impact of drinking water and sanitation on diarrhoeal disease in low- and middle-income settings: systematic review and meta-regression. *Tropical Medicine and International Health*. 19(8): p. 928 - 942. doi: 10.1111/tmi.12331.

Yen, C., Jakob, K., Esona, D., Peckhan, X., Rausch, J.,…LaRussa,(2011), Detection of fecal shedding of rotavirus vaccine in infants following their first dose of pentavalent rotavirus vaccine. *Vaccine*. 29(24): p. 4151-4155. doi: 10.1016/j.vaccine.2011.03.074

APPENDICES

Appendix A: Consent Form - (English)

STUDY TITLE: ROTAVIRUS ASSOCIATED DIARRHOEA AMONG CHILDREN, WITH ACUTE DIARRHOEAL ILLNESS AT MOI TEACHING AND REFERRAL HOSPITAL IN THE CONTEXT OF ROTAVIRUS VACCINATION

Name of Principal Investigator(s): Dr. Cecilia K. Kiilu

Supervisors: Dr. Irene Marete

Dr**.** Edith Apondi

Moi university, Eldoret School of Medicine

This form has been designed to be read and filled by the guardians or parents of the participants of this study

This Informed Consent Form has two parts:

- Information Sheet – to provide an explanation of the study and its purpose.
- Certificate of Consent – to append your signature if you agree to your child's participation.

Part I: Information Sheet

Introduction:

You are being requested to allow your child to be included as a participant in this study. The information given below will provide a brief explanation about the study scope and purpose. Questions and clarifications are welcome. In the event you agree for your child to participate in this study, a copy of the signed form will be issued to you.

Participation in this study is voluntary and will not alter the quality of services offered to you. You are free to drop out of the study at any time during the study or after and one is free to request any information given to be destroyed and hence not to be used in the study. In case there is any new pertinent information regarding the study and its effect to you, you shall be notified.

Purpose of the study:

The purpose of the study is to find out the number of children with rotavirus associated diarrhoea and what stands out about their characteristics both socio-demographic and clinical.

Type of Research Project/Intervention:

There will be a questionnaire administered to you and a subsequent stool test carried out on the child's stool, at the bed side and the results issued to you and the child's clinician.

Why has my child been identified to Participate in this study?

Your child has been selected since he/she has an acute diarrhoeal illness and is aged two years or below. This group is commonly affected by diarrhoea in our country.

How long will the study last?

You will be in this study only during this period of interaction.

What will happen to my child and me during the study?

During this study, you will be asked questions on the questionnaire and your child will be examined and his/her stool tested for rotavirus. The stool sample shall be disposed appropriately immediately after the test has been carried out.

We are requesting your participation to help us learn more about rotavirus associated diarrhoea in our region.

The questions asked shall be regarding your child and the family socio-economic situation. There is also a section on the environmental characteristics of your home. These questions are private and shall be asked in a private environment. Information gathered shall be kept confidential and your identity will not be written on any of the forms nor revealed.

There are no risks or side effects to participating in the study.

Benefits to taking part in the study

a) The possible benefits to you from this study are the free rotavirus stool test
b) There are no financial benefits or gifts offered on participation.
c) The possible benefits to society may include identification of the gaps in prevention of diarrhoea in children and advice on interventional areas.

Contacts:

In case of any question or clarifications please contact – Dr. Cecilia Kiilu – 0720809106 – principle investigator

OR Supervisors – Dr Irene Marete - 0720458695 and Dr Edith Apondi - 0721818157

You may contact Institutional Review Ethics Committee (IREC) 053 33471 Ext.3008. IREC is a group of people that reviews studies for safety and to protect the rights of study subjects.

Privacy and confidentiality of information:

All reasonable efforts will be made to keep your protected information (private and confidential. Protected Information is information that is, or has been, collected or maintained and can be linked back to you. Using or sharing ("disclosure") of such information must follow National privacy guidelines. By signing the consent document for this study, you are giving permission ("authorization") for the uses and disclosures of your personal information. A decision to take part in this research means that you agree to let the research team use and share your Protected Information as described below.

As part of the study, Dr. Cecilia Kiilu and her study team may share the results of your information provided in the questionnaire. These may be study or non-study related. They may also share portions of your medical record, with the groups named below:

- The National Bioethics. Committee,
- The Institutional Review and Ethics Committee,
- The supervisors of this study.

The study results will be retained in your research record for at least six years after the study is completed. At that time, the research information not already in your medical record will be disposed by incineration. Any research information entered into your medical record will be kept indefinitely.

Unless otherwise indicated, this permission to use or share your Personal Information does not have an expiration date. If you decide to withdraw your permission, we ask that you contact Dr. Cecilia Kiilu in writing and let her know that you are withdrawing your permission. The mailing address is P. O. BOX 4606, ELDORET. At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for the purposes of reporting and research quality.

Your treatment, payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part. You will receive a copy of this form after it is signed.

Part II: Consent of Subject:

I have read or have had read to me the description of the research study. The investigator or his/her representative has explained the study to me and has answered all of the questions I

have at this time. I have been told of the potential risks, discomforts and side effects as well as the possible benefits (if any) of the study. I freely volunteer to take part in this study.

_____	_____	_____
Participant's parent	Signature of parent or Guardian	Date & Time
_____	_____	_____
Name of Representative/Witness	Relationship to Subject	Date and Time
_____	_____	_____
Name of person Obtaining Consent	Signature of person Obtaining Consent	Date and Time
_____	_____	_____
Printed name of Investigator	Signature of Investigator	Date and time

Appendix B: Consent Form - (Kiswahili)

FOMU YA IDHINI:

KICHWA: ROTAVIRUS ASSOCIATED DIARRHOEA AMONG CHILDREN, WITH ACUTE DIARRHOEAL ILLNESS AT MOI TEACHING AND REFERRAL HOSPITAL IN THE CONTEXT OF ROTAVIRUS VACCINATION

MTAFITI MKUU: Dr. Cecilia K. Kiilu

WASIMAMIZI: Dr. Irene Marete

Dr**.** Edith Apondi

Moi university, Eldoret School of Medicine

Fomu hii imetayarishwa kwa nia ya kusomwa na kujazwa na wazazi au wasimamizi wa watoto wanaohusika katika utafiti huu.

Fomu hii ina sehemu mbili:

- Sehemu ya maelezo.
- Sehemu ya makubaliano na sahihi.

Sehemu 1: maelezo:

Unaombwa kutoa ruhusa kwa mtoto wako kuhusika katika utafiti huu. Maelezo yafuatayo yataweza kufafanua minaajili ya utafiti huu. Unaruhusiwa kuuliza swali au maelezo zaidi kwa ufafanuzi zaidi. Iwapo utakubali kuhusishwa katika utafiti huu utakabidhiwa na nakala ya fomu hii. Kuhusishwa kwa mtoto wako katika utafiti huu ni kwa hiari. Unaweza kusitisha kuhusishwa katika utafiti huu kwa wakati wowote. Huduma ya afya unayopata haitabadilishwa kwa vyocyote vile.

Ikiwa kutakuwa na ugunduzi wowote unaoweza kukufaidi au kukudhuru katika utafiti huu, basi tutawasiliana nawe kukufahamisha.

Madhumuni ya utafiti huu:

Utafiti huu unafanywa kwa minaajili ya kutambua idadi ya watoto wanaougua na ugonjwa wa kuendesha unaosababishwa na virusi vya rotavirus na kutambua jinsia yao ya kifedha, kisociologia na kiafya.

Aina ya utafiti:

Katika utafiti huu, utaulizwa maswali na majibu yako kuandikwa katika fomu ya maswali. Sampuli ya kinyesi cha mtoto wako itapimwa kutambua virusi vya rotavirus. . Sampuli hiyo haitahifadhiwa ila itatupwa kwa jinsi inayostahili.

Sababu ya kuchaguliwa kwa mtoto wako:

Mtoto wako amechaguliwa kwa sababu ako katika umri wa miaka miwili kuenda chini na ana ugonjwa wa kuendesha. Watoto katika umri huu ndio hupata ugonjwa wa kuendesha kwa wingi katika nchi hii yetu.

Utafiti huu utaendeshwa kwa muda wa miezi sita. Mtoto wako atahesabika katika utafiti huu kwa leo tu

Katika muda huu, utaulizwaa maswali na mtoto kufanyiwa kipimo cha rotavirus. Maswali hayo yatahusisha sehemu ya makaazi, unapotoa maji ya matumizi na pia mapato yako ya kifedha kwa mwezi.

Maswali haya ni ya siri na yataulizwa katika chumba kilicho tengwa kuwezesha usiri huu kudumishwa. Habari zote zutakazopatikana katika utafiti huu hazitafichua jina au chochote kinachoweza kufanya wewe au mtoto huyu kutambulike kwa njia yoyote.

Hakutakuwa na faida ya moja kwa moja ya kushiriki katika utafiti huu bali na matokeo ya kipimo hicho cha rotavirus.Kushiriki katika utafiti huu ni kwa hiari na una uhuru wa kusitisha kushiriki kwa utafiti huu kwa wakati wowote na kuondoka.

Hakuna hatari yoyote katika kuhusika kwa utafiti huu. Weka sahihi au alama yoyote ya kuonyesha kwamba umekubali kuhusika katika utafiti huu.

Manufaa ya kuhusishwa katika utafiti huu:

a) Kipimo cha bure cha rotavirus.
b) Hakuna malipo yoyote.
c) Jamii yetu kwa jumla itafaidika kutokana na habari itakayopatikana katika utafiti huu.

Mawasiliano:

Ikiwa ungependa kuwasiliana na mtafiti mkuu au wasimamizi wake, wasiliana nao kupitia sime nambari zao zikiwa:

Dr. Cecilia Kiilu – 0720809106 – mtafiti mkuu OR wasimamizi – Dr Irene Marete - 0720458695 and Dr Edith Apondi - 0721818157

Unaweza kuwasiliana na shirika la Institutional Review Ethics Committee (IREC) 053 33471 Ext.3008 ambalo linatetea na kuhakikisha haki za mhusika yeyote katika utafiti huu hazijadhulumiwa.

Usiri wa habari zinazopokelewa:

Habari zozote utakazotoa katika utafiti huu zitawekwa kwa usiri mkubwa. Habari zote zutakazopatikana katika utafiti huu hazitafichua jina au chochote kinachoweza kufanya wewe au mtoto huyu kutambulike kwa njia yoyote. Habari hiyo itaweza tu kutolewa kwa wasimamizi, shirika la IREC na shirika la National Bioethics Committee.

Kutolewa kwa habari hiyo itafuata sheria iliyowekwa na National privacy guidelines.

Kuweka sahihi katika fomu hii inatukabidhi ruhusa kutumia habari unazotupatia kwa jinsi tuluoieleza hapa.

Ugunduzi wa utafiti huu, utawekwa kwa muda wa miaka sita. Baada ya muda huu, habari hiyo itawezwa kuchomwa kwa usiri. Habari iliyo katika maktaba ya hospitali inayohusu matibabu na ugonjwa wa mtoto zitaendelea kuwekwa katika maktaba hiyo, kwa muda usiojulikana.

Ikiwa utaamua kujitoa katika utafiti huu, tafadhali wasiliana na mtafiti mkuu Dr. Cecilia K. Kiilu kwa sanduku la posta 4606 eldoret. Tutasitisha kuchukua habari zaidi kwako.Abari utakayokuwa umetupatia itatumika kwenye utafiti huu.

Huduma ya matibabu kwa mtoto wako haitasitishwa ama kubadilishwa hata usipohusika katika utafiti huu.

Sehemu 2: Makubaliano:

Mimi nimeweza kusoma na kuelewa sehemu ya maelezo ya utafiti huu.mtafiti mkuu au msaidizi wake amenieleza kinaga ubaga na kujibu maswali yangu yote. Nimeelezewa manufaa na hatari zitakkazokuwa katika utafiti huu. Nimejitolea kwa hiari kuhusishwa katika utafiti huu.

_____	_____	_____
Jina ya mzazi au msimamizi wake	Uhusiano na mtoto	Sahihi. Tarehe na saa
_____	_____	_____
Jina la anayechukua idhini	Sahihi yake	Tarehe na Saa
_____	_____	_____
Mtafiti mkuu	Sahihi yake	Tarehe na Saa

Appendix C: Questionnaire

Instructions for use:

- Fill in responses in the columns on the right.
- Special instructions written in italics:

INTERVIEWEE NUMBER:	
DATE OF INTERVIEW:	
TIME OF INTERVIEW:	
INTERVIEWERS NAME AND SIGNATURE:	

1.0 SOCIO-DEMOGRAPHIC DATA:

1.1 CHILD'S DATA:

1.1.1 DATE OF BIRTH: _____

1.1.2 GENDER: MALE ☐ FEMALE ☐

1.1.3 RESIDENCE: COUNTY_____ SUB-COUNTY_____

WARD_____ VILLAGE_____

1.2 RESPONDENTS RELATIONSHIP TO CHILD:

FATHER ☐ MOTHER ☐ GUARDIAN ☐

RELATIVE ☐ NOT RELATED ☐

2.0 CLINICAL EXAMINATION:

2.1 VITALS:

2.1.1 PULSE:_____per minute

2.1.2 RESPIRATORY RATE:_____ per minute

2.1.3 TEMPERATURE:_____degrees centigrade

2.1.4 OXYGEN SATURATION:_____%

2.1.5 BLOOD PRESSURE:_____mmhg

2.2 GROWTH AND NUTRITIONAL ASSESSMENT:

2.2.1.WEIGHT – CURRENT(grams) 2.2.2 -BIRTH WEIGHT (grams)	☐
2.2.3 HEIGHT (cm)	
2.2.4 MID UPPER ARM CIRCUMFERENCE: (cm)	
2.2.5 HEAD CIRCUMFERENCE(cm)	

2.3 DEHYDRATION ASSESSMENT:

PARAMETER	**FINDINGS**
2.3.1LEVEL OF CONSCIOUSNESS (AVPU) *(tick appropriately)*	ALERT ☐ VERBAL RESPONSE ☐ PAIN RESPONSE ☐ UNCONSCIOUS ☐
2.3.2 CAPILLARY REFILL IN SECONDS	
2.3.3 SKIN TURGOR IN SECONDS	
2.3.4 PULSE DESCRIPTION	PRESENT OR ABSENT ☐ RATE ☐ VOLUME (LOW OR NORMAL) ☐
2.3.5 EXTREMITIES TEMPERATURE *(indicate appropriate one)*	COLD ☐ WARM ☐
2.3.6 MUCOUS MEMBRANES *(indicate appropriate one)*	DRY ☐ MOIST ☐
2.3.7 FONTANELLE ASSESSMENT *(indicate appropriate one)*	SUNKEN ☐ FLAT ☐

	FULL ☐
2.3.8 SUNKEN EYES *(indicate appropriate one)*	YES ☐ NO ☐
2.3.9 ABILITY TO DRINK *(indicate appropriate one)*	YES ☐ NO ☐

3.0 DURING THIS VISIT:

3.1 DIAGNOSIS: _____

3.2 CO – MORBIDITIES: _____

3.3 OTHER CONCURRENT CHRONIC ILLNESSES:

_____

_____

3.4 ANY KNOWN CONGENITAL ANOMALIES:_____

4. PAST MEDICAL HISTORY:

4.1 NATAL HISTORY:

4.1.1 GESTATION AT BIRTH	TERM ☐ PRETERM ☐

4.2 HOSPITAL VISITS IN THE LAST SIX MONTHS:

TOTAL NUMBER OF VISITS: _____

NUMBER OF FOR VISITS PER DIAGNOSIS:	DIARRHOEAL ILLNESS _____ RESPIRATORY ILLNESS _____ FEBRILE ILLNESS _____

	OTHERS_____ *Select and indicate one appropriately*

4.3 NUMBER OF ADMISSIONS FOR DIARRHOEA SINCE BIRTH:

NUMBER OF ADMISSIONS FOR DIARRHOEAL ILLNESSES FOR THE LAST SIX MONTHS: _____

NUMBER OF HOSPITAL VISITS FOR DIARRHOEAL ILLNESS _____

4.4 PLACE OF TREATMENT SOUGHT PRIOR TO THIS VISIT FOR THIS ONGOING ILLNESS:

FACILITY:	NAME:
PUBLIC FACILITY – (Health center, Dispensary, health post, district hospital, subdistrict, MTRH)	*(Indicate the facility and the order of visits)*
PRIVATE FACILITY – (clinic, hospital, pharmacy)	
NONE	
NON CONVENTIONAL TREATMENT *(herbal, traditional home remedies,)*	

4.5 Treatment used during this diarrhoeal illness if any:

ORS ☐ ZINC ☐ HOMEMADE REHYDRATION SOLUTION ☐

INCREASED LIQUID DIET ☐ NONE ☐

(select as appropriate)

5.0 VACCINATION:

(TICK APPROPRIATELY)

5.1 CLINIC CARD PRESENT?_____

HOSPITAL RECORD verified?_____

BCG SCAR SEEN_____

5.2VACCINE	DATE of administration: *(indicate appropriately as received with a tick below)*
5.2.1 BCG	
5.2.2 OPV 0 1 2 3	
5.2.3 PENTAVALLENT 1 2 3	
5.2.4 PNEUMOCOCCAL VACCINE 1 2 3	
5.2.5 ROTAVIRUS 1 2 (if pentavalent) 3 *Indicate the type received*	
5.1.6 MEASLES 1 2	
5.1.7 VITAMIN A *Indicate number of doses given on clinic visits*	

5.1.8.OTHERS: *Specify*	

6. ENVIRONMENTAL HEALTH INDICATORS:

6.1 TYPE OF RESIDENCE: (*tick ppropriately*)

6.1.1 RENTAL:	☐
6.1.2 SELF OWNED :	☐

6.1.3 PERMANENT	☐
6.1.4 SEMI – PERMANENT	☐
6.1.5 MUD HOUSE	☐
6.1.6 MABATI HOUSE	☐
6.1.7 STREET FAMILY	☐
6.1.8 OTHERS (specify)	

6.2 NUMBER OF RESIDENTS IN THE HOUSE:_____

NUMBER OF ROOMS IN THE HOUSE:_____

6.3 WATER AND SANITATION:

6.3.1 SOURCES OF WATER: *(tick appropriately)*

SOURCE:	TREATED:		UNTREATED:
	BOILED	CHEMICAL	

WELL			
TAP			
SPRING			
WATER VENDOR			
RIVER			
RAIN WATER			

6.3.2 TOILET FACILITIES: *(tick appropriate one)*

TYPE OF FACILITIES AVAILABLE FOR RESIDENCE:

WATER CLOSET (Flush Toilet)	
LATRINE	
PORTABLE LATRINE	
IMPROVISED PIT	
NONE	

6.3.3 AREA OF DISPOSAL OF CHILD'S LAST STOOL YESTERDAY:

(tick appropriate one)

LATRINE	
SOLID WASTE (With other household waste)	
OPEN AREA	
DRAIN	
MUNICIPAL BINS	

7.0 FEEDING:

7.1 Did the child breastfeed in the last 24 hours? YES ☐ NO ☐

7.2 Did he/she take any other milk type? YES ☐ NO ☐

7.2.1 If yes, was the milk diluted? YES ☐ NO ☐

7.3 Did the child take any of the following foods?

☐

A. Grains, roots, tubers

B. Legumes and nuts ☐

C. Dairy products ☐

D. Fresh foods ☐

E. Fruits ☐

F. Vegetables ☐

G. Oils and fats ☐

7.4 Has the child ever breastfed? YES ☐ NO ☐

7.5 Age at introduction of complementary feeds_____ months

7.6 Age at weaning _____months

7.7 Age at replacement feeds _____months

8.0 FAMILY INFORMATION:

8.1.1 FAMILY TYPE: (polygamous, monogamous, polyandrous, single parent mother, single parent father, guardian)	*(indicate appropriate one)*
8.1.2 FAMILY SIZE: total number of children and parents.	

8.4 CARE ENVIRONMENT:

PLACE OF CARE: *(tick appropriate one)*

PARENTAL HOME:	
DAYCARE:	
CHILDREN'S HOME:	
OTHER CARETAKERS (neighbor, relatives)	

PRIMARY CARE TAKER*: (Parent, Guardian, House help Other children, Neighbour, Relative)* _____

9. HIV STATUS *(tick appropriate one)*

STATUS:	TREATMENT MODALITY:
POSITIVE	
SERO EXPOSED	
NEGATIVE	
UNKNOWN	

10. COMMENTS:

_____
_____
_____
_____

11. Antigen test results.

A. Positive
B. Negative

Appendix D: IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2015/108
Approval Number: 0001441

28th July, 2015

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
28 JUL 2015
APPROVED
P.O. Box 4606-30100 ELDORET

Dr. Cecilia Katuu Kiilu,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Kiilu,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Rotavirus Associated Diarrhea among Children, with Acute Diarrheal illness at Moi Teaching and Referral Hospital in the Context of Rotavirus Vaccination."

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1441*** on 28th July, 2015. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 27th July, 2016. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

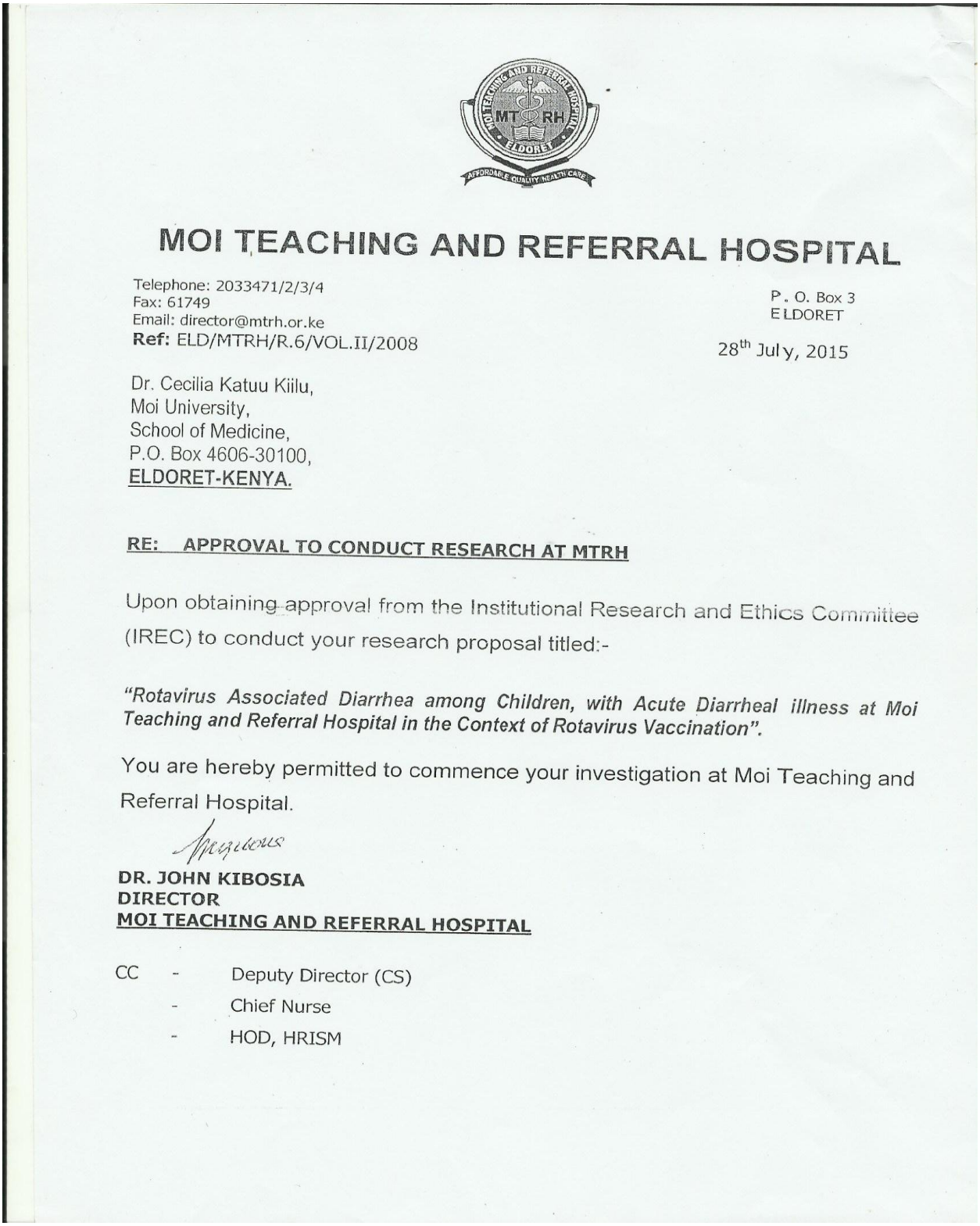
You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc Director - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix E: Hospital Permission Letter



MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3
ELDORET

28th July, 2015

Dr. Cecilia Katuu Kiilu,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Rotavirus Associated Diarrhea among Children, with Acute Diarrheal illness at Moi Teaching and Referral Hospital in the Context of Rotavirus Vaccination".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. JOHN KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

Appendix F: Ascent Form - (ENGLISH)

STUDY TITLE: ROTAVIRUS ASSOCIATED DIARRHOEA AMONG CHILDREN, WITH ACUTE DIARRHOEAL ILLNESS AT MOI TEACHING AND REFERRAL HOSPITAL IN THE CONTEXT OF ROTAVIRUS VACCINATION

Name of Principal Investigator(s): Dr. Cecilia K. Kiilu

Supervisors: Dr. Irene Marete

Dr**.** Edith Apondi

Moi university, Eldoret School of Medicine

This form has been designed to be read and filled by the guardians or parents of the participants of this study

This Informed Consent Form has two parts:

• Information Sheet – to provide an explanation of the study and its purpose.

• Certificate of Consent – to append your signature if you agree to your childs participation.

Part I: Information Sheet

Introduction:

You are being requested to allow your child to be included as a participant in this study. The information given below will provide a brief explanation about the study scope and purpose. Questions and clarifications are welcome. In the event you agree for your child to participate in this study, a copy of the signed form will be issued to you.

Participation in this study is voluntary and will not alter the quality of services offered to you. You are free to drop out of the study at any time during the study or after and one is free to request any information given to be destroyed and hence not to be used in the study. In case there is any new pertinent information regarding the study and its effect to you, you shall be notified.

Purpose of the study:

The purpose of the study is to find out the number of children with rotavirus associated diarrhoea and what stands out about their characteristics both socio-demographic and clinical.

Type of Research Project/Intervention:

There will be a questionnaire administered to you and a subsequent stool test carried out on the child's stool, at the bed side and the results issued to you and the child's clinician.

Why has my child been identified to Participate in this study?

Your child has been selected since she/he has an acute diarrhoeal illness and is aged two years or below. This group is commonly affected by diarrhoea in our country.

How long will the study last?

You will be in this study only during this period of interaction.

What will happen to my child and I during the study?

During this study, you will be asked questions on the questionnaire and your child will be examined and his/her stool tested for rotavirus. The stool sample shall be disposed appropriately immediately after the test has been carried out.

We are requesting your participation to help us learn more about rotavirus associated diarrhoea in our region. The questions asked shall be regarding your child and the family economic and situation. There is also a section on the environmental characteristics of your home. These questions are private and shall be asked in a private environment. Information gathered shall be kept confidential and your identity will not be writed on any of the forms nor revealed.

There are no risks or side effects to participating in the study.

Benefits to taking part in the study.

- The possible benefits to you from this study are the free rotavirus stool test
- There are no financial benefits or gifts offered on participation.
- The possible benefits to society may include identification of the gaps in prevention of diarrhoea in children and advice on interventional areas.

Contacts:

In case of any question or clarifications please contact – Dr. Cecilia Kiilu – 0720809106 – principle investigator OR Supervisors – Dr Irene Marete - 0720458695 and Dr Edith Apondi - 0721818157

You may contact Institutional Review Ethics Committee (IREC) 053 33471 Ext.3008. IREC is a group of people that reviews studies for safety and to protect the rights of study subjects.

Privacy and confidentiality of information:

All reasonable efforts will be made to keep your protected information (private and confidential. Protected Information is information that is, or has been, collected or maintained and can be linked back to you. Using or sharing ("disclosure") of such information must follow National privacy guidelines. By signing the consent document for this study, you are giving permission ("authorization") for the uses and disclosures of your personal information. A decision to take part in this research means that you agree to let the research team use and share your Protected Information as described below.

As part of the study, Dr. Cecilia Kiilu and her study team may share the results of your information provided in the questionnaire. These may be study or non-study related. They may also share portions of your medical record, with the groups named below:

- The National Bioethics. Committee,
- The Institutional Review and Ethics Committee,
- The supervisors of this study.

The study results will be retained in your research record for at least six years after the study is completed. At that time, the research information not already in your medical record will be disposed by incineration. Any research information entered into your medical record will be kept indefinitely.

Unless otherwise indicated, this permission to use or share your Personal Information does not have an expiration date. If you decide to withdraw your permission, we ask that you contact Dr. Cecilia Kiilu in writing and let her know that you are withdrawing your permission. The mailing address is P. O. BOX 4606, ELDORET. At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for the purposes of reporting and research quality.

Your treatment, payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part. You will receive a copy of this form after it is signed.

Part II: Consent of Subject:

I have read or have had read to me the description of the research study. The investigator or his/her representative has explained the study to me and has answered all of the questions I have at this time. I have been told of the potential risks, discomforts and side effects as well as the possible benefits (if any) of the study. I freely volunteer to have my child take part in this study.

_____	_____	_____
Participant's parent	Signature of parent or Guardian	Date & Time

_____	_____	_____
Parent or guardian of participant's parent	Signature:	Date and time:
_____	_____	_____
Name of person Obtaining Consent	Signature of person obtaining Consent	Date and Time
_____	_____	_____
Printed name of Investigator	Signature of Investigator	Date and time

Appendix G: Ascent Form - (KISWAHILI)

FOMU YA IDHINI:

KICHWA: ROTAVIRUS ASSOCIATED DIARRHOEA AMONG CHILDREN, WITH ACUTE DIARRHOEAL ILLNESS AT MOI TEACHING AND REFERRAL HOSPITAL IN THE CONTEXT OF ROTAVIRUS VACCINATION

MTAFITI MKUU: Dr. Cecilia K. Kiilu

WASIMAMIZI: Dr. Irene Marete

Dr**.** Edith Apondi

Moi university, Eldoret School of Medicine

Fomu hii imetayarishwa kwa nia ya kusomwa na kujazwa na wazazi au wasimamizi wa watoto wanaohusika katika utafiti huu.

Fomu hii ina sehemu mbili:

- Sehemu ya maelezo.
- Sehemu ya makubaliano na sahihi.

Sehemu 1: maelezo:

Unaombwa kutoa ruhusa kwa mtoto wako kuhusika katika utafiti huu. Maelezo yafuatayo yataweza kufafanua minaajili ya utafiti huu. Unaruhusiwa kuuliza swali au maelezo zaidi kwa ufafanuzi zaidi. Iwapo utakubali kuhusishwa katika utafiti huu utakabidhiwa na nakala ya fomu hii. Kuhusishwa kwa mtoto wako katika utafiti huu ni kwa hiari. Unaweza kusitisha kuhusishwa katika utafiti huu kwa wakati wowote. Huduma ya afya unayopata haitabadilishwa kwa vyocyote vile.

Ikiwa kutakuwa na ugunduzi wowote unaoweza kukufaidi au kukudhuru katika utafiti huu, basi tutawasiliana nawe kukufahamisha.

Madhumuni ya utafiti huu:

Utafiti huu unafanywa kwa minaajili ya kutambua idadi ya watoto wanaougua na ugonjwa wa kuendesha unaosababishwa na virusi vya rotavirus na kutambua jinsia yao ya kifedha, kisociologia na kiafya.

Aina ya utafiti:

Katika utafiti huu, utaulizwa maswali na majibu yako kuandikwa katika fomu ya maswali. Sampuli ya kinyesi cha mtoto wako itapimwa kutambua virusi vya rotavirus.. Sampuli hiyo haitahifadhiwa ila itatupwa kwa jinsi inayostahili.

Sababu ya kuchaguliwa kwa mtoto wako:

Mtoto wako amechaguliwa kwa sababu ako katika umri wa miaka miwili kuenda chini na ana ugonjwa wa kuendesha. Watoto katika umri huu ndio hupata ugonjwa wa kuendesha kwa wingi katika nchi hii yetu.

Utafiti huu utaendeshwa kwa muda wa miezi sita. Mtoto wako atahesabika katika utafiti huu kwa leo tu

Katika muda huu, utaulizwaa maswali na mtoto kufanyiwa kipimo cha rotavirus. Maswali hayo yatahusisha sehemu ya makaazi, unapotoa maji ya matumizi na pia mapato yako ya kifedha kwa mwezi.

Maswali haya ni ya siri na yataulizwa katika chumba kilicho tengwa kuwezesha usiri huu kudumishwa. Habari zote zutakazopatikana katika utafiti huu hazitafichua jina au chochote kinachoweza kufanya wewe au mtoto huyu kutambulike kwa njia yoyote.

Hakutakuwa na faida ya moja kwa moja ya kushiriki katika utafiti huu bali na matokeo ya kipimo hicho cha rotavirus.Kushiriki katika utafiti huu ni kwa hiari na una uhuru wa kusitisha kushiriki kwa utafiti huu kwa wakati wowote na kuondoka.

Hakuna hatari yoyote katika kuhusika kwa utafiti huu. Weka sahihi au alama yoyote ya kuonyesha kwamba umekubali kuhusika katika utafiti huu.

Manufaa ya kuhusishwa katika utafiti huu:

- Kipimo cha bure cha rotavirus.
- Hakuna malipo yoyote.
- Jamii yetu kwa jumla itafaidika kutokana na habari itakayopatikana katika utafiti huu.

Mawasiliano:

Ikiwa ungependa kuwasiliana na mtafiti mkuu au wasimamizi wake, wasiliana nao kupitia sime nambari zao zikiwa:

Dr. Cecilia Kiilu – 0720809106 – mtafiti mkuu OR wasimamizi – Dr Irene Marete - 0720458695 and Dr Edith Apondi - 0721818157

Unaweza kuwasiliana na shirika la Institutional Review Ethics Committee (IREC) 053 33471 Ext.3008 ambalo linatetea na kuhakikisha haki za mhusika yeyote katika utafiti huu hazijadhulumiwa.

Usiri wa habari zinazopokelewa:

Habari zozote utakazotoa katika utafiti huu zitawekwa kwa usiri mkubwa. Habari zote zutakazopatikana katika utafiti huu hazitafichua jina au chochote kinachoweza kufanya wewe au mtoto huyu kutambulike kwa njia yoyote. Habari hiyo itaweza tu kutolewa kwa wasimamizi, shirika la IREC na shirika la National Bioethics Committee.

Kutolewa kwa habari hiyo itafuata sheria iliyowekwa na National privacy guidelines.

Kuweka sahihi katika fomu hii inatukabidhi ruhusa kutumia habari unazotupatia kwa jinsi tuluoieleza hapa.

Ugunduzi wa utafiti huu, utawekwa kwa muda wa miaka sita. Baada ya muda huu, habari hiyo itawezwa kuchomwa kwa usiri. Habari iliyo katika maktaba ya hospitali inayohusu matibabu na ugonjwa wa mtoto zitaendelea kuwekwa katika maktaba hiyo, kwa muda usiojulikana.

Ikiwa utaamua kujitoa katika utafiti huu, tafadhali wasiliana na mtafiti mkuu Dr. Cecilia K. Kiilu kwa sanduku la posta 4606 eldoret. Tutasitisha kuchukua habari zaidi kwako.Abari utakayokuwa umetupatia itatumika kwenye utafiti huu.

Huduma ya matibabu kwa mtoto wako haitasitishwa ama kubadilishwa hata usipohusika katika utafiti huu.

Sehemu 2: makubaliano:

Mimi nimeweza kusoma na kuelewa sehemu ya maelezo ya utafiti huu.mtafiti mkuu au msaidizi wake amenieleza kinaga ubaga na kujibu maswali yangu yote. Nimeelezewa manufaa na hatari zitakkazokuwa katika utafiti huu. Nimejitolea kwa hiari kuhusishwa katika utafiti huu.

_____	_____	_____
Jina ya mzazi au msimamizi wake	Uhusiano na mtoto	Sahihi. Tarehe na saa
_____	_____	_____
Jina la mzazi au msimamizi wa mzazi wa mtoto	Sahihi	Tarehe na saa:
_____	_____	_____
Jina la anayechukua idhini	Sahihi yake	Tarehe na Saa
_____	_____	_____
Mtafiti mkuu	Sahihi yake	Tarehe na Saa

Appendix H: Bristol Stool Chart

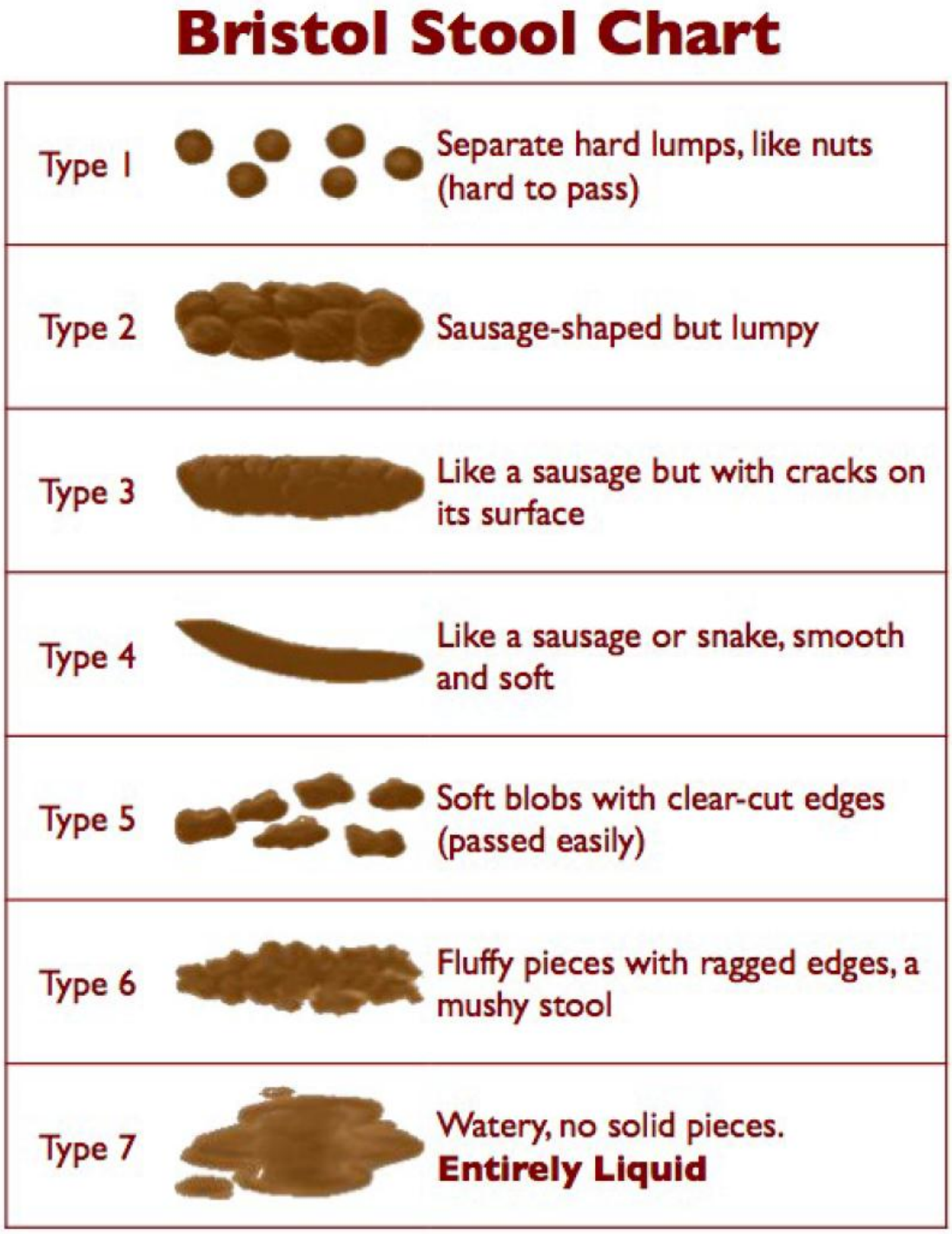


Figure 1: Bristol stool chart(S. J. Lewis, 1997).

Appendix I: Rotavirus Structure

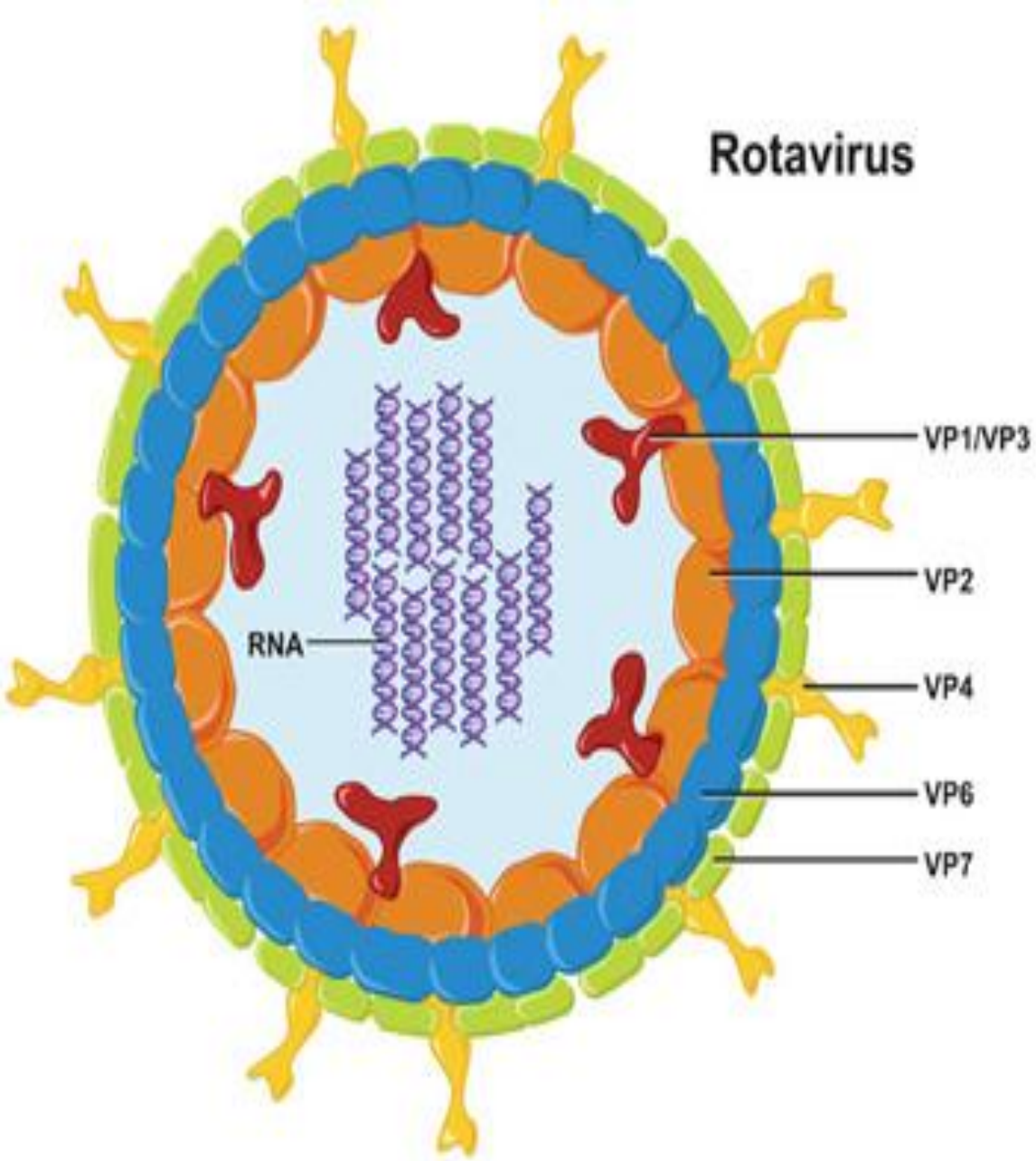


Figure 2: structure of the Rotavirus (NIH, 2010)

Appendix J: Laboratory Procedures

J.1 Stool sample collection method

The participants diaper shall be lined with a plastic wrapper before he/she has passed any stool. Once he/she has passed stool the sample shall be collected into a poly pot for stool collection using a sterile wooden collection stick and gloved hands. For this study a standard wrapped tongue depressor shall be used. About 3 ml of liquid stool shall be collected. Out of this amount about two drops shall be mixed with the test solution provided and the remaining amount stored in the fridge.

J.2 Rotavirus antigen detection test(BIOTEC, 2009)

This test literature has been adopted from the Manufacturer's instructions on CERTEST (BIOTEC, 2009).

Principle of the test: The test to be used is a qualitative immunochromatographic assay. It is used to determine the presence of rotavirus in stool by assessment of the antigen. The membrane at the test kit is coated with monoclonal antibodies on the test band region, against viral antigens. Once the sample reacts with the colored conjugate (red microspheres for the rotavirus), there is a noted color change. The sample moves up the membrane via capillary action, the colored particles migrate and a red line appears in conjunction with a green line that is the control (BIOTEC, 2009).

Specimen collection and preparation: One requires 1ml of fluid diluents and a small quantity of stool. The stool sample is collected in clean containers and the test done immediately after collection. If test is not immediately available, then the sample can be collected and refrigerated at 2-4^{0}C for a day prior to testing. For storage beyond 1 day, refrigeration should be at -20^{0}C. The sample is then thawed to room temperature then tested. Once collected, the small stool sample is mixed with 1ml of diluents, shaken to get good dispersion then a drop of the mixture placed on the test kit sample region.(BIOTEC, 2009)

Reading the results: The color change can vary in intensity depending on viral concentration.**Positive -** two lines, one green and another red, appear for the control and positive rotavirus respectively. **Negative –** one green line appears for the control only. Any other form of results is invalid (BIOTEC, 2009).

Handling the test kit: Test must be done in 2 hours or less after opening the pack. One should also not put a large quantity of stool since it could interfere with the test results by giving additional brown bands (BIOTEC, 2009).

Sensitivity and Specificity: According to the manufacturer, sensitivity is at 100% and specificity at 98%.(BIOTEC, 2009)

J.3 Special considerations:

Due to the highly infectious nature of the rotavirus, strict adherence to protection during handling of the samples shall be employed. These are:

- Use of protective clothing by the individual collecting the stool sample and carrying out the test. These shall be in form of a lab coat and a pair of clean gloves to be discarded after single use for each participant sample collection and testing.
- The surfaces being used shall be cleaned with soap and jik water and disinfected with surface disinfectant before and after every test procedure.
- There shall be thorough hand washing before and after every patient interaction both by the principal investigator and the research assistant.
- Disposal of the waste material shall be done according the infection control protocols set out by the hospital, with all the waste being disposed in the highly infectious waste red colored container.