CHARACTERIZATION OF ROTAVIRUS AMONG CHILDREN UNDER FIVE YEARS WITH GASTROENTERITIS AT KENYATTA NATIONAL HOSPITAL, KENYA

JEREMIAH OMARI ZABLON

MASTER OF SCIENCE

(Molecular Medicine)

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AGRICULTURE AND TECHNOLOGY

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Characterization of Rotavirus among Children under five Years with Gastroenteritis at Kenyatta National Hospital, Kenya

Jeremiah Omari Zablon

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature…… Date……

Jeremiah Omari Zablon

This research thesis has been submitted for examination with our approval as supervisors.

Signature…… Date……

Dr. Raphael Lihana, PhD

KEMRI, Kenya.

Signature…… Date……

Dr. Steven Ger Nyanjom, PhD

JKUAT, Kenya.

DEDICATION

I would like to dedicate this work to my parents who have always supported me in all endeavors of my studies. My siblings for the support they have given me in the pursuit of my academic dreams

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I would like to give special thanks to God Almighty for the gift of life and opportunity to reach this level in my Academic journey.

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ABBREVIATIONS AND ACRONYMS

CDC	Centers for Diseases Control and Prevention
cDNA	Complementary DNA
CVR	Centre for Virus Research
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
EIA	Enzyme-linked Immunosorbent Assay
ERC	Ethical Review Committee
GE	Gastroenteritis
IgG	Immunoglobulin G
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
ORT	Oral Rehydration Therapy
PCR	Polymerase Chain Reaction
PBS	Phosphate Buffer Saline
RT PCR	Reverse Transcriptase Polymerase Reaction Chain
RNA	Ribonucleic Acid
RVA	Rotavirus group A
UNICEF	United Nations International Children Emergency Fund

ABSTRACT

Severe diarrhea is a common occurrence among children below the age of five years worldwide, and the major cause remains infection from rotavirus. The mortality from rotaviruses stands at an estimated 215,000 annually worldwide; 200,000 of these being in Africa alone. The main objective of this study was to characterize the strains of rotavirus among children with gastroenteritis below five years at Kenyatta National Hospital Nairobi County. The research design was cross-sectional, and the sample size was 355 participants. The study participants were identified during clinical examination by clinicians in outpatient and inpatient departments. Stool samples were collected and tested for rotavirus using Enzyme Linked Immuno-assay, one step multiplex qRT-PCR genotyping assay and whole genome sequencing using next-generation sequencing. The prevalence of rotavirus was 16.34% (58/355). Rotavirus infection among males was 10.42% (37/355) as compared to those of females 5.91% (21/355). The distribution of Rotavirus between outpatient and inpatient was 9.30% (33/355) outpatient while 7.04% (25/355) inpatient. There was no statistical significance of rotavirus infection between gender, inpatient, and outpatients. The most prevalent G-type being G1 48.3% (28/58), followed by G2 22.41% (13/58), G3 15.51%% (9/58), and G9 5.17%% (3/58) with mixed infections which included G1, 2, 5.17% (3/58) and G2, 3, 1.72% (1/58). The P-type, P [8] 46.55% (27/58) was most prevalent followed by P4 24.13% (14/58) and P [6] 20.68% (12/58). There were mixed infection which includes P [4, 8] 5.17% (3/58), and P [4, 6] 1.72% (1/58). The G-P combination showed that G1 P [8] 41.37% (24/58) was more prevalent followed by G2 P [4] 22.41%, (13/55) G3 P [6] 15.51% (9/58) and G9 [P8] 5.17% (3/58). The mixed infections included G1, 2 P [4, 8] 5.17% (3/58) and G3, 2 P [4, 6] 1.72% (1/58). The study revealed the prevalence of rotavirus has decreases to 16.34% and the most prevalent genotype was G1P[8]. There was no statistical difference in rotavirus infection in regard with gender, inpatient and outpatient. Rotavirus infection affected more males than females. The recommendation of the study is to increase vaccination of rotavirus among children to reduce gastroenteritis caused by rotavirus and frequent surveillance to monitor emerging genotypes.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The gastroenteritis caused by rotavirus among children has been a global problem since it was reported in 1973 by Bishop and co-workers in Australia (Patton, 2012). The infection caused by rotavirus is common in acute gastroenteritis among young children and is associated with substantial morbidity and mortality. Besides, infection by rotavirus is the leading cause of diarrheoa in developing countries with vaccination showing to be the best method for controlling the disease (Dennehy *et al*., 2015; Kapikian, 2001). Rotavirus has eight different species, group A to H. The common rotavirus strain causing acute gastroenteritis among children below five years worldwide, is Group A rotaviruses (RV-A).

Global mortality rates caused by Rotavirus group A infection was estimated to at 453,000 in 2008 among children below five years of age annually and majority being from Africa (Tate *et al*., 2012). In 2013 the annual deaths had declined by almost half to 215,000 and majority of being from India and Africa (Tate *et al*., 2016). According to WHO (2012), Rotavirus infection in Kenya from stands at 27%. In the last decade progress has been made in research in disease burden and prevalence of rotavirus hence the decline in mortality rates. Rotavirus vaccines available in the market and recommended by World Health Organization (WHO) include; monovalent Rotarix (GlaxoSmithKline Biologicals, Belgium) and the pentavalent RotaTeq (Merck, USA). The vaccines have been introduced to most national immunization programs as recommended by WHO 2009 (Mason *et al*., 2013). In July 2014, monovalent Rotarix was introduced in Kenya national immunization program (Wandera *et al*., 2017).

The introduction of the Rotavirus vaccine in North America, specifically in the United States, saw a sharp decline in hospitalization rates (Tate *et al*., 2011). The programs which are done by public health in the USA to monitor gastroenteritis, focus on the provision of oral rehydration therapy for those children who test positive for rotavirus

infection, while prevention of the disease is through the administration of vaccines (Diggle, 2007). The prevalence of rotavirus has declined in countries that have introduced rotavirus vaccine into their routine childhood immunization requirements (Jiang *et al*., 2010). Infection by rotavirus causes inflammation of the gastrointestinal tract increasing fluids in the small intestine. The buildup of fluids then leads to acute diarrhea and vomiting (Tate *et al*., 2016). The incubation period after infection is about two days; vomiting often will follow the onset of acute symptoms and up to eight days of unrelenting diarrhea which leads to dehydration. The fecal-oral route is key to the transmission of the Rotavirus mainly as a result of contaminated hands, surfaces and objects (Dennehy *et al*., 2000; Tate *et al*., 2016), the study will be nested in the World Health Organization surveillance program at Kenyatta National Hospital. The surveillance program by WHO started in 1997.

1.2 Statement of the problem

There is increased death among children below five years old as a result of diarrhea, which is mostly due to rotavirus. Most children in the world are exposed to rotavirus infection at least once by the time they reach five years of age (Bernstein, 2009). This results in developing immunity, thus reducing the severity of the disease in subsequent infections. There is a high burden of hospitalization due to rotavirus infection, which strains the limited facilities in developing countries with restricted resources (Tate *et al*., 2009). Management of rotavirus disease is often attained by access to medical care, this however remains a great challenge in Kenya. Rotavirus infection results both in high mortality and morbidity rates in Kenya. Moreover, hospitalizations due to rotavirus put the strain on already scarce resources which could be used to manage other conditions. In general, time spent on care of patients has resulted in a significant loss to the economy of the country. (Tate *et al*., 2011). Characterization of rotavirus will assist in designing future vaccines as well as allowing improvement of the current vaccine through determination of circulating genotypes of rotavirus. These are due to the serotypes- specific immunity which plays a role in protection against the rotavirus

infection (Parashar *et al.*, 1998). Precisely, the infection caused by Rotavirus group A (RVA) in Kenya is estimated to cause 8,000 deaths and 27% hospitalizations due to RVA among children below five years (WHO, 2016).

1.3 Justification of the Study

The reduction and prevention of rotavirus will reduce mortality, reduce hospital burden due to RVA and reduce working hours spent in taking care of sick children due to rotavirus. Following the introduction of rotavirus vaccine in July 2014 by the Ministry of Health in Kenya, there is a need to conduct studies on genotypes circulating post vaccination among children with symptoms of gastroenteritis (Wandera *et al*., 2017). Furthermore, the prevalence of rotavirus genotype needs to be monitored whether there are emerging genotypes against the current vaccine is less effective (Tate *et al*., 2016). The study also provided information as to whether the vaccine helps in reducing the prevalence of diarrhea infections and other related infections among the children to reduce cases of hospital admissions. Rotavirus genotypes change through the accumulation of point mutation and genetic re-assortments leading to the emergence of a new genotype. This study will help determine genotypes circulating post vaccination will help determine the most prevalent genotype and whether if the serotype antigens are included in the current vaccine which will determine the efficacy of the vaccine. This study is nested in the WHO rotavirus surveillance program in order to determine the prevalence and the impact of the vaccine.

1.4 Research Questions

1. What is the prevalence of rotavirus among children with gastroenteritis at Kenyatta National Hospital?
2. What are the demographic characteristics of children suffering from Rotavirus infection at Kenyatta National Hospital?
3. Which are the genotypes of rotavirus circulating among children with gastroenteritis at Kenyatta National Hospital?

4. What is the phylogenetic relationship of Rotavirus genotypes at Kenyatta National Hospital?

1.5 Objectives

1.5.1 General Objective

To characterize rotavirus strains among children under five years with gastroenteritis at Kenyatta National Hospital in Nairobi, Kenya.

1.5.2 Specific Objectives

1) To determine the prevalence of rotavirus among children under five years suffering from gastroenteritis in Kenyatta National Hospital.
2) To determine the demographic characteristics of children suffering from Rotavirus in Kenyatta National Hospital.
3) To determine Rotavirus genotypes among children under five years suffering from gastroenteritis in Kenyatta National Hospital.
4) To determine the phylogeny relationship of Rotavirus genotypes in Kenyatta National Hospital.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of Rotavirus

Rotaviruses are non-enveloped double-stranded RNA (dsRNA) virus and belongs to the family *Reoviridae*, and is the principal agent in gastroenteritis, which causes diarrhea among infants (Parashar *et al*., 2003). It is characterized by segmented double-stranded RNA (dsRNA) genomes enclosed together with multi-shelled virus particles (Kapikian *et al*., 1996). The virus particles have a diameter of up to 76.5nm (Patton, 1995). Rotavirus has been grouped to ten different species (A-J) based on the antigenic difference of VP6. Rotavirus group A is common cause of infection among children and it is classified to different genotypes as per RNA segments of 7 and 4 (VP7 and VP4 respectively). Rotavirus classification uses dual structural proteins for classification of the glycoprotein VP7 and the protease-sensitive protein VP4. These define the G serotype and P serotypes respectively and are found on the surface (Patton *et al*., 1995). There has been 28 P- types and G-types which have been identified worldwide (Nokes *et al* 2010; Santos *et al* 2005. The genotyping of Rotavirus using all eleven genomic segments has been created using whole genome nomenclature following Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, which collate with the following genes VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5,respectively (Matthijnssens *et al*.,2012).

2.2 Genome organization of Rotavirus

Rotavirus has 11 genome segments which include structural proteins VP1, VP2, VP3, VP4, VP6 and VP7, and nonstructural protein NSP1to NSP5 (Matthijnssens *et al*., 2008). The viral structural proteins VP1, VP2, VP3, VP4, VP6, and VP7 constitute the rotavirus genome (figure 2.1) and they are part of the structural proteins that form the virion (the virus particle). The cells infected by Rotavirus produce nonstructural proteins which include NSP1, NSP2, NSP3, NSP4, and NSP5.The proteins in a mature virus particle determine host specificity, cell entry and enzymatic activity required by the virus. The protein VP1 at the core of the virus particle is an RNA polymerase while VP2

binds the RNA genome and forms the core layer of the virion (Tate *et al*., 2012). The viral capping enzyme VP3 (guanylyltransferase) is used in the post-translation modification of mRNA catalyzes the process that forms the 5' cap. The molecules that bind to surface cells are bound by VP4 and are called drives and receptors that assist the virus to gain entry into the cell. Also, a protein that is highly antigenic and used for identification of the virus is VP6. The structure protein VP7 is a glycoprotein that is involved in immunity to infection (Desselberger, 2014). The protein NSP3 which is bound to viral mRNA which plays the role of shutting down cellular protein synthesis. The NSP4 which is a viral enterotoxin induces diarrhea. Consequently, genome segment 11 of rotavirus A and virus-infected cells encode the NSP5 which then piles up in the viroplasm (Prasad and Chiu, 1994).

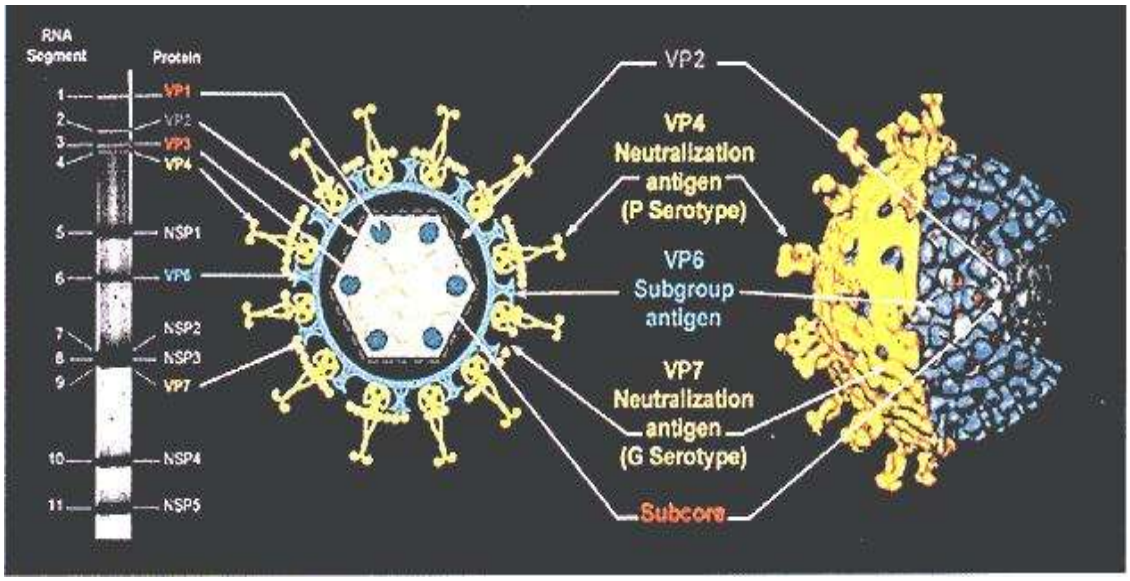


Figure 2.1: Structure of Rotavirus *(*Parashar *et al.*, 1998*).*

2.3 Global Prevalence of Rotavirus

The infection of rotavirus usually occurs globally among children from the age of 3 to 5 years, in 2003 rotavirus infected 114 million children globally of which 24 million outpatient and 2.3 hospitalization cases. The infection due to rotavirus in 2013 that occurred in India was 47,100 which accounted for 22% of the global deaths due to Rotavirus. The mortality rates due to rotavirus in the same year in four countries like India, Nigeria, Pakistan, and the Democratic Republic of Congo accounted for approximately half of the global deaths due to Rotavirus (Tate *et al.,* 2016). A study published in Kenya the year 2017 the overall prevalence was 31.5%, while the prevalence among neonates was 6%, children from 13 to 24 months prevalence was 39.4% and children with 12 months were 37.2% (Odditt . *et al*. 2014; Agutu*et al*., 2017)

2.4 Geographical distribution of Rotavirus Genotypes

The distribution of rotavirus genotypes globally G1P[8], 31.2%; G2P[4], 13%;G3P[8],10.7%; G9P[8],10.2%;G4P[8],5.0% and G12P[8],2.75% (Doro *et al.,* 2014). The stains of Rotavirus of medical importance in 1990 and 2000s G9P[8] and G12P[8]. The endemic worldwide strains included G1P[8], G2P[4]G3P[8] and G4P[8] (Matthijnssens and Van Ranst, 2012). Regionally the genotypes which are prevalent and are of medical importance include G1P[8], 20.4-47.7%;G2P[4],10-29.2%;G3P[8],2.6-34.4%;G4P[8],15.5%;G9P[8],7.9%-16.6%10.2%; and G12P[8],0.1-10% (Doro *et al*., 2014).The distribution of group A rotavirus in a study conducted in Kenya from 2009 to 2017 indicated the most prevalent strains to include; G1P[8], 28% ; G9P[8], 12%; G8P[4], 7%, G1P[4], 5%; G9P[4], 4%and G12P[6], in 2012 there was a shift G9P[8] to G1 P[8 detected (Wander. *et al*. 2017; Nokes., *et al* 2011; Agutu*et al* 2017).

2.5 Laboratory Diagnosis of Rotavirus

The laboratory techniques used in detection of these viruses involve such methods as employing electron microscopy, virus isolation in cell culture , polyacrylamide gel

electrophoresis (PAGE) of the virus segment, enzyme immunoassay, immune chromatographic coupled with RT PCR and qRT-PCR (Freeman *et al*.,2008; Guatam *et al.*,2014). The methods are further discussed in details below.

2.5.1 Rotavirus Electron Microscopy

These involve the direct examination of stool samples with an electron microscope after negative staining with phosphotungstic acid (Esona and Guatam., 2015). The sample is prepared by resuspension with phosphate buffer then observed under the electron microscope. The detection limit of the virus is 10^7 particles of the virus per ml of stool (Guatam, *et al*., 2014).

2.5.2 Rotavirus Culture

Animal and human rotaviruses are successfully cultured through the use of primary and transformed monkey kidney cells or by using trypsin for proteolytic activation of the virus before infection (Esona and Guatam., 2015). Animal strains of rotavirus used as laboratory models including RRV, SA11-4F, UK, and OSU grow well in continuous cell lines, reaching titers of between 10^7 to 10^8 plaque-forming units (PFU) per milliliter of medium. The culture-adapted human rotavirus, compared to most animal strains, average titers 1- to 3-logs lower. The culture of RVs from fecal matter must employ several blind passages in primary cells before they can grow in continuous cell lines. Clinical isolates only need to be adapted to the cell culture, and they will experience a growth spurt as good as any other prototypic culture-adapted human RV strains including Wa, DS-1, ST3, and P (Arnold *et al.*, 2009).

2.5.3 Rotavirus Antigen Detection

The Enzyme-linked Immunosorbent Assay (EIA) is commonly used in Kenyatta Immunology lab (Pereira *et al*., 2011). The EIA method uses Rotavirus specific antibodies to capture rotavirus antigen onto wells of plastic plates. Beside the EIA method having a highly sensitive and specificity, it is ideal and can easily be applied in a 96-well plate format for large sample volumes (Esona and Guatam, 2015). The results which are in terms of the Optical Density (OD) can be input using a standard plate

reader and then analyzed using computer analysis programs (WHO, 2008). Latex agglutination also can be used in place of the EIA method, and here latex particles that have an Anti-Rotavirus antibodies coating are employed. In consulting rooms where rapid near-patient testing are desired, immunochromatographic methods are preferred. The importance of Rotaviruses in clinical settings has promoted the commercialization of antigen detection methods with data on the sensitivity and specificity of the different methods being readily available (WHO, 2008).

2.5.4 Molecular Identification of Rotavirus

Amplification using RT-PCR of the rotavirus genes, VP7 and VP4, has been developed for the detection of Rotavirus (Iturriza *et al*., 2008). These genes, the VP7 and VP4, are used for genotyping rotavirus strains, in particular, G-P genotypes, and are very important not only for surveillance studies to determine circulating strains but also for vaccine development (Kapikian *et al*., 1996).The method will be used in the study during identification and genotyping.

2.5.5 Electropherotyping of Rotavirus

Studies carried out on migration patterns of rotavirus genomic dsRNA segments have allowed the classification of the virus into long and short electrotypes which helps identification of Rotavirus using a gel; in a process referred to as electropherotyping (Kapikian *et al*. 1996). As this Rotavirus can be classified into different groups. Based on their gene distribution, group A, B, and C each have embossed patterns (figure 2.2) (WHO, 2008).

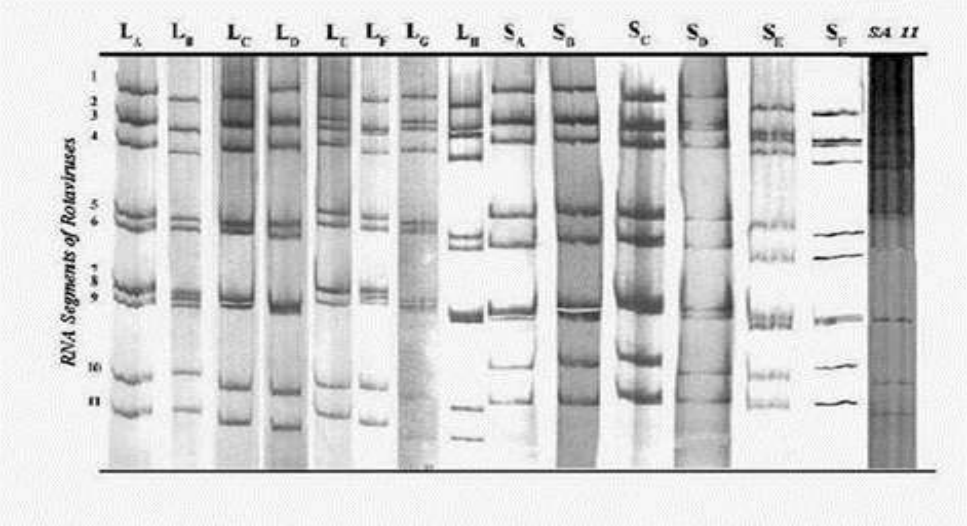


Figure 2.2: Different Migration Patterns of Rotavirus genotypes (Kapikian *at el,* 1996)

2.6 Transmission of Rotavirus

Rotavirus is highly contagious, and the infective dose is as few particles at ten particles of the virus. The virus can survive in the environment for more than ten days and on human hands four hours. The standard route of transmission of rotavirus is fecal-oral route, direct contact with the Rotavirus (Cárcamo *et al*, 2005; Lundgren and Svenson *et al.*, 2001). It can also be transmitted through fecally contaminated water or food (Steele *et al*., 2011). The improvement of hand hygiene in hospital decreases the incidences in health associated infections. Transmission via aerosol might be possible because evidence of airborne spread of Rotavirus gastroenteritis has been reported (Cárcamo *at el*, 2005; Lundgren and Svenson *et al.*, 2001).

2.7 Pathogenesis of Rotavirus

2.7.1 Viral entry and replication

The entry and replication occurs in the mature, non- dividing enterocytes in the middle and tip of the villi enteroendocrine of small intestines (Cárcamo *et al.*, 2005; Lundgren and Svenson *et al.*, 2001). When the rotavirus enters the host cell like the epithelial cells,

an interaction occurs of virions with cell surface molecules from the host cell. The virus VP4 protein attaches to the epithelial cell lining in the gut (Haselhorst *et al*., 2009) and while the spikes from VP4 and VP7 are shed, the rest of the particles enter the cell cytoplasm converting the triple layer to a double layer particle (Lawton *et al*., 1997). The double layer becomes transcriptionally active producing positive-strand RNAs (mRNAs) which acts as templates in synthesizing new dsRNA genomic segments (Lopez *et al*., 2005). The virus-encoded structural and nonstructural proteins accumulate and lead to the formation of viroplasms and contributing to dsRNA synthesis and virus replication. The newly formed double layer assembles into endoplasmic reticulum lumen where it acquires the envelope. The envelope later is replaced by the outer layer of VP4 and VP7 (Calderon *et al*., 2012). The release of the mature virions from infected cells occurs by cell lysis or a non-classical Golgi apparatus- independent, vesicular transport pathway (Lopez *et al*., 2005). The new infection cycle starts again with the new viruses infecting healthy cells (Matthijnssens *et al*., 2008). The infected epithelial cells secrete chloride due to the simulation by the enterotoxin, NSP4. These lead to increased water secretion into the gut along the concentration gradient (Lundgren and Svenson *et al.*, 2001; Richardson *et al*., 1998). The lysis of infected epithelial cells and fluids from the stomach fill the gut. The exit from the gut results in diarrhea (Calderon *et al*., 2012) (Fig 2.3).

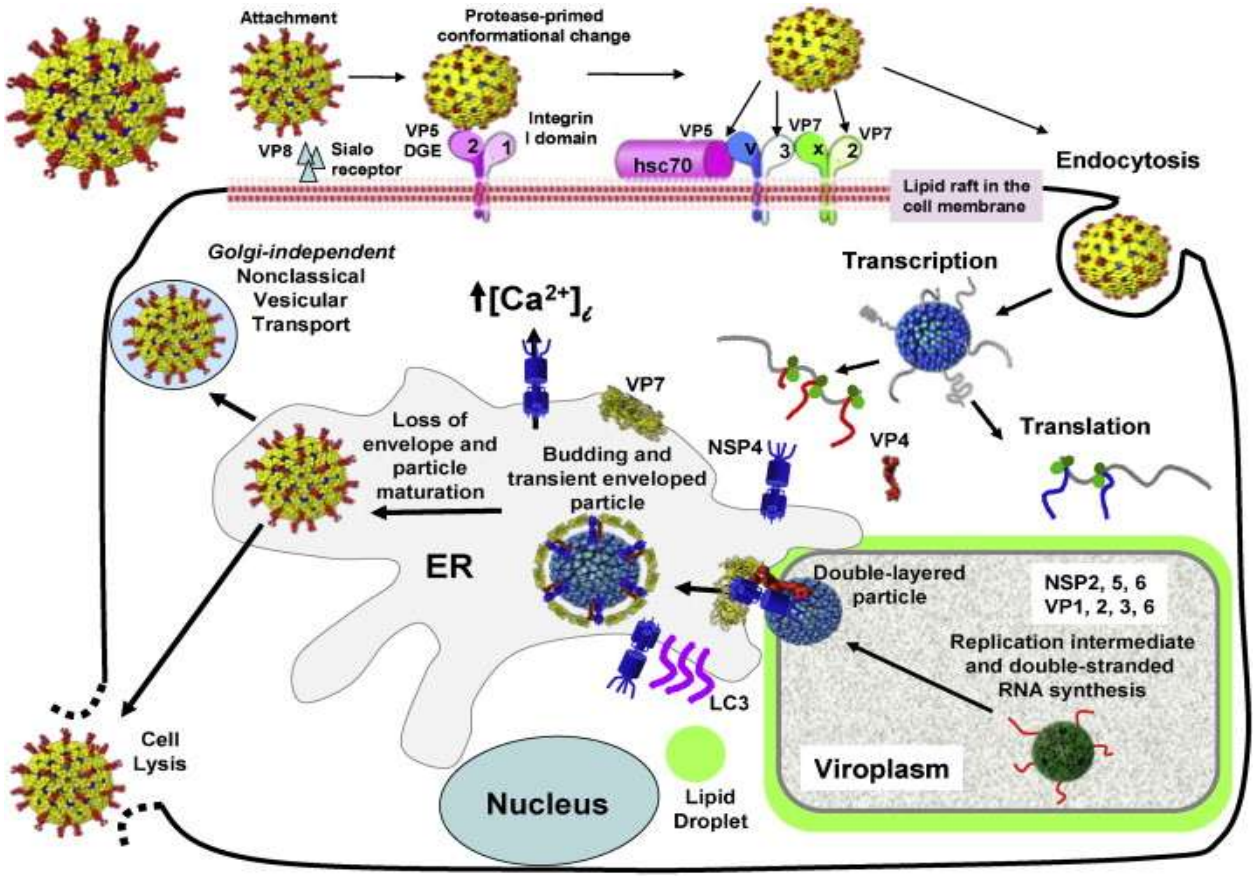


Figure 2.3: Rotavirus replication cycle (Desselberger, 2014).

2.7.2 Gastroenteritis symptoms

The difference between bacteria gastroenteritis to Rotavirus gastroenteritis is non bloody diarrhea which happens short span and limited inflammation response. The diarrhea caused by rotavirus is referred to as non-inflammatory. The mechanism of diarrhea can be due to secretory effects of NSP4 which causes activation of enteric nervous system or by osmotic diarrhea due to malabsorption because of damaged or death of enterocyte (Morris and Estes,.,2001). The infection can cause lead to secretion of hydroxytryptamine that activates the signaling pathway that induces vomiting and diarrhea (Hagbom *et al*., 2011). Histological studies carried to determine effect of rotavirus infection and replication to duodenal mucosa have shown disruption of normal cellular homeostasis. These will lead to reducing and atrophy of villi loss of microvilli,

and distended endoplasmic reticulum and mitochondrial swelling in enterocytes. Observation was made in infected calves and piglets showed that blunting of intestinal villi and enterocyte vacuolization which can be similar to infected children (Estes and Greenberg, 2013). The expression of NSP4 has been shown in the cell culture studies in mice and viral replication can takeover cellular pathway that might lead to cell death or extrusion from the epithelium of the intestine. Other studies have proposed virus mediated apoptosis, NSP4 mediated mislocalization at the tight junction protein ZO-1 and the binding to the basement membrane causes disruption of cellular homeostasis (Hyser *et al*., 2010).

The mechanism of reduced epithelial absorption due to Rotavirus infection have been proposed. The infected enterocytes are lost and NSP4 mediated sodium-coupled solute symporters that are involved in reabsorption of large volumes under physiological conditions do not work. Sufficient number of enterocytes remain intact with sodium-glucose cotransporter 1 hence rotavirus does not affect all enterocytes which explains the efficacy of oral rehydration (Svensson *et al.,* 2016).

Rotavirus infection leads to increase of calcium levels which is mediated by NSP4. The calcium levels induce production of 5- HT from enteroendocrine cells which are calcium dependent. The production of 5- HT activates the enteric nerves that innervate the small intestine and eventually leads to increased intestinal motility (Svensson *et al.,* 2016).

The infection by Rotavirus and release of NSP4- dependent 5-HT release can also activate vagal nerve that protects regions of the brain that is associated with nausea and vomiting. The vagal nerves contains neurons which project from the gut to the brain which contain a pathway in detection of emetic stimuli and generation of vomiting (Andrews *et al*., 1992).

Fever and malaise are commonly associated with Rotavirus infection. Hypothalamus regulates temperature and fever is an acute response to Rotavirus infection, other symptoms which accompany fever include; depression, inactivity, sleepiness and low intake of food and water. The inflammatory cytokines play a critical role in induction of fever (Brodal, 2010).

2.7.3 Immune response to Rotavirus

Immunological and non-immunological factors determine susceptibility to rotavirus infection. The innate immunity recognizes Rotavirus infection by pattern recognition receptors (PRRs) in the enterocytes. The PRR recognition plays a role in detection of probable ATP-dependent RNA helicases DDX58 which induces helicase C domain-containing protein 1 which activates type I and type III interferon responses (Lin *et al* 2016). The PRRs are speculated to have a role in age dependent resistance to rotavirus disease in experimental mice and are expressed in higher levels in adult mice. The NSP1 and other viral proteins can inhibit the signaling pathway of activated PRRs which explains poor innate immune responses to rotaviruses (Lopez *et al.,* 2016). The inhibition of STAT1 activation can be facilitated by NSP1 hence blocking interferon responses. The combination of cytokines like IL-18 and IL-22 have been proposed in clearance of Rotavirus infection (Lin *et al* 2016).

The adaptive immune responses like CD8 T cells and B cells can mediate rotavirus clearance but very low levels of CD8 T cells have been detected among children as compared to adults (Jaimes *et al.,* 2002).

2.8 Management of Rotavirus Gastroenteritis

It involves controlling symptoms, especially dehydration (Diggle *et al*., 2007). The management of dehydration is critical since its mismanagement in children can cause death due to severe dehydration. The treatment of dehydration includes oral rehydration. The dosage of oral rehydration depends on the severity of diarrhea (Tablang, 2014). In case of prolonged diarrhea, some of the recommendations put forward by both WHO (2008) and UNICEF (2016) include the use of low-osmolarity oral rehydration and zinc supplements (WHO, 2008). When infection is severe, hospitalization is required and fluids administered intravenously or through Nasogastric incubation while monitoring the child's electrolytes and blood sugar levels (Jiang *et al*., 2010).

2.9 Prevention of Rotavirus Infections

The most critical step in preventing rotavirus infections is through practicing hygiene standards. Improved hygiene standards and clean water are difficult to achieve in resource-limited countries. The ideal method is vaccination, evidence from data comparing morbidity in countries with weak healthcare systems and those with advanced healthcare systems still shows that the prevalence of disease in those industrialized countries is low compared the developing countries which do not have rotavirus vaccine (Dennehy *et al*., 2013). The best and effective method for prevention is the vaccine (Parashar *et al*., 2003). Two Rotavirus vaccines have been introduced namely; Rotarix (GlaxoSmithKline) and RotaTeq (Merck Sharp & Dohme) (Tate *et al*. 2011). The vaccines have shown a good response in the prevention of rotavirus. (Dennehy *et al*., 2015; Tate *et al*., 2011).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Site

The study was done at Kenyatta National Hospital (KNH). The hospital is a teaching and referral hospital in Kenya. It serves as the pediatric emergency for referral cases and pediatric outpatients services for residents of Nairobi.

3.2 Study Design

The study design was a cross-sectional descriptive study carried out in the year 2017.

3.3 Study Population

The study population was children below five years with symptoms of gastroenteritis.

3.3.1 Inclusion Criteria

1) The children whose parents or guardians consented.
2) Children below five years of age
3) Those who presented with acute diarrhea for not more than seven days
4) Those who had experienced three episodes of watery stools in 24 hours.

3.3.2 Exclusion Criteria

1) Children whose parents refuse to consent.

3.4 Sample Size Determination

The sample size was determined as previously described (Naing *et al*., 2006).The size was calculated as per the statistical formula:

$n = Z^2 \underline{P(I-P)}/d^2$

Where;

n = Minimum sample size

Z- = Standard error for confidence interval at 95% = (1.96)

P= Estimated prevalence for rotavirus infection among children in Kenya (31.5%) (Agutu *et al*., 2017)

d= Precision of the estimate required (1-α= 0.05)

Hence, $n = 1.96^2 \times 0.31(1-0.31)/0.05^2$

$n=334$

The minimum sample size was 334.

3.5 Sampling Method

The sampling method used was systematic random sampling of those who meet the inclusion criteria until the sample size was reached. A total of 149 inpatients and 206 outpatient were sampled. The demographic data captured using a simple reporting form. Demographic data of sex, age, inpatient, outpatient, and vaccine status was collected.

3.6 Sample collection

The stool specimen collected approximately 20g were collected into sterile containers by the guardians of patients. Specimens were transported in a cool box to Kenyatta National Hospital Immunology Laboratory at 4°C and stored at -20 ° C till further use.

3.7 Laboratory Procedures

3.7.1 Rotavirus Enzyme-Linked Immunosorbent Assay

The samples were processed and tested using ELISA Prospect TM which was optimized using WHO guideline 2008. The stool sample was diluted in a ratio of 0.1g of solid feces (small pea sized portion) or approximately 100 µl of liquid to 1000 µl of phosphate buffered saline. The diluted samples were vortexed (whirlimixer brand) and centrifuged (DT5-6A) at 10,000 × *g* at 4°C for 15 min, then 100µl of diluted samples were added to the microwell plate (WHO, 2016). A known positive sample from the kit and negative provided in the kit were used for both positive and negative control respectively. One hundred microliters of horse-radish peroxidase conjugate were then added to all the wells; the plate was then sealed and incubated at room temperature for 60 minutes. The microwell plate was washed and aspirated five times with diluted wash buffer, the microwell plate being blotted after each wash on a clean absorbent towel to remove excess buffer. One hundred of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate was then added the plate covered and further incubated at room temperature for 10 minutes upon which the reaction was stopped by addition of 50 µl 2 M of sulphuric acid was added to each well to stop the reaction. The optical density of each well was read using ELX 800 universal microplate reader at 450 nm against the reference range of 620 to 650 nm. The cut-off value was determined according to the manufacturer instruction by addition of the value 0.2 of the optical density of negative control well. Therefore samples whose optical density exceeded the cut off (0.5) value were considered positive, while samples whose optical densities were below the cut off were considered negative (Raboni *et al*., 2002).

3.7.2 RNA Extraction

The samples were diluted with phosphate buffered saline (PBS) to make 10% suspension. The RNA was extracted using the Kingfisher extraction machine (Thermo Scientific, Waltham MA) as per the manufacturer's instruction (Slavica *et al*., 2016). Besides, the samples were spiked with XenoRNA a 50 µl volume of 10% PBS suspension prepared as a quality control measure (Slavica *et al*., 2016). The

concentration of the RNA was checked using the Qubit machine (Thermo Scientific, Waltham MA)

3.7.3 NSP3 RT-PCR

The RNA was amplified for NSP3 RT-PCR. The master mix was prepared by adding 25 µl nuclease free water, 7.5 µl deoxynucleoside triphosphates (dNTP), 2.5 mM Mn(OAc)2, 0.1 U rTth polymerase, 500 nM (each) forward NVP3-FDeg (5'-ACC ATC TWC ACR TRA CCC TC-3'), reverse primer NVP3-R1 (5'-GGT CAC ATA ACG CCC CTA TA-3'), and probe NVP3-Probe (5'-ATG AGC ACA ATA GTT AAA AGC TAA CAC TGT CAA-3'), 150 nM probe, and 5 µl of un-denatured RNA extract. The mixture was then placed in a microAmp fast Optical 96- well reaction plates (Applied Biosystems, Inc., Foster City, CA). The template was denaturated, and the amplification of RT and PCR was carried out using the ABI 7500 Fast real-time PCR system (Applied Biosystems, Inc., Foster City, CA). The thermocycling was then undertaken as follows; a 5-minute hold at 95°C in order to denature the dsRNA template, a 30-minute hold at 50°C for RT, 1 minute at 95°C, and 45 cycles of 15 seconds at 95°C and 1 minute at 60°C. A positive test result occurred for the case in which a sigmoidal amplification curve intersected the threshold before 45 cycles. The positive and negative control reactions gave expected results. Resulting samples were then compared to both the positive and the negative controls (Slavica *et al*., 2013; Freeman *et al*. 2008)

3.7.4 One-step RT-PCR genotyping

The extracted RNA was reverse transcribed into cDNA using a one-step RT-PCR kit (Qiagen) according to the manufacturer's instruction (Freeman *et al*., 2008). The primer set of a cocktail of six forward and three reverse primers were used (Table 3.1). The amplification of the VP4 sequences produced 500 bp. The VP7 sequences were also amplified resulting in 400 bp (Leen-Jan *et al*. 2009). Temperatures of 95°C for a total period of 5 minutes was then used for the denaturation process. The reverse transcription steps for the RT-PCR was concurrently done at the following conditions: 50°C for 30 minutes: denaturation step of 5 minutes at temperatures of 95°C, 40 cycles of PCR for

the temperature 94°C was carried out for 30 seconds, for 50°C it was done for 30 seconds, and a temperature of 72°C for 45 seconds, and a final incubation was carried out at 72°C for 7 minutes. Labeling of the amplified material was possible since all reverse primers carry a biotin moiety at the 5′ end (Leen-Jan *et al*., 2009). The amplified RNA was analyzed using analyzed with the tape station.

Table 3.1: Primers used for singleplex genotyping

Target	**Sequence (5'-3')**	**Polarity**	**Gene**	**Nucleotide Position**	**Amplicon size**
VP4uF	TGGYTTCVCTCATTTATA GACA	+	VP4	11-32	
P4 R5	GCATYCCTACAAGTCTATTAYTAG	-	VP4	508-485	497
P 6 R2	ACCATCGAGTAGTGGYTCTATYGTTG	-	VP4	210-185	199
P8 -R2	GYGGTTCAAYAGCAACKACT	-	VP4	350-330	339
P9-4T-1	TGAGACATGCAATTGGAG	-	VP4	402-385	391
P10-5T-1	ATCATAGTTAGTAGTCGG	-	VP4	594-575	583
Vp7R	AACTTGCCACCATYATYTTCC	-	VP7	914-932	158
9T-1	TCTTGTCAAAGCAATAATG	-	VP7	176-195	244
9con1-L	TAGCTCCTTTAATGTATGGTAT	+	VP7	37-59	464

3.7.5 One-step multiplex RT-PCR genotyping

The assay was set in four wells to detect the genotypes together with the vaccine strains for Rotateq and Rotarix. The primers were designed for each of VP7 genotypes G1, G2, G3, G4, G9, G12; as well as for the VP4 genotypes: (P[4], P[6], P[8]) (Slavica *et al*., 2016). The RNA was run using the OneStep RT-PCR kit (Qiagen) in four different reaction wells using multiplex qRT-PCR assays. The master mix was then added in the assay wells 1,2, 3 and 4. The master mix contained; 5 µL of 5X EZ buffer, 2.4 µM dNTPs, 2.5 mM Mn(OC)2, 2.5 U/µL rTth polymerase or 5.0 U/µL rTth polymerase for well 4, forward primer, reverse primer and probe at final concentrations that was specific for each component assay. 2 µL of template, which is water free of RNA or nuclease, was added to form a 25 µL final reaction volume. Preparation and tests for both the samples and controls were then done in duplicates. The PCR processes included denaturation of dsRNA for 5 min at a temperature of 95 ^{O}C; two reverse transcription processes: the first for 30 min at 50 ^{O}C and another for 1 min at 95 ^{O}C and finally 45 amplification cycles consisting of 15 sec at 95 ^{O}C and another for 1 min at 60 ^{O}C. The results were then analyzed with ct value compared with the positive and the negative cut off value (Slavica *et al*., 2016; Gautam *et al*., 2014). The descriptions of details of primer designs and probes are displayed in table 3.2

Table 3.2: Primers and Probes used for multiplex qRT-PCR rotavirus genotyping assay (Slavica *et al*., 2016).

Target-real time assays	Nucleotides sequences (5'-3')	Fluorophore-quencher (5′-3′)	Amplicon size
RotaTeq® VP6	FPb-GCGGCGTTATTTCCAAATGCACAG RPc-CGTCGGCAAGCACTGATTCACAAA Probe-ATCACGCAACAGTAGGACTCACGCTT	HEX-BHQ1	99
Xeno-IPC Rotarix® NSP2		TR-BHQ2	
G12	FP-TAG{C}TCYTTTTRATGTATGGTAT RPc-CGTCCARTCRGGRTCAGTTATTTCAGTC ARTTTTGAG{C}TYYAATAAATGGCAGYAYG	FAM-BHQ1	309
WELL 2 G9	FPb-TAG{C}TCYTTTTRATGTATGGTAT RPc-CAGAGTATYYTTCCATTCHGTATCTCC Probe-CCACARTT{C}TAA{C}CTTTYTGATATCA	HEX-BHQ1	302
	FPb-ACCATCTWCACRTRACCCTC RPc-GGTCACATAACGCCCCTATA	TR-BHQ2	318
NSP3	ProbeATGAGCACAATAGT″T″AAAAGCTAACACTGTCAA FPbTAG{C}TCYTTTTRATGTATGGTAT RPc-ATAGWGTAT{C}TTTCCATTCAKTGTC	FAM-BHQ1	319
G4	ProbeTGTAGTATTRT{C}AKTATTAK{C}GAATGCRC		
G3	Pb-TAG{C}TCYTTTTRATGTATGGTAT RPc-TTGCAGTGTAG″C″GTCRTAYTTC G3-Pe-TGTATTAYC{C}AACTGAAG″C″WGCAACAG	Cy5-BHQ3	459
G2	FPb-TAG{C}TCYTTTTRATGTATGGTAT RPcCARTYGGCCAT{C}CTTTAGTTA Probee-CCAATAACGGGRT{C}ACTAGACGCTGT	TR-BHQ2	352
P4	FPb-GG″C″TATAAAATGGG'C'CTTCGCT RPc-ATYACYMTGACTACTAGCTTAAACATTTCG	TR-BHQ2	459
P6	FPb-GG″C″TATAAAATGGG'C'CTTCGTCT RPc-AAGCAAYCCAAAYATCAGTTTTATTG	FAM-BHQ1	297
P8	P-consensus-FPb-GG″C″TATAAAATGG″C″TTCGCT RPc-ACTTCCAYTTAT{C}TGAATCRTTWCT	Cy5-BHQ3	424

3.7.6 Next Generation Sequencing

The stool samples were suspended in 30% v/v in phosphate buffered saline and extracted by Magna pure compact (Roche Applied Science) Instrument in line with instructions from the manufacturer. The extracted dsRNA was treated with DNase I (Life Technologies) to remove DNA from samples. Single-stranded RNA was separated from double-stranded rotavirus RNA by LiCl precipitation. The cDNA library was generated from rotavirus RNA with a commercial kit (NEBNext Ultra RNA Lib Prep, New England Biolabs, Boston, MA) according to manufacturer's instructions (Matthijnssens *et al*., 2008). The library was enriched by PCR using primers listed below table 3.3 (Dung, *et al*. 2017). Next-generation sequencing was carried out on a MiSeq sequencer using the Illumina MiSeq reagent kit v.2 with 500 cycles, and the standard 250 bp paired-end reads method. Contigs were assembled from the obtained sequence using the *de novo* assembly command and guided assembly with default parameters in CLC Genomics Workbench 7.0.4 software (http://www.clcbio.com/products/clc-genomics-workbench/). The genotypes were determined according to the guidelines of the RCWG (Matthijnssens *et al.*, 2008) using the online genotyping tool RotaC (http://rotac.regatools.be) (Matthijnssens *et al.*, 2008) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 3.3 Primers used for rotavirus genome enrichment amplification

Primer name	Primer sequence 5' 3.'
FR20RV	GCCGGAGCTCTGCAGATATC
FR26RVN	GCCGGAGCTCTGCAGATAT
FR26RV-VP7-F	CCGGAGCTCTGCAGATATCGGCTTTAAAAG
FR26RV-VP7-R	GCCGGAGCTCTGCAGATATCGGTCACATCATA
FR26RV-VP4-F	GCCGGAGCTCTGCAGATATCGGCTATAAAATG
FR26RV-VP4-R	GCCGGAGCTCTGCAGATATCGGTCACATCCTC
FR26RV-VP4-F2	GCCGGAGCTCTGCAGATATCTGGCTTCGCCAT
FR26RV-VP4-R2	GCCGGAGCTCTGCAGATATCATTTCGGACCAT
FR26RV-VP6-F	GCCGGAGCTCTGCAGATATCGGCTTTWAA ACG
FR26RV-VP6-R	GCCGGAGCTCTGCAGATATCGGTCAC ATCCTC
FR26RV-VP1-Fb	GCCGGAGCTCTGCAGATATCGGCTATTAAAGC
FR26RV-VP1-Rb	GCCGGAGCTCTGCAGATATCGGTCACATCT
FR26RV-VP2-Fc	GCCGGAGCTCTGCAGATATCGGCTATTAAAGG
FR26RV-VP2-Rc	GCCGGAGCTCTGCAGATATCGTCATATCTCCA
FR26RV-VP3-Fe	GCCGGAGCTCTGCAGATATCGGCTWTTAAAGC
FR26RV-VP3-Re	GCCGGAGCTCTGCAGATATCGGTCACATCATG
FR26RV-NSP1-F	GCCGGAGCTCTGCAGATATCGGCTTTTTTTTATG
FR26RV-NSP1-R	GCCGGAGCTCTGCAGATATCGGTCACATTTTATGC
FR26RV-NSP2-F	GCCGGAGCTCTGCAGATATCGGCTTTTAAAGC
FR26RV-NSP2-R	GCCGGAGCTCTGCAGATATCGGTCACATAAGC
FR26RV-NSP5-F1	GCCGGAGCTCTGCAGATATCGGCTTTTAAAGCGCT
FR26RV-NSP5-F2	GCCGGAGCTCTGCAGATATCGGCTTTTAAAGCGCT
FR26RV-NSP5-R1	GCCGGAGCTCTGCAGATATCCTATCTGAATCATC
FR26RV-NSP5-R2	GCCGGAGCTCTGCAGATATCGGTCACAAAACGGGA

3.7.7 Phylogenetic analysis

For each gene, multiple alignments were made by using the Multiple Sequence Comparison by Logic- Expectation (MUSCLE) algorithm implemented in MEGA6 software. Once aligned, the DNA Model Test program implemented in MEGA version 6 was used to identify the optimal evolutionary models that best fit the sequence datasets. Using Corrected Akaike Information Criterion (AICc). With these models, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support. Nucleotide and amino acid distance matrices were prepared using the *p*-distance algorithm of MEGA 6 software (Koichiro *et al*., 2013).

3.7 Data Analysis and Management

Collected samples were assigned laboratory numbers which were used the processing. The demographic data was keyed in to excel and stored in a password-protected document. The results were analyzed according to the presence and absence of the virus using the RT- PCR method and Ct values were tabulated and compared to a positive and negative control to determine a positive and negative samples. Elisa samples were analyzed using the optical densities measurement to determine a positive and negative sample. The cut off was calculated using the manufacture instruction to determine the cut off value. The prevalence of rotavirus in regards to age, sex, inpatient, and outpatient clinics was presented in proportions. Chi-square was used to analyze the differences in the distribution of rotavirus concerning gender, age, and inpatient or outpatient status at 95% confidence interval. The data was presented using charts, graphs, and tables Rotavirus positive samples were genotyped using multiplex genotyping assay and the Ct values were compared with positive control to determine the genotypes. Samples with high viral load were sequenced using next generation sequencing and the nucleotide and amino acid sequences were analyzed using MEGA, and the phylogenetic tree was drawn.

3.9 Ethical Considerations

The study was nested within the World Health Organization (WHO) rotavirus surveillance which started from 2007 to 2019 which was approved by the ministry the year 2007. The study was approved by the Ethical Review Committee (ERC) at the Kenyatta National Hospital Registration No: P372/07/2017 (Appendix 5). The study involved children whose parents voluntarily signed informed consent. Personal information was not used in processing the samples. Only study assigned numbers were used to process and analyze the samples.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics

Samples were collected from 355 children presenting gastroenteritis and met the WHO rotavirus criteria. More samples were collected as compared to the sample size calculated to account for the errors in sampling and sufficient sample for further processing. The vaccination rate indicated nearly all children were vaccinated except 29 whose guardians would not remember or records did not indicate if they had been vaccinated. The majority of children sampled came from Nairobi County from the following towns Kangware, Mukuru, Kibera, Githurai, Ongata Rongai and Jamuhuri. The vaccination coverage among the children who were sampled was 91.83% (326/355). The children who were not vaccinated were 8.16% (29/35). The males were 63.09% (224/355) as compared to female who were 36.90% (131/355). The inpatient were 41.97% (149/355) compared to outpatient 58.02% (206/355) (Table 4.1).

Table 4.1 Rotavirus Infection distribution in regard to gender, inpatient and outpatient at KNH.

	Positive	**Negative**	**Totals**
Male	37	187	224
Female	21	110	131
	58	**297**	**355**
Inpatient	25	124	149
Outpatient	33	173	206
Totals	**58**	**297**	**355**

4.2 Overall prevalence of rotavirus

The prevalence of rotavirus was 16.34 % (58/355). Rotavirus group A infections among males were 21.46% (37/58) compared to 12.18% (21/58) females who were positive. The distribution of rotavirus group A between outpatient and inpatient showed that; 56.89% (33/58) were inpatient while 43.10% (25/58) were outpatient. The distribution was analyzed by chi square to show the difference in prevalence in regard to gender, inpatient and outpatient. The distribution showed no difference at 95 confidence interval since p< .05. The chi-square statistic was 0.6980 and P value was 0.873472. The most affected age group was 7 to 12 months 42.25% (25/58) followed by 13 to 18 months 18.30% (12/58), then 19 to 24 months 18.30% (9/58), 0 to 6 months 11.63% (6/58), 25 to 30 months 8.45% (5/58) and finally >31 months 2.2% (1/58). The median age was 5 months. (Figure 4.1) (Table 4.2).

Table 4.2: Rotavirus distribution between age groups at KNH

Age in months	No. children infected	Percentage
0 to 6	6	11.83%
7 to 12	25	42.25%
13 to 18	12	21.12%
19 to 24	9	14.80%
25 to 30	5	8.45%
>31	1	2.25%
Totals	58	

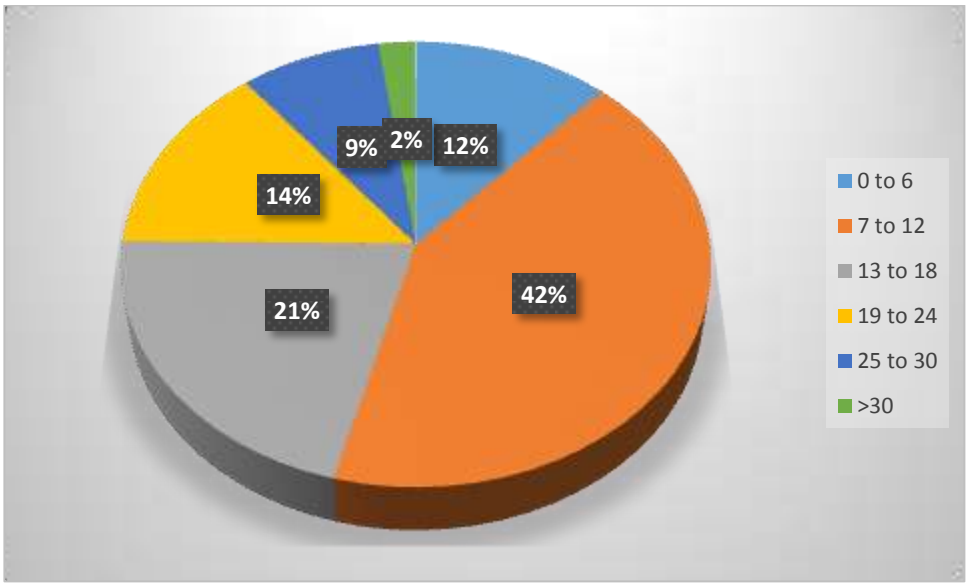


Figure 4.1: Rotavirus distribution in various age groups at KNH

4.2 Rotavirus genotypes

The prevalence of VP7 and VP4 genotypes were as follows; VP7 G-type was G1 48.27% (28/58), followed by G2 22.4% (13/58), G3 15.51%% (9/58), and G9 5.17%% (3/58) while there was some mixed infection which included G1, 2 5,17%(3/58) and G2,3 1.72% (1/58). The P-type P[8] 46.55% (27/58) was prevalent followed P4 24.13% (14/58) and P[6] 20.68% (12/58). There were mixed infection which includes P[4,8] 5.17% (3/58), and P[4,6] 1.72% (1/58). The G-P combination showed that G1P[8] 41.37% (24/58) was more prevalent followed by G2P[4] 22.41%, (13/58) G3P[6] 15.51%(9/58) and G9[P8] 5.17%(3/58). The mixed infections detected G1, 2 P [4, 8] 5.17% (3/58) and G3,2 P[4,6] 1.72% (1/58). The detailed report is found in appendix 2 (Table 4.3).

Table 4.3: Rotavirus Genotypes at KNH.

Genotype	No.
G1P[8]	24
G2P[4]	13
G3P[6]	9
G9P[8]	3
G1P[6]	2
G1,2P[4]P[8]	3
G3,2P[6]P[4]	1
G1P[NT]	1
G1,3P[6]	1
G1P4	1

4.3 Next generation sequencing results

NGS was performed on samples with ct values below 25 and sampled from all genotypes. The results generated by NGS from 17 samples were compared with the ones from multiplex genotyping and they all agreed. The 17 samples contained the following genotypes; G1P[8] 8; G3P[6] 2; G9P[8] 2; G2P[4] 3; G1,2P[4,8] 1 and G2,3P[4,6] 1 more details check Appendix 3. The summary is found in table 4.4

Table 4.4: The consensus sequences analyzed by NGS

Sample no.	Genotype By Gold standard	NGS genotype
3724	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3755	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3638	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3994	G3P[6]	G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E6-H2
4054	G2P[4]	G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2
4047	G1,2P[4,8]	G1G2-P[4,8]-I1-R1R2-C1C2-M1M2-A1A2-N1N2-T1T2-E1-H1
3607	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3920	G2P[4]	G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2
3413	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3668	G9P[8]	G9-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3471	G9P[8]	G9-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3920	G2P[4]	G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2
3818	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3753	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1
3946	G2,3P[4,6]	G2G3-P[4,6]-I2-R2-C2-M2-A2-N2-T2-E2-H2
3994	G3P[6]	G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E6-H2
3754	G1P[8]	G1-P[8]-I1-R1-C1-M1-A1-N1-T1- E1-H1

4.4 Phylogenetic analysis

The analysis of VP7 indicated that the G1 samples no: 3607, 3638, 3978, 4047 and 3818 belonged to lineage 1; while 3113, 3724 and 3754 belonged to lineage II. The samples in which were grouped to G2 included 3920,4019, and 4047 which belonged to lineage II, while 3948 belonged to lineage I. The G9 group included 3471 which included lineage I and 3498 which belonged lineage III. The G3 samples were 3946, 3994 and 4009 which belonged to lineage I (Figure 4.2).

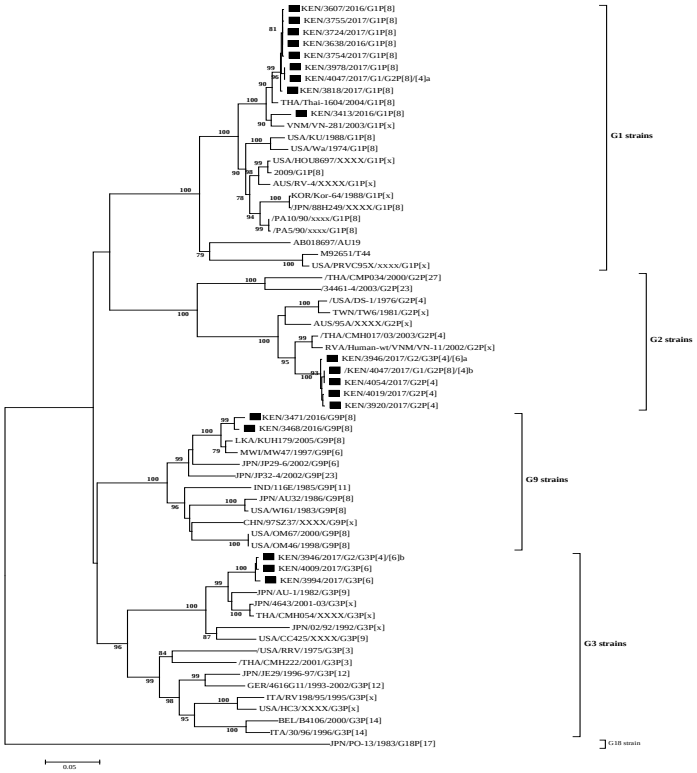


Figure 4.2: Genetic relationship of nucleotide sequence of VP7 from KNH

The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site,

maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The VP4 gene analysis grouped P [8] which had the following samples belonging to lineage II; 3968, 3755, 3607, 3978, 4047 and 3618 while lineage II included 3413, 3471, 3724 and 3754. The P [4] samples in lineage III were 4054 and 4047, lineage I 3946 and 4019 belonged to lineage II. Finally the P [6] had 3946, 3994 and 4009 being grouped to lineage I (Figure 4.3).

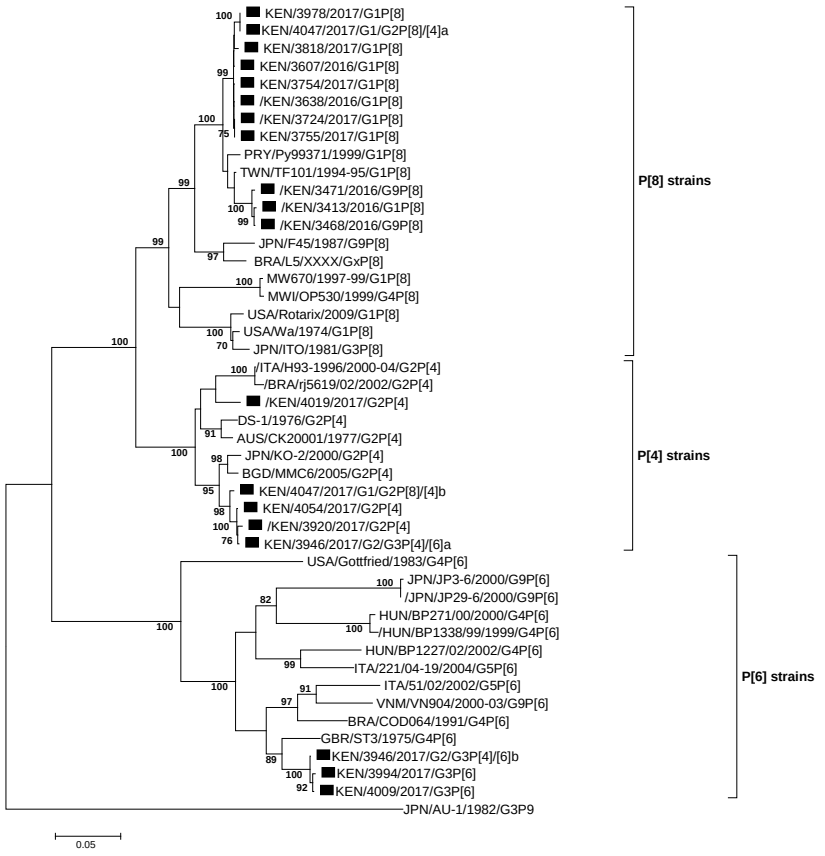
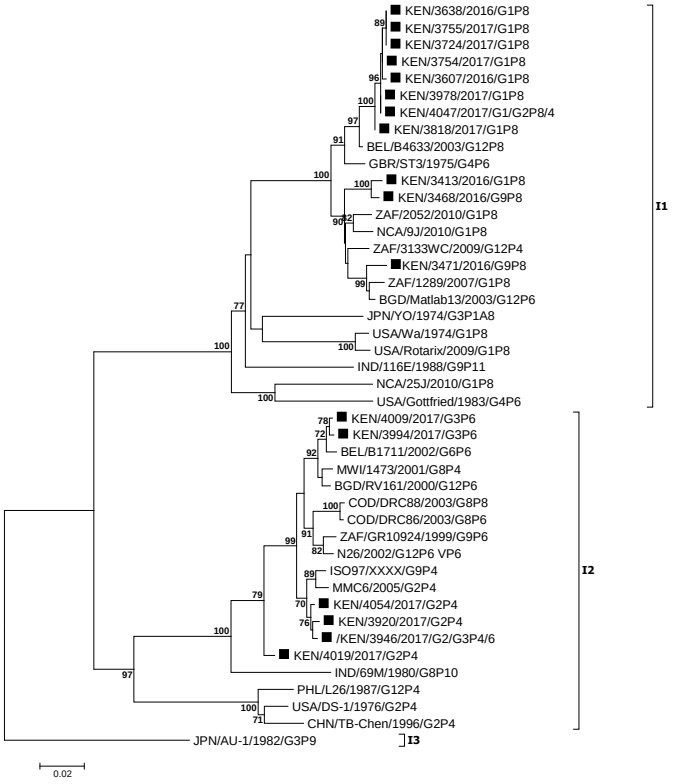


Figure: 4.3 Genetic relationship of nucleotide sequence of VP4 from KNH.

The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each

branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

When VP6 was analyzed it showed that samples 3724, 3754, 3607, 3979, 4047, 3818 and 3471 belonged to group I1 while 4079, 3994, 4054, 3820 and 3945 belonged to group I2. When the nucleotide (amino acid) homologies of VP6 gene sequences of the Kenyan strains with Wa-like backbone were compared with cognate gene sequences of strains belonging to previously VP6 genotypes, all of them were more closely related to strains in the I1 and I2 genotypes, respectively. Similarly, Kenyan strains with DS-1-like genetic backbone were more closely related to strains in the I1 and I2 genotypes, respectively (Figure 4.4).



The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The analysis of the nucleotide (amino acid) homologies of the VP1 gene sequences of the Kenyan strains with Wa-like backbone. The samples 3818, 3978, 4047, 3607, 3754, 3638, 3754, 3638, 3724,3755, 3413, 3468, 3176 and 3471 belonged to group R1 while 4009, 3994, 3964, 4019, 3920, 4054 and 4047 belonged to R2 group (Figure 4.5)

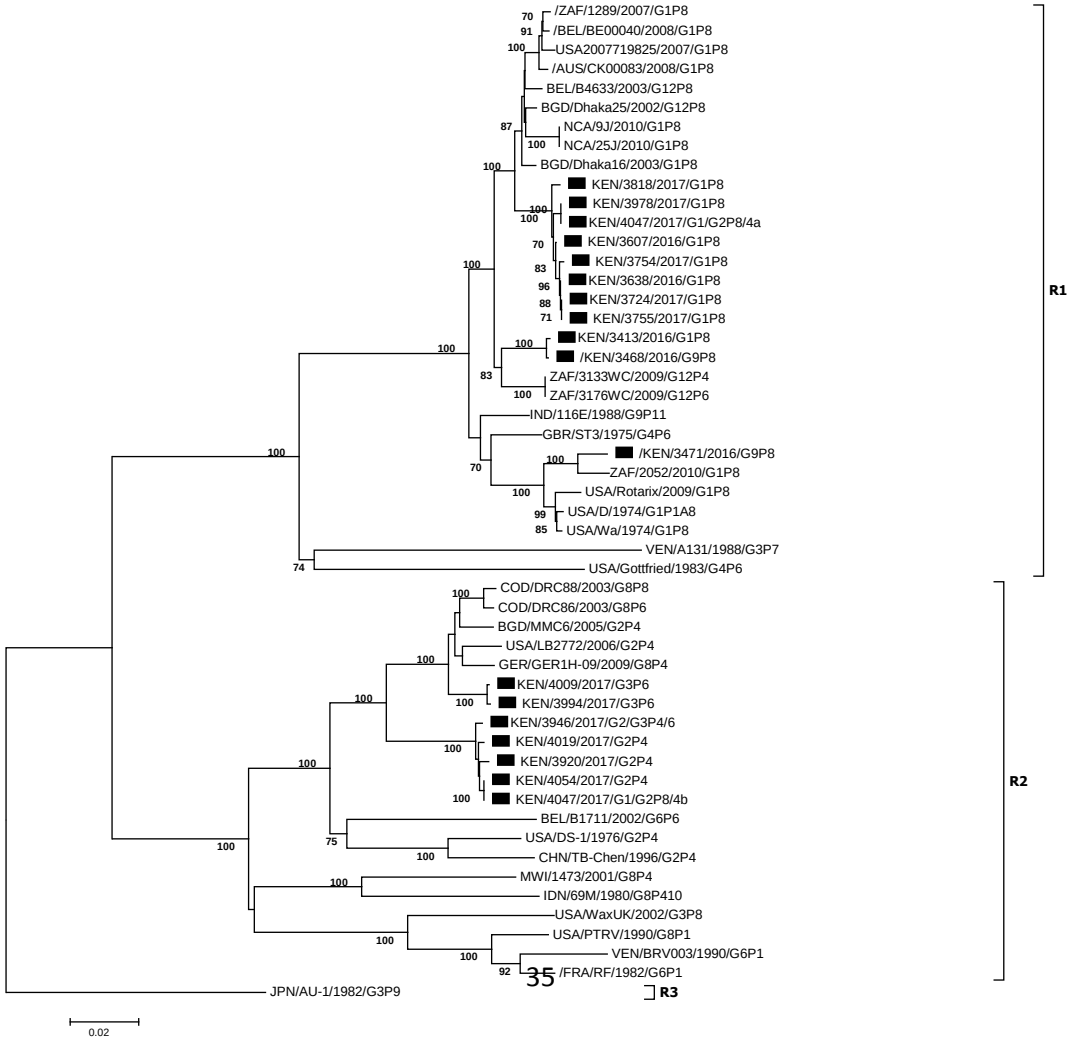


Figure 4.5: Genetic relationship of nucleotide sequence of VP1 from KNH.The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site**,** maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The analysis of VP2 showed that the samples which belonged to group C1 included; 3724, 3755, 3754, 3638, 3607, 3818, 3978, 4047, 3471 and 3413. The samples which belonged to group C2 included; 4019, 3920, 3946, 4054, 4047, 4009 and 3994 (Figure 4.6).

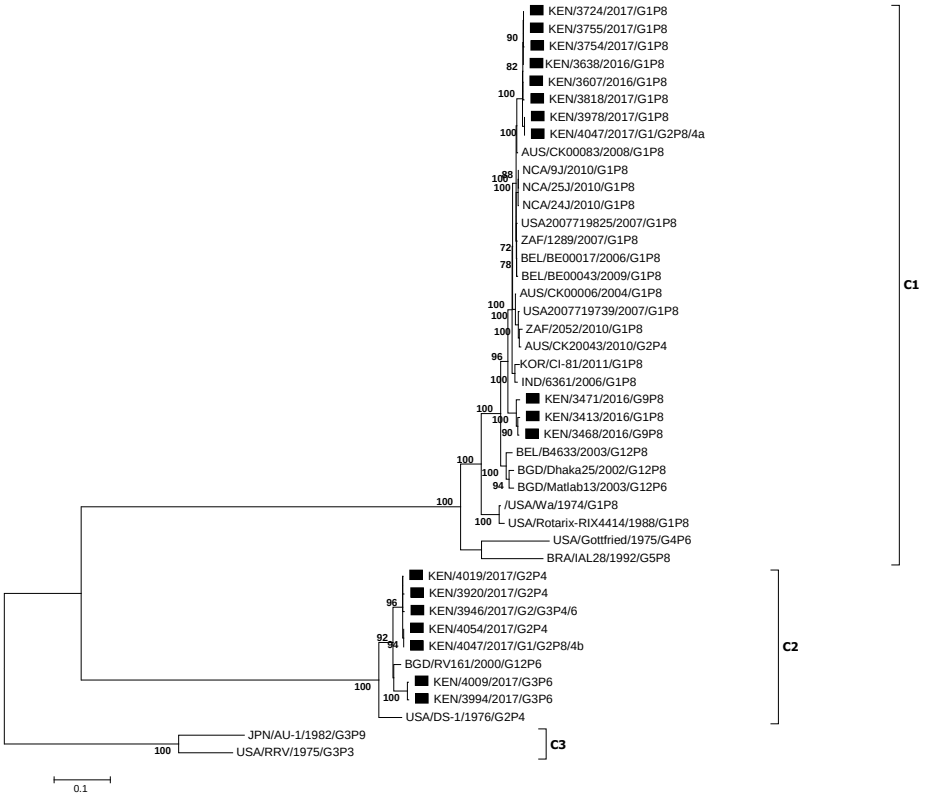


Figure: 4.6: Genetic relationship of nucleotide sequence of VP2.

The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

In the analysis of VP3 gene, the samples which belonged to group M1 included; 3724, 3755, 3638, 3754, 3607, 3978, 4047, 3818, 3971 3413 and 3468. The group M2 samples are as follows; 3920, 4054, 3946, 4047 and 4019 (Figure 4.7).

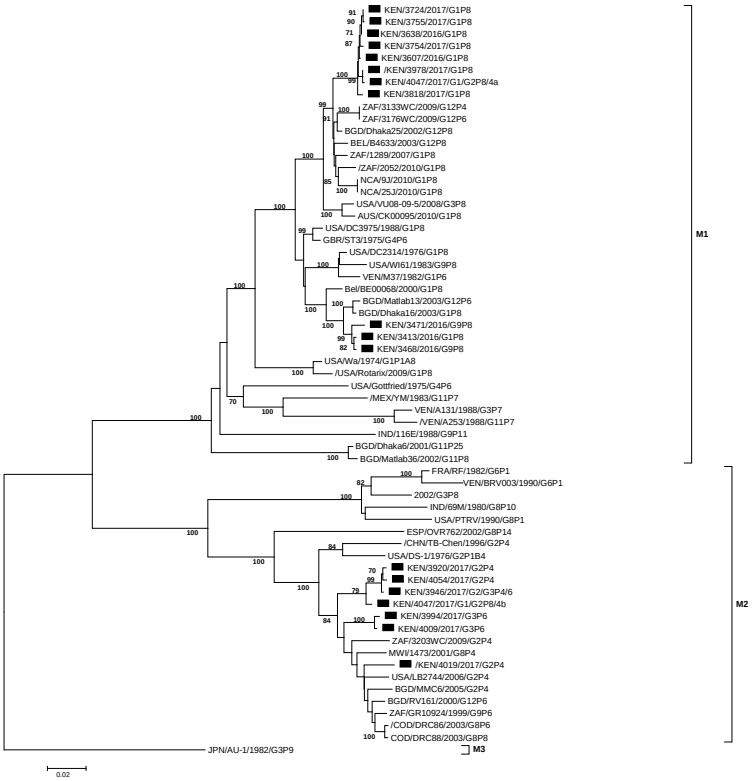


Figure 4.7 Genetic relationship of nucleotide sequence of VP3 from KNH.

The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support

Phylogenetic analysis of NSP1 gene nucleotide sequence demonstrated that the Kenyan strains with Wa-like genetic backbone. The samples belonged to two groups that A1 and A2, A1 samples included 3724, 3755, 3754, 3638, 3607, 3978, 4047, 3471, and 3413. While the samples belonged to gour A2 included; 3920, 4019, 4047, 4054 and 3946 (Figure 4.8).

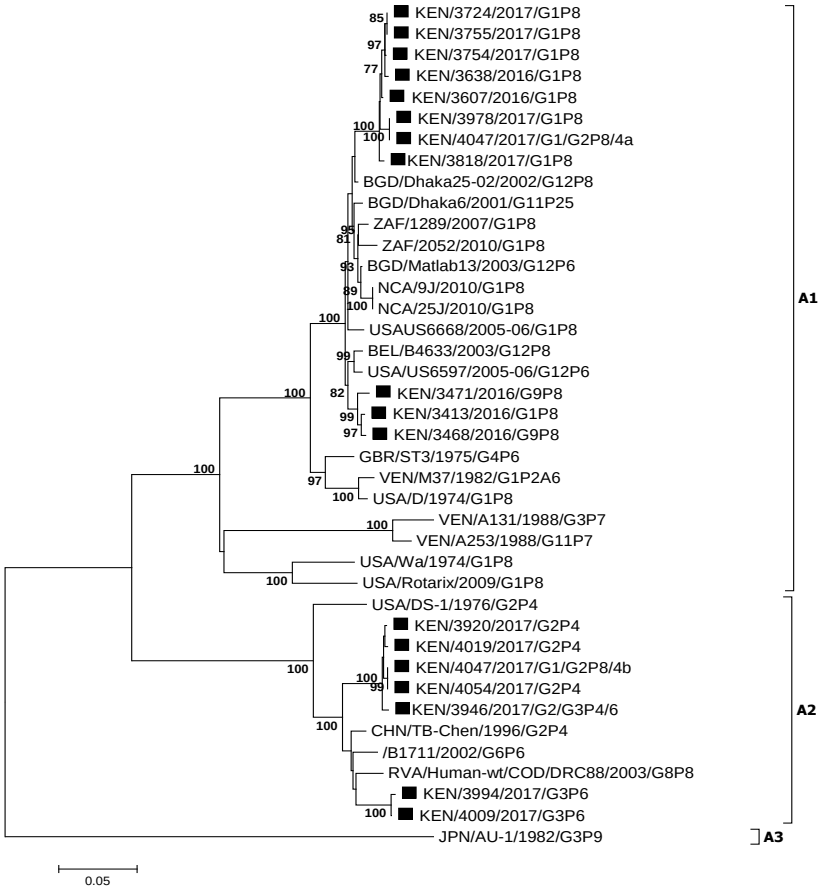


Figure 4.8: Genetic relationship of nucleotide sequence of NSP1 from KNH.
The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The analysis of NSP2 gene showed that samples which were grouped to N1 included 3607, 3755, 3638, 3724, 3754, 3818, 4047, 3878, 3474, 3413 and 3468. The N2 group had the following samples; 4054, 3920, 3946, 4009, 3494 and 4019 (Figure 4.9).

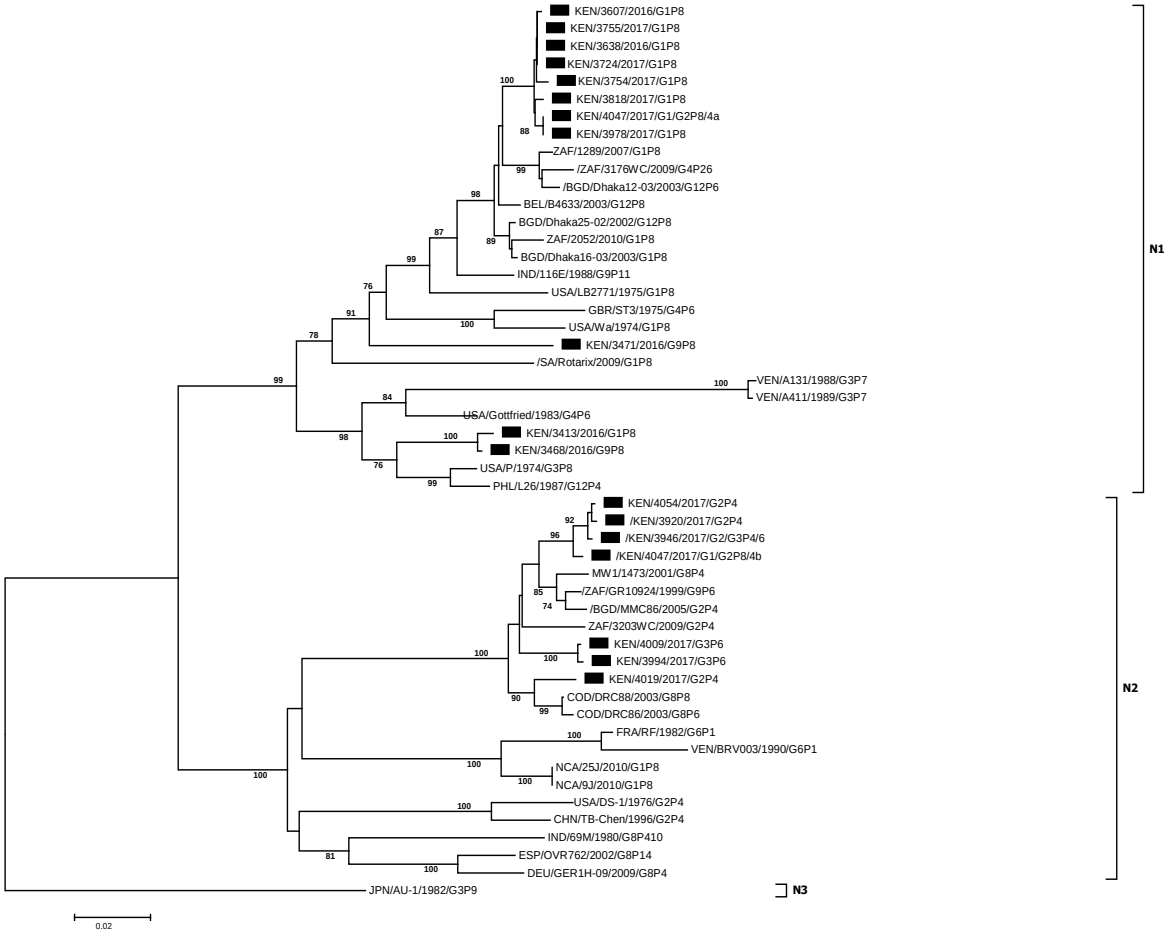


Figure: 4.9: Genetic relationship of nucleotide sequence of NSP2 from KNH. The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The analysis of NSP3 gene also grouped the samples into group E1 and E2. The samples which were grouped to E1 included; 3413, 3468, 3818, 3754, 3978, 4047, 3724, 3638, and 3755 while the E2 group included; 3946, 4054, 3920, 4019, 4009 and 3994 (Figure 4.10).

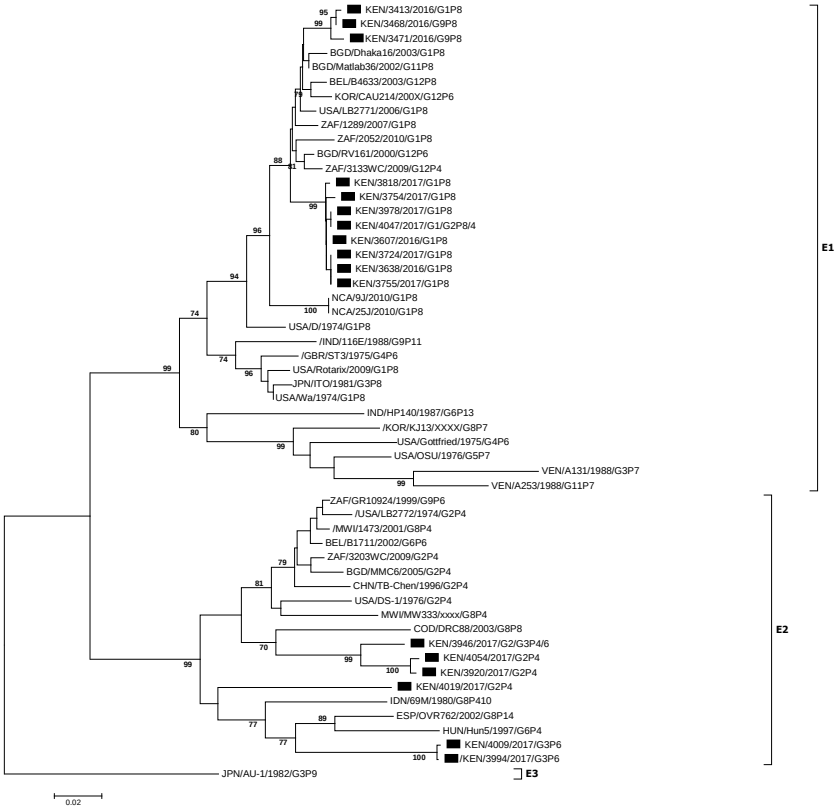


Figure 4.10: Genetic relationship of nucleotide sequence of NSP3 at KNH**.** The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site**,** maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The NSP4 gene also had only two groups T1 and T2. The samples which belonged to T1 included 3638, 3755, 3724, 3754, 3607, 3978, 4042, 3818, 3471, 3113 and 3468. The T2 group included; 4054, 4047, 3920, 3946, 4019, 4009 and 3994 (Figure 4.11).

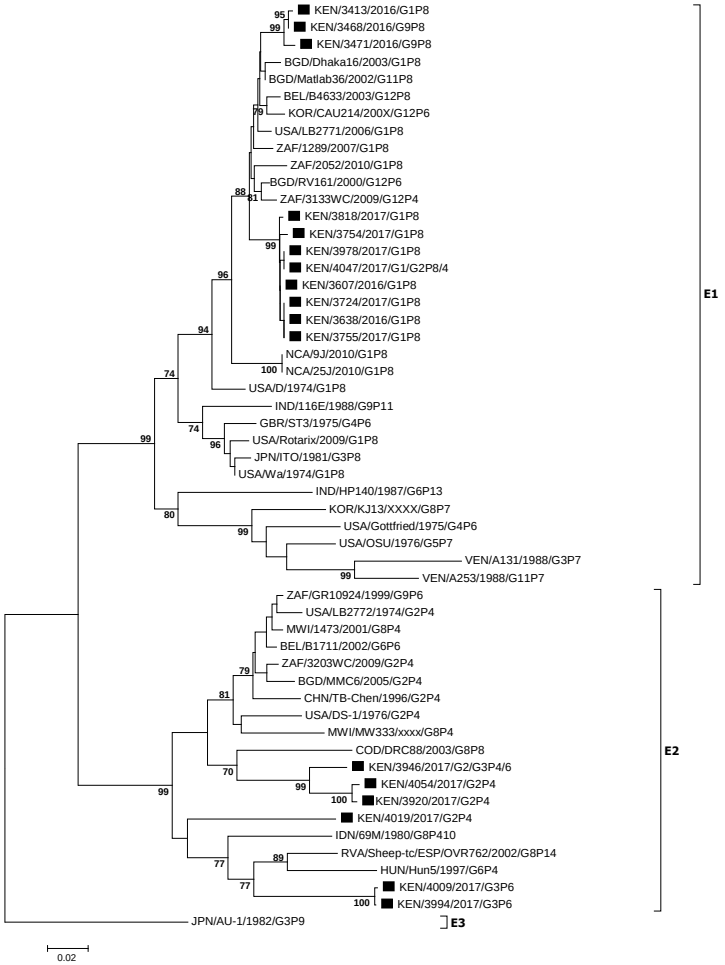


Figure 4.11: Genetic relationship of nucleotide sequence of NSP4 at KNH.

The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

The analysis of NSP5 gene grouped the samples into H1 group and H2 group. The samples which belonged to group H1 included; 3413, 3468, 3818, 3724, 3607, 3638, 3755, 4047, 3978 and 3471, while the H2 group included; 4019, 4009, 3994, 4054, 3920 and 3946 (Figure 4.12)

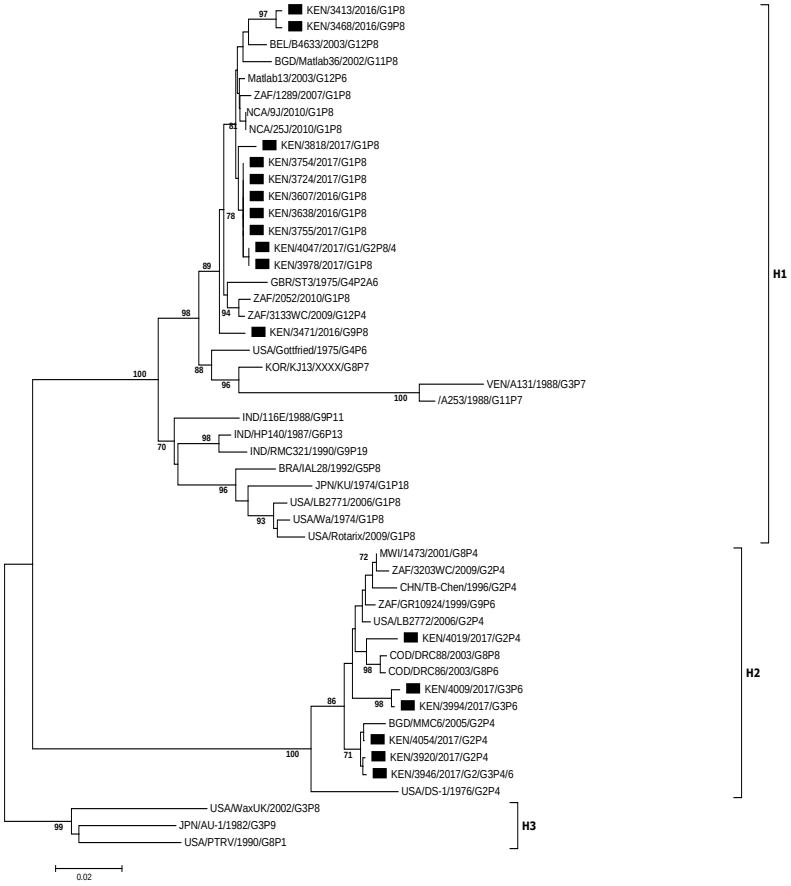


Figure 4.12: Genetic relationship of nucleotide sequence of NSP5 at KNH. The black squares represent samples collected at KNH with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site, maximum-likelihood trees were constructed using MEGA 6 with 500 bootstrap replicates to estimate branch support.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Rotavirus infections have been reported to be leading cause of acute gastroenteritis in the world among children under five years old and approximately 5% of children deaths annually. In the years 2015 Rotavirus caused 215,000 deaths among children under five years and close to 80% of the deaths came from south Asia and Africa (Tate *et al*., 2016; Tate *et al.,* 2012). The risk factors associated to Rotavirus infection include early complimentary feeding, dehydration and nutritional status. The global prevalence of rotavirus in hospital based prevalence ranges between 5 to 56%. Rotavirus prevalence in Kenya between 2006 and 2008 was at 40% among children under five years hospitalized (Mwenda *et al.,* 2010; WHO, 2008). A similar study which was carried in the year 2012 to 2013 reported the prevalence of rotavirus to be at 23% among children under five years (Raini *et al*., 2015). The prevalence of Rotavirus of the study was 16.34% which is a decrease in Rotavirus infection. A review conducted in Kenya from 1975 to 2005 showed the prevalence of rotavirus to be between 11 to 56.2% (Kiulia *et al*., 2008).

Another study conducted at Kenyatta National Hospital to check the prevalence of rotavirus in 2007 showed the prevalence to be at 59% (Osano e*t al*., 2011). The prevalence in Kiambu County was found to be at 36.6% by a similar study (Wandera *et al*., 2017). The study indicated the decrease in prevalence from 56% in previous studies to 16.34% as indicated by this study (Kiulia *et al*., 2008). This can be attributed to the introduction of the rotavirus vaccine Rotarix® in Kenya in July of 2014 (Wandera, *et al* 2017). However prevalence of rotavirus remains high in Kenya and other east African countries despite the introduction of the vaccine (Wandera *et al*., 2017; Tate *et al.*, 2016). Increased vaccine coverage can be encouraged to decrease the infection further. According to these study the children between the ages of 7 to 12 years were more affected followed by 13 to 18 months. Rotavirus infection among males was more

compared to those of females. The outpatient with infection were more compared to inpatients. The study had similar conclusions to a study done by Surajudeen *et al* (2011) which indicated that males were more affected than females.

In another study done in Mukuru in Kenya post vaccination, it indicated age categories most affected age group is between 6 to 24 months (Raini *et al.,* 2015). In Rwanda similar study was done 2014-2015 and similar conclusions that most affected groups were 6 months to 24 months (Ngabo *et al*., 2016). The study carried by Odiit *et al*., (2014) indicated that the most affected age group was 13 to 24 months just as this study found. This is due to the wadding of maternal antibodies resulting in children becoming more vulnerable as their own immune system slowly builds antibodies (Wandera *et al., 2*017). The effective way to reduce prevalence of Rotavirus through vaccination. The effectiveness on the vaccine on study carried in Rwanda showed the vaccine is less effective as compared to developed countries like USA (Umesh *et al.,* 2016). The efficacy of Rotavirus vaccine in Africa in reducing rotavirus disease burden is 65% (Armah *et al*., 2010).

The study showed a decrease in prevalence and no new genotypes as compared from previous studies carried out across the country (Kiulia *et al*., 2008; Nokes *et al*., 2011; Mwenda *et al*., 2010; van Hoek *et al*., 2012). There was no difference in infection rates among gender, inpatients and out patients. This shows that the rotavirus infection is distributed evenly and no group is affected more than the other. The chi-square analysis statistic was 0.6980 and P value was 0.873472 showed no association relationship Rotavirus of genotypes in relation to age and sex as indicated by the study. This can be attributed to infection of various genotypes being random. This study indicated the main G1 genotype was G1 G2 and G3, and the P type were P4, P8 and P6. These genotypes are very common across species. The G-P combination showed the most prevalent was The G-P combination showed that G1P[8] was more prevalent followed by G2P[4], G3P[6] and G9[P8]. The mixed infections detected G1, 2 P [4, 8] and G3,2 P[4,6]. The study findings are compared to other studies carried Wandera, *et al* (2017) and Agutu *et al,* (2017) in Kenya, the most prevalent VP7 is G1 followed by G9, G8, G2 and G12, no

new genotypes have been detected. In the VP4 gene diversity the P[8] was the most prevalent followed by P[4] and P [6]. In the comparison of G-P combinations in the studies mentioned above, the top three most prevalent were; G1P[8]; G9P[8] and G8P[4]. This can be attributed to the genotype being more in circulation in the environment. The other genotypes detected included; G1P [4] ; G8P [8] G9P [4]; G12 P [4] G9P [6] ; G8P [4] ; G3P [8] G3P [6] ; G3 P[4]; G2 P [8]; G2P [4]; G1P [4]; GI P[6] (Wander. *et al.*,2017; Nokes., *et al.*, 2011; Agutu *et al.*, 2017). The globally prevalent genotypes are; G1P[8],; G2P[4]; G3P[8]; G9P[8]; G4P[8] and G12P[8] (Doro *et al*., 2014) with the G1P[8] and G9P[8] being the most prevalent both globally and in Kenya while G8P[4], which is among Kenya's most prevalent genotypes, was not prevalent globally.

The genotype diversity in the African continent showed that; G1P[8]; G2P[4], G3P[8], G4P[8], G9P[8], were common (Doro *et al*., 2014). The G1P[8] and G9P[8] are common in Kenya, Africa and globally. Some genotypes like G2P[4] and G3P[8] were common in Africa but detected in relatively small numbers in Kenya (Doro *et al*., 2014; Wander. *et al* 2017; Nokes., *et al* 2011; Agutu *et al* 2017). The unusual strains detected in Kenya included; G1P[4], G8P[8], G12P[4] and G1P[6] (Doro *et al*., 2014).The Rotarix® vaccine protects against GIP[8] which was the most prevalent genotype. The unusual genotypes which have been detected in Africa include G8P[6], G10, and G12 P[6] (Armah *et al*., 2003). The genotypes in circulating in Kenya are similar to the genotypes in Tanzania which include G9, G1, G3, P[8], P[6],and P[4] (Nyang'ao *et al*., 2010). Study carried in Nairobi informal settlement it showed G1P[8] to be the most prevalent affecting 40% of the infections (Raini *et al*., 2015).

The NGS results from the study have similar findings as those of studies carried around the globe where G1P[8] is the dominant genotype. (Santos and Hoshino 2005; Matthijnssens and Van Ranst 2012). Studies carried out in Belgium and Australia in pre-vaccination period showed that G1P[8] was the dominant genotype detected. The rotavirus genotype distribution geographically fluctuates from time to time in the absence of the vaccination (Kirkwood 2010; Zeller *et al*. 2010). The genotypes from

G1P[8], G3P[8] and G1G2P[4,8] were found to have a genetic constellation belonging to Wa-like which were identical to the ones isolated in Nicaragua, Japan and Belgium while the G2P[4], G12P[6], G8P[4] and G9P[4] had genetic constellations that were DS-1 like, similar to the ones isolated in Bangladesh, Malawi and India. The results showed no difference in other studies carried out during both pre and post vaccination which showed that pre-vaccine G1P[8] strains had a typical Wa-like genetic constellation (Buttery *et al*., 2011; Drummond *et al.,* 2012; Magagula *et al.,* 2015 and Zeller *et al.,* 2010). This can be attributed to the vaccine stimulating the immune system to offer protection as well as to the vaccine coverage. The G1P[8] backbone strains detected after vaccine introduction was DS-1 like genotype constellation which is associated with G2P[4] strains, the G9 and G12 strains and with G8P[4] or G8P[6] strains in Africa (Buttery *et al*., 2011; Drummond *et al.,* 2012). The studies from Africa and Asia show similarity of a typical DS-1 like G1P[8] which showed distinct phylogenetic clustering patterns in 11 genome segments (Kuzuya *et al*., 2014; Fujii *et al*., 2014; Yamamoto *et al*., 2014; Komoto *et al*., 2015; Komoto *et al*.,2016 and Nakagomi *et al* 2017). There has been evidence of frequent reassortment between DS-1 and Wa-like Strains in Malawi in G1P[8] (Bar-Zeev *et al*., 2016)

5.2 Conclusions

1. The most affected age group was 7 to 12 months followed by 12 to 24 months then 4 to 6 months 25 to 60 months and finally 0 to 3 months, more males were affected compared to females and the outpatients were more compared to inpatients.
2. The prevalence of Rotavirus was at 16.34% among children with gastroenteritis at KNH.
3. The most prevalent genotypes, G-type was G1,G2, G3,and G9 respectively, while there was some mixed infection which included G1, 2 and G2,3. The P-type P[8] was prevalent followed P4 and P[6] . There were mixed infection which includes P[4,8] and P[4,6 . The G-P combination showed that G1P[8] was

more prevalent followed by G2P[4], G3P[6] and G9[P8]. The mixed infections detected G1, 2 P [4, 8] and G3,2 P[4,6]

4. The Phylogenetic analysis showed that the genotypes from G1P[8], G3P[8] and G1G2P[4,8] were found to have a genetic constellation belonging to Wa-like which were identical to the ones isolated in Nicaragua, Japan and Belgium while the G2P[4], G12P[6], G8P[4] and G9P[4] had genetic constellations that were DS-1 like, similar to the ones isolated in Bangladesh, Malawi and India.

5.3 Recommendations

1. Country wide prevalence studies be done to monitor the efficacy of the vaccine.
2. Genotyping studies be carried out annually in order to detect new strains as soon as they emerge.
3. Further studies on the serotypes of rotavirus causing disease in the post vaccine era should be pursued.
4. Vaccine evaluation studies should be carried out in Kenya to evaluate the efficacy of the rotavirus vaccine.

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APPENDICES

Appendix I: Informed Consent information Form

CONSENT FORM (GUARDIAN / SUBJECT)

Study title

Molecular characterization of rotavirus strains among the children under five years with gastroenteritis at KNH, Nairobi.

INVESTIGATORS

PRINCIPAL INVESTIGATOR

1. Jeremiah Omari Zablon, Kenyatta National Hospital Lab medicine department & Jomo Kenyatta University of Agriculture & Technology
   Contact: +254724374080, Email: Jzablon2000@yahoo.com

Supervisors

Dr. Raphael Lihana (KEMRI): **Mobile:** 0733-735 562 **Email**: lihanaraphael@gmail.com

Dr. Steven Ger Nyanjom (JKUAT), **Mobile:** 0721593457 **Email**: snyanjom@gmail.com

WHY IS THE STUDY BEING DONE?

The research we are carrying will look at the type of the virus known as 'rotavirus' causing diarrhea in children under 5 years old. The study will also investigate the specific type a rotavirus strain present in diarrheal stool samples obtained from children under 5 years admitted at KNH hospital or seeking pediatric clinical services at KNH outpatient clinics. So our recruitment site is Kenyatta National Hospital Nairobi. The results of this study will be shared among all collaborating institutions (Kenya Medical Research Institute (KEMRI, Kenyatta National Hospital and Jomo Kenyatta University of Agriculture and Technology) and other invited stakeholders including the Ministry of Health (MoH) officials who will take the necessary interventions. The study will have a long team benefit to the general population, as the results will provide information on circulating rotavirus strain(s) in this region and highlight the importance of rotavirus epidemiology among children. Please feel free to ask any question about the study or what will happen when your child participates in the study and possible risks and benefit.

HOW MANY PEOPLE PARTICIPATE IN THE STUDY?

A total of 355 of children who present with symptoms of gastroenteritis

WHAT DOES THE STUDY INVOLVE?

The study involves random collection of diarrheal stool samples among children less than 5 years presenting with gastroenteritis from KNH pediatric wards and pediatric clinics. You will be provided with sterile stool container to collect the stool sample (one teaspoon or 5mls) during the visit. Ward patients will be given containers in which

stools will be collected in the morning. Samples will be processed and stored frozen at Immunology Laboratory, Kenyatta National Hospital for later immunoassay laboratory experiments to detect samples infected with rotavirus and confirmation of the infecting rotavirus strain(s) at partner institutions (KEMRI and CDC Atlanta).

CONSENT FOR COLLECTION, STORAGE AND TRANSPORTATION OF SAMPLES FROM KENYATTA NATIONAL HOSPITAL TO CENTER FOR VIRUS RESEARCH (KEMRI) AND CDC ATLANTA (*PLEASE CHECK ONE BOX ONLY).*

YES ☐ NO ☐

HOW LONG WILL SAMPLE COLLECTION TAKE?
It will take approximately 6 months to g*et al*l stool samples (355 stool samples)

WHAT ARE THE RISKS FOR THE STUDY?
You will collect the stool in containers that will be provided to you during the normal defecation process. This will therefore not subject you to any pain.

ARE THERE BENEFITS FOR TAKING PART IN THE STUDY?
Subjects will not have any direct benefits. However, the results of the study will be shared with KEMRI and other relevant authorities, who will take measures to advice general public and take necessary actions. As already stated, the results that will be obtained may provide useful information on the circulating rotavirus strain(s) in this region and highlight the importance of rotavirus prevention and control using appropriate vaccines and other interventions.

WHAT ABOUT CONFIDENTIALITY?
All the information obtained will be strictly confidential. Study unique code identifiers will be assigned to participants' study tools (questionnaires, study requisition forms) and samples to maintain confidentiality. Only the principal investigator, co-investigators, officials in Kenyatta National Hospital and if need be, appointed authority from Kenyatta National Hospital University of Nairobi Ethics Committee (KNH/UoN-ERC) will be able to access the information. You will remain anonymous during and after study, being identified only by the personal identification Numbers (PIN) assigned to you. The results obtained will be made available to the health care givers if you give a written permission.

WHAT ARE THE COSTS?
You do not pay anything to participate in this study.

SUMMARY OF YOUR RIGHTS AS A PARTICIPANT IN A RESEARCH STUDY

This study is absolutely voluntary, you will be free to withdraw from the study at any point and will not be penalized in any way, and you are not also waiving any of your legal rights by signing this informed consent document.

CONTACT INFORMATION

The following person will be available for contact in event of any research related questions or comments.

Principal Investigator: Jeremiah Omari Zablon, Kenyatta National Hospital

Telephone: 0724374080: Email:Jzablon2000@yahoo.com

And in case of complaints regarding rights of participation, one can always contact the secretary, KNH/UoN-ERC on Tel: +254-020- 2726300 Ext 44355, Email: uonknh_erc@uonbi.ac.ke

PARTICIPANT CONSENT FORM

I have read the consent information form and conditions of this project. All my questions have been answered and I hereby give my voluntary consent.

CONSENT FOR STOOL COLLECTION GIVEN YES ☐ NO ☐

SIGNATURE OF PARTICIPANT_____DATE_____

PRINTED NAME OF PERTICIPANT_____

Name and signature of the witness_____

*Only in case the subject is not literate and thumb is used. *Must not be the person-collecting specimen

SIGNATURE OF THE PERSON OBTAINING CONSENT_____DATE_____

DATE PRINTED NAME OF THE PERSON OBTAINING CONSENT

(*Must be the principal investigator or individual who has been designated in the checklist to obtain consent*)

SIGNATURE OF THE PRINCIPAL INVESTIGATOR OR DESIGNEE_____DATE_____

Verbal Consent Information Form

Hello, my name is Mr. Jeremiah Omari Zablon (0724374080, Jzablon2000@yahoo.com) the principal investigator for this study from Kenyatta National Hospital, Nairobi County. My supervisors are: Dr. Steven Ger Nyanjom (JKUAT) **:** 0721593457 **Email**: snyanjom@gmail.com and Dr. Raphael Lihana (KEMRI): **Mobile:** 0733735562 **Email**: lihanaraphael@gmail.com

Your child has been chosen at random to participate in the study to look at rotavirus infection among children below five years. This will take approximately 20 minutes of your time. If your child is having diarrhea and you agree your child to be enrolled in this

study, you shall answer a few questions. After answering them, you will be given a container and requested to go to the toilet with your child and collect one teaspoonful of stool (5mls) into the provided stool container. The stool will be tested for rotavirus and results will be given to your doctor for your child to be treated. You will not pay for the test.

Participating in this study does not benefit you financially or any other way. If you feel you have not been treated well during the study, or have questions concerning your child's rights during the study period please call The Secretary/ Chairperson KNH-UoN ERC on Tel No. 2726300 Ext 44355

To participate in this research is absolutely voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to stop.

I certify that I have consented the participant (code no.)…….

Researcher's name:……...

Signature:…….....

Date:……

Consent Form Explanation (Swahili)

KARATASI YA KUFAHAMISHA IDHINI (WATU WAZIMA)

KICHWA:

Uchunguzi waainaya virusi vya rota vinavyosababisha ugonjwa wakuhara katika maeneoya Kenyatta.

WACHUNGUZI:

MCHUNGUZI MKUU

1. Jeremiah Omari Zablon

WASAIDIZI

1. Dr. Steven Ger Nyanjom (JKUAT)
2. Dr. Raphael Lihana (KEMRI)

MADHUMUNI

Mradi huu unachunguza aina ya virusi vyarota vinavyosababisha ugonjwa wakuharisha katika maeneo ya Nairobi. Sampuli zitakusanywa kutoka hospitali kuu ya Kenyatta kutoka kwa wagonjwa amabao wanakuja hosiptalini kupata matibabu. Matokeo ya uchunguzi huu yatakayowasilishwa katika kituo cha utafiti wa magonjwa mbalmbali ya binadamu (Kenya Medical Research Institute- KEMRI), kitachokua jukumu kufuatiana na matokeo ya utafiti ni kuelimisha na kusaidia uma kwa njia mbalimbali. Matokeo ya kazi hii pia yata faidi jamii kwa jumla kwa kutujulisha aina ya virusi vya rota katika maeneo ya Nairobi.

IDADI YA WASHIRIKI

Utafiti huu utahusisha washiriki 355 ambao watakua ni watoto wachanga wasio zidi umri wa miaka mitano wana dalili ya kuwa na virusi vya rota.

TARATIBU ZA UTAFITI

Utafiti unafanywa kwa wagonjwa wanao pata matibabu katika hosipitali kuu ya Kenyatta. Unahusisha uchunguzi wa vinyesi vinavyotolewa na wagonjwa wanaohisi maumivu ya tumbo yanayosababisha ugonjwa wa kuharisha. Utapewa chupa utakayotumia kuweka kenyesi cha asubuhi cha mtoto wako na utakileta zaharatini. Vinyesi vitakusanywa nakuhifadhiwa. Baadaye sampuli hizi zitasafirishwa hadi mahabara ya hospitali ya Kenyatta kisha baadaye chuo cha utafiti cha KEMRI na CDC Altanta, Marekani.

IDHINI YA KUIFADHI NA KUSAFIRISHA SAMPULI KUTOKA HOSIPITALI YA MUKURU KWA NJENGA HADI CHUO CHA UTAFITI CHA KEMRI

NDIO ☐ LA ☐

CHAGUA MOJA

NI MUDA GANI NITADUMU KWA MRADI?

Mradi huu utachukua miezi nane.

MADHARA

Hakuna uchungu wowote mtoto wako atakaohisi. Utaweka kinyesi cha mtoto wako ndani ya chupa wakati wakuenda haja kubwa kama kawaida.

FAIDA

Hakuna faida yoyote yakibinafsi utapata. Yamuhimu nikwamba majibu ya utafiti yatatumiwa baada ya idhini ya mshirika, kuelimishwa na kusaidia uma. Matokeo ya kazi hii pia yatafaidi jamii kwa jumla kwa kutujulisha aina ya virusi vya rota katika wagonjwa wanao kuja hospitalini Kenyatta, Nairobi. Udhabiti wa mahabara wa virusi vya rota utafanywa bila malipo na mtoto wako kupewa majibu ya udhabiti ndio daktari wako atatumia kumtibu mtoto wako.

SIRI YA HALI YAKO

Matokeo ya utafiti yatawekwa kwa siri. Mchunguzi, wasaidizi wachunguzi, maafisa ambao wahahusika kutoka hosipali ya Kenyatta na kutapokokua na haja atakaye idhinishwa na KNH/UoN-ERC ndio pekee watakua na idhini ya kuyafikia. Hakuna jina litakalochapishwa popote wakati hata baada ya uchunguzi kukamilika. Utajulikana kwa nambari ya siri utakayopewa baada ya idhini yako yakushiriki.

GHARAMA

Hakuna ada au gharama yeyote utatozwa ili mtoto wako ashiriki katika mradi huu.

MUHKTASARI JUU YA HAKI ZAKO KAMA MSHIRIKI KATIKA MRADI

Kushiriki kwa mtoto wako katika mradi huu ni kwa hiari yako unaweza kujiondoa wakati wowote bila kupoteza faida yeyote inayotokana na mradi huu. Vilevile hutahujumu haki za mtoto wako kwa kutia kidole kwenye stakabadhi hii.

HABARI ZINZPATIKANA

Maswali yoyote kuhusu mradi huu yatastahili kuelekezwa kwa

Mchunguzi mkuu: Jeremiah Omari Zablon-Kenyatta National Hospital Lab medicine

Simu:0724374080

Parua Pepe:Jzablon2000@yahoo.com

Pia maswali yeyote kuhuzi wachunguzi wa mradi huu au haki zako au mtoto wako kukandamiza utaelekaza kwa:

KNH/UoN: Karani

Nambayasimu : (254-020) 2726300 Ext 44355

Pepe: uonknh_erc@uonbi.ac.ke

IDHINI YA MUHUSIKA

Nimesoma fomu hii na mashariti ya mradi huu.

Maswali yangu yamejibiwa na kwahvyo natoa idhini ya kushiriki kwa mradi huu na sampuli ya mtoto wangu kuchululiwa.

Sahihi ya mshiriki_____Tarehe-_____

Jina_____Tarehe_____

Jina la shahidi_____Sahihi_____

*Kwa wale washiriki wasiojua kusoma nakuandika *Lazima awe mtuhuru

Sahihiyaanayechukuaidhini_____Tarehe_____

Jina_____Tarehe_____

Lazima awe mtafiti /mchunguzi amamtu aliyepe wajukumu la kupewaidhini)

Sahihi ya mchunguzi mkuu

_____Tarehe_____

Appendix II: Details of Genotyping Results

No.	Sample ID	Age (Months)	Sex	Address	Elisa	Vaccination	NSP3-qRT-PCR C_T values	VP7	VP4	VP7	VP4	VP7/VP4
1	3418	9	Male	3C	Negative	NO	√ (37.97)			G2		
2	3473	1	Male	3A	Negative	NO	Negative					
3	3478	4	Male	3A	Negative	NO	Negative					
4	3532	4	Male	PEU	Negative	NO	√ (32.06)					
5	3534	20	Male	PEU	Negative	NO	Negative					
6	3610	13	Female	PEU	Negative	NO	√ (34.94)					
7	3621	15	Male	PEU	Negative	NO	Negative					
8	3638						√ (17.68)	G1	P[8]	G1, 2		G1P[8]
9	3639						√ (29.739)		P[4]	G2		G2P[4]
10	4005	24	Male	3A	Negative	Yes	Negative					
11	4008	14	Female	3C	Negative	Yes	Negative					
12	4012	13	Male	3B	Negative	Yes	Negative					
13	4015	12	Male	3B	Negative	Yes	Negative					

14	4016		Female	3D	Negative	Yes	Negative
15	4017	12	Female	3A	Negative	Yes	Negative
16	4018	9	Male	PEU	Negative	Yes	Negative
17	4020	12	Female	PEU	Negative	Yes	Negative
18	4021	19	Male	PEU	Negative	Yes	Negative
19	4022	16	Male	PEU	Negative	Yes	Negative
20	4023	13	Female	PEU	Negative	Yes	Negative
21	4029	24	Male	PEU	Negative	Yes	Negative
22	4031		Female	3A	Negative	Yes	Negative
23	4036	27	Female	3D	Negative	Yes	Negative
24	4040	10	Male	3B	Negative	Yes	Negative
25	4041		Male	3C	Negative	Yes	Negative
26	4044	10	Male	3A	Negative	Yes	Negative
27	4049	18	Female	3B	Negative	Yes	Negative
28	4050	19	Male	PEU	Negative	Yes	Negative

29	4053	9	Male	PEU	Negative	Yes	Negative						
30	4057	9	Male	3D	Negative	Yes	Negative						
31	4059	6	Female	PEU	Negative	Yes	Negative						
32	4062		Male	PEU	Negative	Yes	Negative						
33	3707	16	Male	3A	Negative	Yes	Negative						
34	3719	24	Female	3C	Negative	Yes	Negative						
35	3720	12	Female	3B	Negative	Yes	Negative						
36	3721	12	Female	3C	Negative	Yes	Negative						
37	3723	12	Female	3C	Negative	Yes	Negative						
38	3724	16	Male	3C	Positive	Yes	√ (16.72)	G1	P[8]	G1	P[8]	G1P[8]	
39	3735	5	Male	3C	Negative	Yes	Negative						
40	3739	3	Female	3C	Negative	Yes	√ (36.51)						
41	3740	10	Male	3B	Negative	Yes	√ (31.24)						
42	3741	9	Male	3B	Negative	Yes	Negative						
43	3744	9	Male	3A	Negative	Yes	Negative						

44	3748	10	Female	3C	Negative	Yes	√ (31.24)	G1	P[8]		G1P[8]
45	3749	7	Male	PEU	Negative	NO	Negative				
46	3750	36	Male	PEU	Negative	NO	Negative				
47	3765	5	Male	3C	Negative	Yes	Negative				
48	3767	12	Female	3D	Negative	Yes	Negative				
49	3768	3	Female	3C	Negative	Yes	Negative				
50	3769	16	Female	3B	Negative	Yes	Negative				
51	3773	12	Female	PEU	Negative	NO	Negative				
52	3811	13	Male	PEU	Negative	Yes	Negative				
53	3812	15	Male	3C	Negative	Yes	Negative				
54	3816		Male	3D	Negative	NO	Negative				
55	3829	19	Male	3C	Negative	Yes	Negative				
56	3831	7	Male	PEU	Negative	Yes	√ (24.88)	G3	P[6]	P[6]	G3P[6]
57	3858	3	Female	PEU	Negative	Yes	Negative				
58	3888	10	Male	PEU	Negative	Yes	Negative				

59	3940	15	Male	3A	Negative		Negative					
60	3941	13	Female	PEU	Negative	Yes	√ (27.11)	G2	P[4]	G2	P[4]	G2P[4]
61	3946	9	Male	PEU	Negative	Yes	√ (27.87)	G3,2	P[4] [6]	G2, 3	P[4]P [6]	G3,2P[4]P[6]
62	3952	9	Male	3C	Negative	Yes	√ (24.53)	G3	P[6]		P6	G3P[6]
63	3956	24	Male	PEU	Negative	Yes	Negative					
64	3966	3	Male	3A	Negative	Yes	Negative					
65	4001	3	Male	3D	Negative	Yes	Negative					
66	4015	12	Male	3D	Negative	Yes	Negative					
67	4020	2	Female	PEU	Negative	Yes	Negative					
68	4042	11	Male	3C	Negative	Yes	Negative					
69	4043	27	Female	3C	Negative	Yes	Negative					
70	4052	9	Male	PEU	Negative	Yes	Negative					
71	4055	7	Male	PEU	Negative	Yes	Negative					
72	4057	9	Male	3D	Negative	Yes	Negative					
73	4059	6	Female	PEU	Negative	Yes	Negative					

74	3901	20	Male	PEU	Negative	Yes	Negative					
75	3902	6	Male	PEU	Negative	Yes	Negative					
76	3903	8	Female	3C	Negative	Yes	√ (31.78)		P[6]			
77	3904	10	Male	3D	Negative	Yes	Negative					
78	3905	12	Female	PEU	Negative	Yes	Negative					
79	3908	14	Female	3D	Negative	Yes	Negative					
80	3927	16	Female	PEU	Negative	Yes	Negative					
81	3928	24	Male	3B	Negative	Yes	Negative					
82	3929	15	Female	PEU	Negative	Yes	√ (34.77)					
83	3930	24	Male	PEU	Negative	Yes	Negative					
84	3939	24	Female	PEU	Negative	Yes	Negative					
85	3936		Male	PEU	Negative	Yes	Negative					
86	3941	13	Female	PEU	Negative	Yes	√ (27.11)	G2	P[4]	G2	P[4]	G2P[4]
87	3943	13	Female	PEU	Negative	Yes	√ (27.87)					
88	3752	11	Male	PEU	Negative	Yes	Negative					

89	3943	13	Female	PEU	Negative	Yes	√ (27.87)	G1	P[8]	G1		G1P[8]
90	3952	9	Male	3C	Negative	Yes	√ (24.53)	G3	P[6]		P[6]	G3P[6]
91	3956	24	Male	PEU	Negative	Yes	Negative					
92	3958	8	Male	PEU	Negative	Yes	Negative	G1	P[8]			G1P[8]
93	3979	12	Male	3C	Negative	Yes	√ (30.32)		P[4]	G2		G2P[4]
94	3978	4	Female	PEU	Negative	Yes	√ (18.18)	G1	P[8]	G1, 3	P[8]	G1P[8]
95	3976	12	Male	PEU	Negative	Yes	Negative					
96	3955	24	Male	3A	Negative	Yes	Negative					
97	3960	8	Female	3D	Negative	Yes	Negative					
98	3976	12	Male	PEU	Negative	NO	Negative					
99	3973	12	Male	PEU	Negative	NO	Negative					
100	3972	22	Male	PEU	Negative	NO	Negative					
101	3971	4	Female	PEU	Negative	NO	√ (33.33)					
102	3963	10	Female	PEU	Negative	NO	Negative					
103	3964	24	Male	PEU	Negative	NO	Negative					

104	3965	24	Male	3A	Negative	NO	Negative					
105	3966	3	Male	3A	Negative	NO	Negative					
106	3967	24	Male	PEU	Negative	NO	Negative					
107	3982	14	Male	PEU	Negative	NO	Negative					
108	3984	24	Female	PEU	Negative	NO	√ (35)	G1	P[8]			G1P[8]
109	3985	9	Female	3B	Negative	NO	Negative					
110	3987	24	Male	3D	Negative	NO	√ (24.49)	G1	P[8]	G1	P[8]	G1P[8]
111	3988	14	Male	3D	Negative	NO	√ (30.15)	G2	P[4]	G2		G2P[4]
112	3990	4	Male	PEU	Negative	NO	Negative					
113	3991	11	Male	PEU	Negative	NO	√ (30.15)					
114	3992	8	Female	PEU	Negative	NO	Negative	G3	P[6]			G3P[6]
115	3995	17	Female	3D	Negative	NO	Negative					
116	3994		Male	PEU	Negative	NO	√ (12.35)	G3	P[6]	G3	P[6]	G3P[6]
117	3852	2	Male	3D	Negative	Yes	√ (33.61)					
118	3811	13	Male	PEU	Negative	Yes	Negative					

119	3813		Male	3C	Negative	Yes	√ (32.84)
120	3826	12	Female	3A	Negative	Yes	Negative
121	3827		Male	3A	Negative	Yes	Negative
122	3829	19	Male	3C	Negative	Yes	Negative
123	3830	4	Male	3A	Negative	Yes	Negative
124	3837	8	Female	3A	Negative	Yes	Negative
125	3841	6	Male	3C	Negative	Yes	Negative
126	3854	12	Female	3B	Negative	Yes	Negative
127	3868	9	Male	3B	Negative	Yes	√ (32.86)
128	3886	7	Male	3C	Negative	Yes	Negative
129	3896	12	Female	3C	Negative	Yes	Negative
130	3897	5	Female	3D	Negative	Yes	Negative
131	3899	12	Male	3C	Negative	Yes	Negative
132	3857	9	Male	PEU	Negative	Yes	Negative
133	3872	4	Male	3A	Negative	Yes	Negative

134	3808	17	Female	PEU	Negative	Yes	Negative					
135	3722	8	Male	3B	Positive	Yes	√ (21.11)	G2	P[4]	G2	P[4]	G2P[4]
136	3707	16	Male	3A	Negative	Yes	Negative					
137	3702	4	Female	PEU	Negative	NO	Negative					
138	3720	12	Female	3B	Negative	Yes	Negative					
139	3728	15	Female	3A	Negative	Yes	√ (28.49)					
140	3727	9	Male	3D	Negative	Yes	Negative					
141	3726	9	Male	3D	Negative	Yes	Negative					
142	3725	15	Female	3C	Negative	Yes	√ (29.45)					
143	3739	3C	Female	3C	Negative	Yes	√ (31.06)			G1		
144	3738	13	Male	3C	Positive	Yes	√ (21.19)	G2	P[4]	G2	P[4]	G2P[4]
145	3736	4	Male	3C	Negative	Yes	Negative					
146	3757	13	Male	PEU	Negative	NO	√ (31.92)					
147	3756	24	Female	3D	Negative	Yes	Negative					
148	3763	19	Male	3C	Negative	Yes	Negative					

149	3762	4	Female	3C	Negative	Yes	Negative					
150	3751	9	Male	3C	Negative	Yes	Negative					
151	3770	10	Female	PEU	Negative	NO	Negative					
152	3748	10	Female	3C	Negative	Yes	√ (22.02)	G1	P[8]	G1	P[8]	G1P[8]
153	3746	5	Male	3C	Negative	Yes	Negative					
154	3745	17	Female	3D	Negative	Yes	Negative					
155	3744	9	Male	3A	Negative	Yes	Negative					
156	3743	20	Male	3D	Negative	Yes	Negative					
157	3742	16	Male	PEU	Positive	Yes	√ (12.54)		P[8]	G1	P[8]	G1P[8]
158	3741	9	Male	3C	Negative	Yes	√ (34.63)	G1			P[6]	G1P[6]
159	3740	10	Male	3B	Negative	Yes	Negative					
160	3932	13	Female	3A	Positive	Yes	√ (27.39)	G1				
161	4054	13	Male	9A	Positive	NO	√ (20.0)	G2	P[4]	G2	P[4]	G2P[4]
162	4047	14	Female	PEU	Positive	NO	√ (19.87)	G1,2	P[4][8]	G1, 2,	P[8]	G1,2P[4][8]
163	3607	23	Male	PEU	Positive	Yes	√ (15.85)	G1	P[8]	G1	P[8]	G1P[8]

164	3920	8	Female	PEU	Positive	Yes	√ (18.61)	G2	P[4]	G2	P[4]	G2P[4]
165	3977	12	Male	PEU	Positive	NO	√ (19.65)	G2	P[4]	G2	P[4]	G2P[4]
166	3413	36	Female	3C	Positive	NO	√ (19)	G1	P[8]	G1	P[8]	G1P[8]
167	3468	12	Male	PEU	Positive	NO	√ (16)	G9	P[8]	G9	P[8]	G9P[8]
168	3471	8	Male	PEU	Positive	NO	√ (15)	G9	P[8]	G9	P[8]	G9P[8]
169	4038		Male	3D	Positive	NO	√ (16)	G9	P[8]	G9	P[8]	G9P[8]
170	4011	7	Female	PEU	Positive	NO	√ (24)	G2	P[4]	G2	P[4]	G2P[4]
171	4019	12	Male	3B	Positive	NO	√ (22)	G9,2	P[4][8]	G1,2	P[4]P[8]	G1,2P[4][8]
172	4009	14	Male	3C	Positive	NO	√ (17)	G3	P[6]	G3	P[6]	G3P[6]
173	3755	13	Male	PEU	Positive	Yes	√ (15)	G1,2	P[4][8]	G1,2	P[4]P[8]	G1,2P[4][8]
174	3818	7	Male	3B	Positive	Yes	√ (13)	G3	P[6]	G1,3	P[6]	G1,3P[6]
175	3753	8	Female	3A	Positive	Yes	√ (12)	G1	P[8]	G1	P[8]	G1P[8]
176	4051	12	Female	PEU	Positive	NO	Negative					
177	3754	8	Male	PEU	Positive	NO	√ (16)	G1	P[8]	G1	P[8]	G1P[8]
178	3731	9	Male	3A	Positive	Yes	√ (21)	G2	P[4]	G2		G2P[4]

179	3944	7	Female	5	Positive	NO	√ (19)	G1	P[8]		P[8]	G1P[8]
180	3547	8	Male	PEU	Positive	NO	√ (38)			G1		
181	3993	12	Female	3C	Positive	NO	√ (40)	G1				G1

Appendix III: Next generation Sequencing Data

	DASH Number	Qubit Conc. (ng/mL)	Qubit Conc. (ng/uL)	Illumina Conc. (nM)	Dilution to 4nM	Lib (uL)	Buffer	Genotype RT-PCR	NGS Genotype	Final Genoty
1	3638	75.6	7.6	33.1	8.27	2	14.5	G1P[8]	G1P[8]	G1P[8]
2	3724	627.0	62.7	235.1	58.78	2	115.6	G1P[8]	G1P[8]	G1P[8]
3	3978	112.0	11.2	49.0	12.25	2	22.5	G1P[8]	G1P[8]	G1P[8]
4	3994	510.0	51.0	223.1	55.78	2	109.6	G3P[6]	G3P[6]	G3P[6]
5	4054	245.0	24.5	122.5	30.63	2	59.3	G2P[4]	G2P[4]	G2P[4]
6	4047	34.4	3.4	17.2	4.30	2	6.6	G1,2P[4][8]	G1,2P[4][8] W	G1,2P[
7	3607	468.0	46.8	234.0	58.50	2	115.0	G1P[8]	G1P[8]	G1P[8]
8	3920	448.0	44.8	224.0	56.00	2	110.0	G2P[4]	G2P[4]	G2P[4]
9	3413	101.0	10.1	50.5	12.63	2	23.3	G1P[8]	G1P[8]	G1P[8]
10	3468	580.0	58.0	290.0	72.50	2	143.0	G9P[8]	G9P[8]	G9P[8]
11	3471	844.0	84.4	422.0	105.50	2	209.0	G9P[8]	G9P[8]	G9P[8]
12	4038	7.8	0.8	3.9	0.97	2	-0.1	G9P[8]	Negative	G9P[8]
13	4019	146.0	14.6	73.0	18.25	2	34.5	G1,2P[4][8]	G2P[4]	G1,2P[
14	4009	590.0	59.0	258.1	64.53	2	127.1	G3P[6]	G3P[6]	G3P[6]
15	3755	570.0	57.0	213.8	53.44	2	104.9	G1,2P[4][8]	G1P[8]	G1,2P[
16	3818	276.0	27.6	103.5	25.88	2	49.8	G1P[8]	G1P[8]	G1P[8]
17	3754	189.0	18.9	94.5	23.63	2	45.3	G1P[8]	G1P[8]	G1P[8]

Appendix IV: Publication

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Prevalence of Rotavirus at Kenyatta National Hospital among children under 5 years of age presenting with gastroenteritis

Authors
Jeremiah Omari Zablon[1,2], Shaiva Nathan[4], Raphael Lihana[3], Steven Ger[1] Bakarimwinyi[2], Micah Oyaro[5], Vincent Gitau[2], Titus Gitonyi[2], Felister O[2], Raphael G[2], Roseline O [2], James Nyangao[3]
[1]Jomo Kenyatta University of Agriculture and technology
[2]Kenyatta National Hospital
[3]Kenya Medical Research Institute
[4]Masinde Muliro University of Science and Technology

Abstract
Introduction: *Diarrhea has been one of the principal causes of death and the major cause of diarrhea is rotavirus. The global mortality caused by rotavirus is 453,000 annually and 80% of these deaths occur in developing countries. The effective way to control rotavirus is through vaccination. Two vaccines have been licensed for use which is; Rotateq and Rotarix. The Rota Teq vaccine was introduced in Kenya July 2014.*
Objective: *Determine the prevalence of RVA after the introduction of the vaccine. Samples were collected from children below 5 years of age presenting gastroenteritis whose parents consented to be in the study.*
Method: *The samples were collected from children below 5 years with gastroenteritis and analyzes by ELISA using prospect kit. Data were analyzed by chi-square and prevalence calculated in proportions.*
Results: *The prevalence of RVA was 14% as compared in previous studies which were 27%. The chi-square calculation showed no difference of RVA infection in regard to gender, inpatient, and outpatient. The most affected age group was between 7 to 12 months, followed by 13 to 24 months.*
Conclusion: *There is a decrease in rotavirus prevalence and vaccination campaigns need to be emphasized to decrease the prevalence further.*
Keywords: *Rotavirus, Kenya, Vaccine ELISA.*

Introduction

The principal causes of death among children below 5 years of age in Kenya is diarrhea, which is estimated at 9% and about 27% of under-five diarrhea hospitalization is caused by rotavirus (Liu et al., 2012; Khagayi et al., 2014). Rotavirus has been associated with acute severe diarrhea among children under five years and the major cause of mortality in low and middle-income countries in Asia and sub-Saharan Africa (Fischer et al., 2000; Parashar et al., 2006). The global mortality rate caused by rotavirus group A is estimated at 453,000 annually (Tate et al., 2012). The approximation of 85% of the deaths occurs in Africa and Asia (Parashar et al., 2006). The estimates of deaths caused by rotavirus group A in Kenya include; 68 deaths, 132 hospitalization and 21800 clinic visits per 100,000 children below 5

years old (Nyangao et al., 2010). Rotavirus affects mostly children who age from 6 to 24 months (Cunliffe et al., 1998; Gatheru et al 1993). Studies carried in sub-Sahara Africa in Kenya, Tunisia and Nigeria indicate that 90% of children get infected with rotavirus by the time they reach 2 years (Aminu et al., 2010; Trabelsi et al., 2000; Nyangao.,2010).The classification of rotavirus is a double-stranded segmented RNA (dsRNA) virus which has three concentric layers of proteins. The most effective way of control rotavirus is a safe rotavirus vaccine which will reduce the mortality rates (Rodrigo et al., 2010). There are two rotavirus group A vaccine available; a pentavalent bovine-human reassortant (Rota Teq™) and a monovalent attenuated human (HuRVA) vaccine Rotarix™ which are recommended by world health organization (WHO, 2006). The vaccines have been introduced to the 81 countries as part of their national immunization program as of May 2006. The monovalent vaccine was introduced in Kenya to the national immunization program in July 2014 (Ernest et al., 2017).

The main purpose of the study was to determine the prevalence of rotavirus after the introduction of the vaccine among children with gastroenteritis attending Kenyatta National Hospital.

Materials and Methods

Sample collection

Study site.

The study was carried at Kenyatta national hospital, located in Nairobi County in Kenya. It is the largest referral hospital in Kenya.

Study Population

The study involved children below 5 years of age presenting with gastroenteritis at Kenyatta national hospital.

Inclusion and exclusion criteria

The children who were included in the study were those whose parents or guardians consented. They were below 5 years of age and presented with acute diarrhea for not more than 7 days and had experienced three episodes of watery stools in 24 hours.

Sampling

The sampling method which was used was random and consecutive until the required sample size was reached.

A total of 355fecal samples were collected in the year 2017 from children under the age of 5 years presenting with gastroenteritis who visited Kenyatta National Hospital.

The study was approved by Kenyatta and University of Nairobi Ethical Review board.

Laboratory Method

The Enzyme Immunoassay was used for detection of RVA which was a rapid and sensitive method which has been used since the 1980s (Dennehy *et al*., 1988; Christy *et al*., 1990). The commercial kit which was used was ProSpect™

Rotavirus detection

The preparation of fecal suspension was prepared by suspending 1g of stool sample 0r 100ul rectal swap to 1ml of 0.01M phosphate- buffered saline (PBS). The suspension was vortexed vigorously and centrifuged at 10,000 rpm at 5 minutes. The suspension was tested for RVA antigen by ELISA (Taniguchi et al., 1987) using PROSPECT kit.

Results

A total number of samples collected were 355 from children presenting with gastroenteritis and which met the WHO rotavirus criteria (WHO 2002). The prevalence of rotavirus was 14.36 % (51/355). RVA distribution among gender 33/51 males was positive as compared to 18/51 female who were positive. The distribution of rotavirus group A between outpatient and inpatient showed that 27/51 were inpatient and 24/51 were outpatient. When the distribution was analyzed by chi-square to show the difference in prevalence in regard to gender, inpatient, and outpatient. The distribution showed no difference at 95 confidence interval since $p < .05$. The chi-square statistic was 0.6980 and P value was 0.873472. The results are shown in table 2. The presentation of gastroenteritis in regard to age showed that 7 to 12 months were mostly affected followed by 13 to 24 months. The least affected were from 0 to 3

months. This can be attributed to maternal antibodies. The demographic distribution of the children showed that most children came from Nairobi County and a few came from neighboring counties Like Kiambu, Kajiado, and Machakos. The presentation is presented in figure 1.

Table 1: Elisa Results

	Rotavirus Positive Cases	Gastroenteritis Cases	Total	% positive
Female	18	113	131	13.74
Male	33	191	224	14.73
Total	51	304	355	14.37
Inpatients	27	179	206	13.11
Outpatients	24	125	149	16.11
Totals	51	304	355	14.37

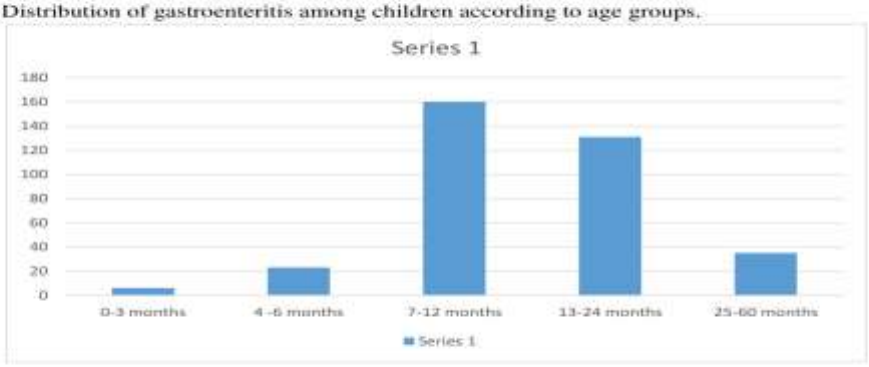
Table 2: Chi-square Results

The factors associated with rotavirus infection were analyzed with chi-square in the table below. The findings showed no difference in regard to age and gender.

Results			
	Positive	Negative	**Row Totals**
Male	33 (32.18) [0.02]	191 (191.82) [0.00]	224
female	18 (18.82) [0.04]	113 (112.18) [0.01]	131
Inpatient	27 (29.59) [0.23]	179 (176.41) [0.04]	206
Outpatient	24 (21.41) [0.31]	125 (127.59) [0.05]	149
Column Totals	102	608	

The Chi-square statistic is 0.6989. The P-value is 0.873474. The result is not significant at p<0.05.

Figure 1: Distribution of gastroenteritis among children according to age groups.



Discussion

The principal objective of the study was to establish the prevalence of RVA among children below the age of 5 years who present with gastroenteritis at Kenyatta National Hospital after the introduction of the vaccine in July 2014 (Wandera et al., 2017). The study showed that RVA prevalence was at 14% among children below 5 years who presented gastroenteritis at Kenyatta National Hospital. The prevalence

shows a decrease in prevalence as compared with studies done in different regions in the earlier years which showed an average prevalence of 27% (Wandera. et al 2017; Nokes., et al 2011; Agutu et al 2017). The review article conducted between 1975 to 2005 on the prevalence of rotavirus in Kenya indicated that the prevalence ranges between 11 to 56 % (Kiulia et al., 2008), while a study carried at Kenyatta national hospital indicated the prevalence to be 56% (Gatinu et al., 2007). The studies carried in other parts of the country like Kiambu County showed the prevalence to be 36. 6% (Apondi et al., 2012). The chi-square analysis showed no difference in distribution among gender and inpatient or outpatient. The gender factor and infection rate agrees with another study finding which looked at risk factor and diarrhea among children (Pennal et al., 2010). The gastroenteritis distribution occurred among all age groups (0 to 59 months) the most affected age group is 7 to 12 months while the least affected being 0 to 3 months. These findings agree with other studies carried out in the country and WHO surveillance team (Wandera et al., 2017; WHO 2012). At the age of 5 months the maternal antibodies which protect the child from rotavirus decline and makes the child vulnerable to infection (Odiit et al., 2014).Research indicated that the most affected children were below 18 months and vaccination will not benefit children above 24 months since they have developed antibodies against rotavirus due to exposure to rotavirus (Wandera et al., 2017; WHO 2012). The main objective of rotavirus vaccination was to prevent infection by inducing immunity before the natural infection occur (Wandera et al., 2017). The vaccination has reduced the prevalence from 27% studies carried before to 14 %. The vaccination campaign should be emphasized and more children vaccinated. Although the Rotarix vaccine protects against GIP[8] that was the most prevalent genotype. This indicates more has to be done for to increase the number of kids get the vaccine and they should get all the vaccine doses. Close monitoring of the vaccination program, the impact of the vaccine and the molecular epidemiology has to done continually to help in public health programs.

Conclusions

The prevalence of Rotavirus was 14% after vaccine introduction.

Acknowledgement

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Appendix v: Ethical Approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/329

3rd November, 2017

Jeremiah Omari Zablon
Reg. No. TM305-0217/2009
J.K.U.A.T

Dear Jeremiah,

REVISED RESEARCH PROPOSAL –CHARACTERIZATION OF ROTAVIRUSES AMONG CHILDREN UNDER 5 YEARS WITH GASTROENTERITIS AT KENYATTA NATIONAL HOSPITAL, KENYA (P372/07/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 3rd November 2017 –2nd November 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
d) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website**http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chairperson, KNH-UoN ERC
Supervisors: Dr. Raphael Lihana (KEMRI), Dr. Stephen Ger Nyanjom (J.K.U.A.T)