PREVALENCE AND GENETIC DIVERSITY OF GROUP A ROTAVIRUSES IN CHILDREN WITH ACUTE GASTROENTERITIS POST VACCINE INTRODUCTION IN KERICHO COUNTY REFERRAL HOSPITAL, KENYA

Beth Khayeli Nyamanga

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DECLARATION AND APPROVAL

DECLARATION

I declare that this thesis is my original work and has not been presented for the conferment of a degree or award of diploma in this or any other University.

SIGNATURE:_____DATE: _____

Beth Khayeli Nyamanga

PGC/MIC/0005/16

APPROVAL

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

SIGNATURE: _____ DATE: _____

Dr. Janeth J. Kombich

Department of Biological Sciences

University of Kabianga

SIGNATURE: _____ DATE:_____

Dr. Raphael W. Lihana

Centre for Virus Research

Kenya Medical Research Institute

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DEDICATION

To my father Mr Stephen Attalo Nyamanga ,loving mum the late Mrs Sarah Mtamai Nyamanga and my baby sister Vivian Musimbi Nyamanga for believing in me and your constant support.

ABSTRACT

Rotavirus infections are the chief source of acute gastroenteritis in children of five years and below causing 600,000 deaths globally and about 80% occurring in Sub-Saharan Africa and South Asia. Rotavirus has caused more than 3908 infant deaths in Kenya. After introducing the vaccine, a limited amount of information on the effects of this in Kericho County has been made available to the public. The study focused on determining the prevalence, demographic characteristics and the diversity of rotavirus strains in children seeking medical attention at the Kericho County Referral Hospital. The study used a hospital-based cross-sectional survey design. Simple random sampling was employed to recruit the study participants. The study population was children aged five years and under seeking medical attention for gastroenteritis. A sample size of 200 stools was collected using sterile stool collection polypots and transferred to the National Rotavirus Laboratory based at The Kenya Medical Research Institute, Nairobi. Demographic characteristics of the participants in the study were obtained using structured questionnaires. Identification of rotavirus-positive samples was done using Enzyme-Linked Immunosorbent Assay (sandwich ELISA). Long and short eletropherotypes were characterized of using Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS-PAGE). Molecular characterization of rotavirus strains was done using Reverse Transcriptase –Polymerase Chain Reaction (RT-PCR) genotyping of G-genotypes and P-genotypes. Among the 200 samples, 23 tested positive for rotavirus when subjected to Enzyme-Linked Immunosorbent Assay (sandwich ELISA). This resulted in a prevalence of 11.5%. Among the twenty three samples, fourteen were from male participants (60.87%) while nine (39.13%) were from female children. All the children had been vaccinated, except for one whose guardian was uncertain. This study confirmed that children above 20 months of age were most affected. SDS-PAGE indicated that all positive samples were long electropherotypes.RT-PCR confirmed G3 genotype as the leading serotype affecting the children. In conclusion, the ROTARIX® vaccine currently in use by the National Immunization Program might not completely protect against rotavirus infections in the age-group being studied. There might be a need for the introduction of a vaccine booster for older children and the inclusion of a multivalent vaccine for emerging strains. The data generated from this study is of benefit to the County Government of Kericho and National Ministry of Health.

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LIST OF SYMBOLS/ABBREVIATIONS

AGMK cells - African Green Monkey (*Cercopithecus aethiops*) Kidney cells.

CDC- Centres for Disease Control and Prevention

cDNA- complementary DNA

dsRNA- Double Stranded Ribonucleic acid

EIA- Enzyme Immuno Assay

ELISA - Enzyme Linked Immunosorbent Assay (EIA)

FDA- Food and Drug Administration

G-Protein - Glycoprotein

HRV- Human rotavirus

MAbs- Monoclonal Antibodies

mRNA- messenger Ribonucleic acid

NSP - Non Structural Proteins

NT- Non- Typable

OD- Optical Density

PAGE- Polyacrylamide Gel Electrophoresis

PABs- Polyclonal Antibodies

PCR- Polymerase Chain Reaction

P-Protein - Protease sensitive protein /Protease activated protein

RNA - Ribonucleic acid

RT-PCR- Reverse Transcriptase Polymerase Chain Reaction

RVGE- Rotavirus Gastroenteritis

SG- Sub Group

ssRNA- Single Stranded Ribonucleic acid

VP- Viral Protein

dH_2O- Deionized water

CHAPTER ONE

INTRODUCTION

1.1. Background to the Study

Acute gastroenteritis (diarrhoea) among children below five years is normally accredited to rotavirus infections (Babalola et al., 2021). Gastroenteritis is an inflammation of the lining of the stomach and intestines.A rotavirus particle is made up of a capsid enclosing a dual stranded RNA. This is a characteristic found in family Reoviridae (Drolet et al., 2022). The rotavirus-RNA genome is comprised of 11 gene sections bounded by three concentric shells.

Rotavirus infections are at least experienced once in most infants around the world before or at the age of five years old. As infections keep recurring, immunity develops thus adults are the least affected by these infections. Between the years 1996 and 2005 the strains that accounted for 90% of rotavirus cases of children five years and below were of group A rotavirus namely G1,G2,G3,G4 and G9 in the US (Chissaque, 2023). The strain G1 accounted for more than 75% of the cases (Mwanga et al., 2020). As of 2016 acute gastroenteritis due to rotavirus resulted in the death of 128,500 infants below the age of 5 years worldwide (Troeger et al., 2018) , 104,733 of the deaths happened in Sub-Saharan Africa (Chissaque, 2023). In Kenya, it is estimated that rotavirus infections account for over 3000 mortalities annually in children under 5 years of age (Mwanga et al., 2020)

Rotavirus strains are categorised in to genotypes centred on the migration of

RNA segments in gel electrophoresis (Tate et al., 2023). The group and subgroup specificity are determined by Viral Protein 6 (VP6) a protein present on the inner capsid (Kgokolo, 2023). Rotavirus has eight different strains grouped alphabetically from Rotavirus A - H in relation to their antigenic and genetic features using the amino acid sequence of VP6 capsid protein (Mendoza, 2023). Groups A to C have been identified as common human pathogens though also found in other animals. Groups D to G exclusively occur in animals not in humans.

Group A contributes to more than 90% of infections thus making it the most prevalent in humans (Omatola & Olaniran, 2022). Rotavirus infections of Group B strains are rare but associated with infections in Asia and China(Tian et al., 2024). Children in Kenya have displayed Group C species of rotavirus strain(J. N. Gikonyo et al., 2020).

Rotavirus A classification is for the most part grounded on two vital proteins on the outer shell which are Viral Protein 7 (VP7) and Viral Protein 4 (VP4) (Saxena, 2023). VP7 being glycoprotein or G-Protein and VP4 being protease sensitive or P -Protein antigenic specificity respectively (Saxena, 2023). These determine distinct variations within this group of the virus are the source of neutralizing antibody which acts against the immune system (Shaw, 2006).

The foundation of classifying group A rotaviruses as G and P genotypes relies greatly on encoding of the outer shell's VP7 and VP4. So far, 35 G and 50 P genotypes have been identified worldwide (Hoque et al., 2020). The most frequent genotypes linked to human infection belong to genotype P that has 20

genotypes often denoted as P[1] to P[20] (El-Senousy et al., 2020).In this group P [4], [6], and [8] consist of more prevalent strains identified in infections found in human beings (Hu et al., 2018). Whereas there are fourteen G genotypes with G-types 1 to 4, that are linked to 80% of human infections (Igwe et al., 2023). It should be noted that other strains are also becoming frequent such as G8 and G9 which have already been described in Kenya (Mwanga et al., 2020).

Rotavirus is stable and remains viable in a locality indefinitely if the area is not disinfected. It infects a large number of species but the strains are antigenically distinct from those that cause infection in people(Sadiq et al., 2022).

Rotavirus is transmitted through the oral faecal pathway. Its particles invade and destroy cells of the epithelial layer that lines the small intestine resulting into gastroenteritis (Hameed et al., 2021).Viral reproduction happens in the small intestine in an area that experiences direct contact with food during digestion that is the villous epithelium (Amimo et al., 2021).

Studies carried out indicate that more than a half of children with acute gastroenteritis often have the rotavirus antigen in their serum (Zweigart et al., 2021). This may cause a reduction of the ability of the intestinal epithelium to absorb products of digestion (Jiang et al., 2002).

Rotavirus has a maturation time of 1 to 4 days (Saha et al., 2021). Scientific indicators of infections differ and rely on whether it is the first infection or a subsequent one. The initial rotavirus infection normally occurs at twelve weeks of age and is termed as the most severe. The infection might not have any

symptoms, may result in limited watery diarrhoea or may cause acute dehydrating diarrhoea accompanied with high temperature and nausea (Florez et al., 2020). The symptoms generally disappear between three to seven days (Rubbenstroth et al., 2020).

The World Health Organization Strategic Advisory Group of Experts endorsed the assimilation of the rotavirus vaccine into the vaccination programs for children that are five years and below in April 2009 (Kumraj et al., 2022). A number of countries in the world did adopt this recommendation and included rotavirus vaccines as part of their paediatric programs. In Kenya this vaccination was introduced on July 2014.The vaccine introduced as part of childhood immunization schedule was the ROTARIX vaccine a monovalent which contained live attenuated G1P[8] strain 89–12. ROTARIX is administered twice, at eight weeks and sixteen weeks(Chilengi et al., 2020).

Time has passed from when WHO initially endorsed vaccination of children to curb rotavirus infections. Information on the impact and severity of disease post vaccine introduction is critical.

It has also been noted through a study by the African Rotavirus Surveillance Network that rotavirus prevalence reduced greatly in countries that were initial users of the vaccine after being permitted by WHO (35% in 2010 to 19% in 2015 for countries using prior to 2013). The drop was less noticeable for countries that introduced rotavirus vaccination after 2013 (44% in 2010 to 25% in 2015). Recent studies have displayed estimations of vaccine efficacy varying from 49% to 86%,

with the highest efficacy detected against severe disease and in younger infants (Burke et al., 2019) . This was also seen in the case of communities of low social economic status in 2012 (Lopman et al., 2012). It would be reasonable to find out in which percentage Kericho County would fall in.

Despite the fact that the rotavirus burden decreased over the past twelve years, it is still the most prevalent source of diarrhoea associated deaths in children of five years and below. It is ranked third after malaria and pneumonia despite vaccine introduction. There is also a great inconsistency in the occurrence and mortality due to rotavirus infections observed in high and low income regions. This calls for more efforts to stop rotavirus spread by improving vaccination programmes and sanitation. (Troeger et al., 2018).

1.2. Statement of the Problem

A study in established that rotavirus caused approximately 130,000 mortalities in children of five years and below were as a result of rotavirus in Africa (Fujii et al., 2019).

Diarrhoea is the cause of nine percent (9%) of mortalities in children five years and below in Kenya making it the leading killer of infants in the country. In the nine percent 3000 deaths are accounted for by rotavirus infections. These deaths have occurred despite the inclusion of ROTARIX as a vaccine into the immunization programme.

The Government of Kenya introduced vaccination for rotavirus in 2014 using ROTARIX. This was incorporated in to the routine national immunization program.

ROTARIX vaccine is monovalent and there are concerns on its effectiveness based on circulating genotypes. Although ROTARIX was highly efficacious for preventing severe rotavirus gastroenteritis there is evidence of children being infected with rotavirus. This then raises concerns as to the effectiveness of the current vaccine in use.

Vaccine introduction normally has an effect on the existing circulating strains thus genotyping and continuous monitoring of the emerging rotavirus strains remains critical in understanding the efficacy of the vaccines.Outcomes for such interventions can vary substantially in different regions depending on other intervening factors such as socio-economic, geographical, cultural beliefs and implementation challenges.

The County of Kericho has unique climatic conditions which make it unique for the study. This iincluding the limited amount of published information on rotavirus and the impact of its vaccines within the county.The current study was intended to give answers to the critical questions of the impact of vaccine introduction in terms of prevalence, disease severity, age shift as a result of vaccination pressure and circulating genotypes against the background of a programmatic monovalent vaccine.

1.3. Objectives of the Study

1.3.1. General Objective

To determine the prevalence and genetic diversity of group A rotaviruses in children with acute gastroenteritis post vaccine introduction in Kericho County

Referral Hospital.

1.3.2. Specific Objectives

1. To determine demographic characteristics of children below five years presenting rotavirus related gastroenteritis post vaccine introduction in Kericho County Referral Hospital.
2. To determine the prevalence of gastroenteritis caused by rotavirus in children below five years post vaccine introduction in Kericho County Referral Hospital.
3. To determine genetic diversity of circulating strains of rotavirus amongst children below five years in post vaccine introduction Kericho County Referral Hospital.

1.4. Research Questions

1. What are the effects of Rotavirus vaccine introduction on the demographic characteristics of children of five years and below presenting with rotavirus related gastroenteritis in Kericho County Referral Hospital?

2. How does the introduction of rotavirus vaccine affect the prevalence of rotavirus gastroenteritis in children below five years in Kericho County Referral Hospital?

3. What are the effects of the introduction of Rotavirus vaccine on genetic diversity of circulating strains of rotavirus amongst children below five years in Kericho County Referral Hospital?

1.5. Justification of the Study

This study investigated the different rotavirus (RV) strains together with the frequency of rotavirus infections among infants that have a history of vaccination against rotavirus and their demographic characteristics.

Generally viruses are known to respond to vaccination pressure in many ways, one of which is mutation which aims to enable them evade the immune system and age shift to untargeted age groups. The significance of the current study was to confirm the effect of vaccine introduction after a decade of useage in an area where little has been published on the rotavirus transmission. ROTARIX being a monovalent vaccine, which means it is based on a single strain of the virus, indicates that it has only one of the very many strains of the attenuated virus. It might provide immunity for the existing strains but with time virus mutates for survival. This ability to mutate and form different starins is due to its segmented genome.This means that vaccine might not cater for the new mutations. It was important to find out whether the introduction of the vaccine had an impact on the infection especially in an area such as Kericho with varied climate and an agricultural background. It was also important to make available such information since it is limited since no such research has been carried out on the county.

1.6. Significance of the Study

The vaccine was first used in 2014 as part of the nation-wide infant immunization programme and after a decade of usage it was important to evaluate the impact on prevalence and genetic diversity of rotavirus infections. Findings here,

suggest indirect effects on older pediatric age groups. These results should encourage countries still considering routine rotavirus vaccine implementation. The study findings will encourage programmes that already introduced rotavirud vaccine to continue to strive for high rotavirus vaccination coverage as the impact was demonstrated to a large extent in the current study. In addition , high vaccine coverage is recommended to build sufficient herd immunity thereby protect unvaccinated cohorts or untargeted groups.

1.7. Scope of the Study

The scope was confined to children five years and below seeking medical attention in Kericho County Referral Hospital and presenting with acute gastroenteritis.

1.8. Assumptions

The verbal information given by the parent/guardians on vaccination status was true in the absence of evidence by vaccination /immunization card.

CHAPTER TWO

LITERATURE REVIEW

2.1. Biology of Rotavirus

A fully functional and transmissible rotavirus particle consists of three protein coats covering a segmented double-stranded RNA genome. It is non-enveloped and, icosahedral in shape. The furthest layer of the three-layered particle comes off when membrane infiltration of the host cell takes place, resulting in a two layered particle controlling the transcription of viral mRNA by action of RNA polymerases found in the virion. Protein synthesis usually takes place with the use of rotavirus mRNAs as templates. Viral mRNAs collect together with proteins within electron rich inclusions known as viroplasms. Viral RNA will then be replicated in the viroplasms, packaged and the double layered particles. It is through budding by the endoplasmic reticulum as a final stage that double layered virions form three layered virions. Mature virions are let out by either cell disintegration or exocytosis (Hassan et al., 2021).

The outermost capsid layer of a rotavirus particle is made up of VP7 and VP4 and the middle layer is made up of VP6. The innermost layer is created by VP2 encompassing two proteins which are VP1 (RNA dependent RNA polymerase) and VP3 (Viral Capping enzyme) (Sadiq et al., 2018a).

Its genome comprises of 11 units which are dual stranded RNA and cypher for six structural proteins (VP1-4 and VP6-7) in addition to six non-structural proteins

(NSP1-6). Non-structural proteins are only made within the host cells after being infected with the virus. VP2 results into the auto assembling of the third innermost layer which encloses the genome; VP1 and VP3. VP6 gene codes for the middle layer and this is arranged into 260 trimers (Ruiz et al., 2009) Figure 2.1 below.

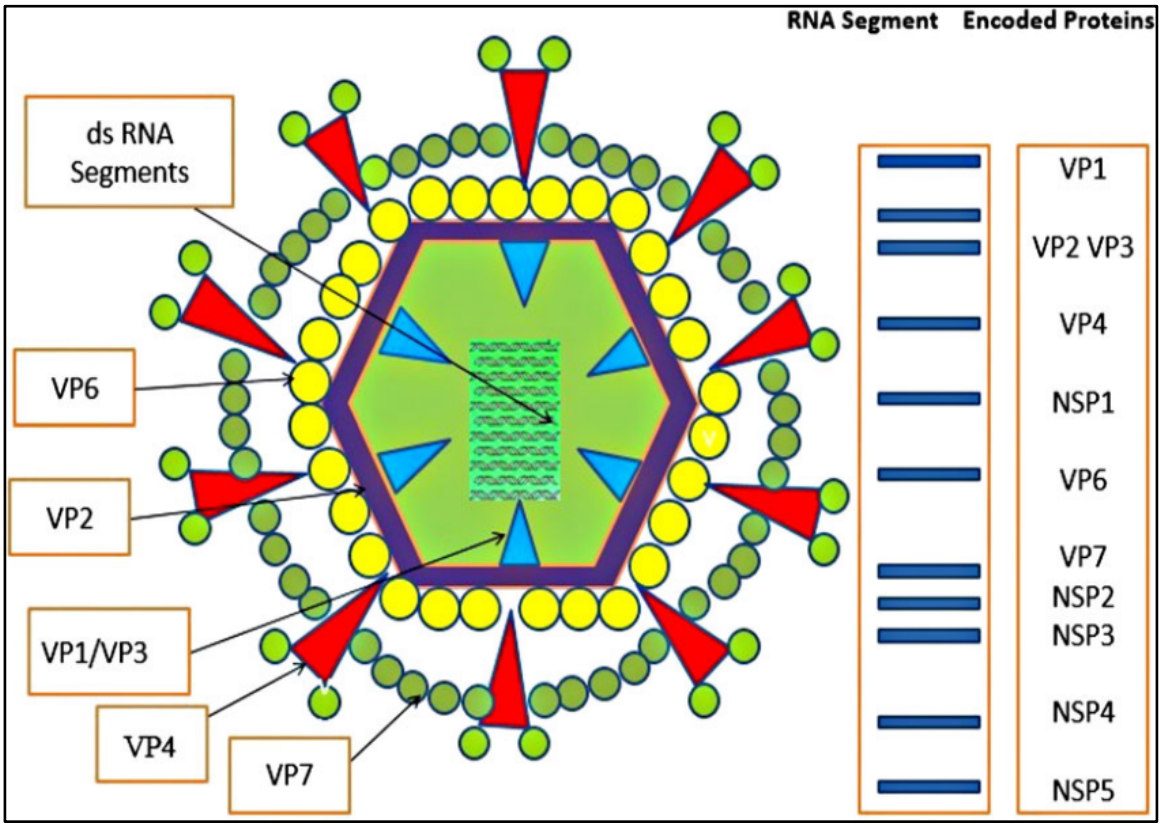

Figure 2.1- The structure of a rotavirus virion and its genome organization (Sadiq et al., 2018b).

2.1.1. Structural Proteins

Viral Protein 1(VP1) is an RNA polymerase enzyme situated at the centre of the particle. This enzyme creates the mRNA transcripts for viral protein synthesis once the particle is in a cell. It also replicates the rotavirus genome for new virions (Asensio-Cob et al., 2023).

The innermost layer of the viral capsid comprises of Viral Protein 2 (VP2). It holds genetic material together with RNA processing enzymes. VP2 is an important cofactor that activates the polymerase hence initiating dsRNA synthesis. It combines packaging with genome duplication while ensuring that VP1 makes dsRNA inside an assembling pre-virion (Xia et al., 2024).

Viral Protein 3 (VP3) is an enzyme named guanylyl transferase which forms the particle's innermost core. This enzyme accelerates 5'cap formation during post transcriptional alteration of mRNA which is responsible in making the viral mRNA stable, by shielding it from nucleases (Dai, 2023).

Viral Protein 4 (VP4) is found as spikes on the particle surface. This protein binds onto the receptors of the host cell resulting in the virus accessing it. It is altered by trypsin into VP5 and VP8. VP4 determines how infectious the particle is (Asensio-Cob et al., 2023).

Viral Protein 6 (VP6) makes up the greater part of the capsid and is extremely antigenic. This is usually used to classify rotavirus strains. VP6 is a major

component in laboratory detections of rotavirus A infection. It mediates specificity and considered the most immunogenic rotavirus protein. However prompts no neutralizing antibodies (Abs) (Dellis et al., 2024).

The virion's outermost surface is composed of a glycoprotein which is Viral Protein 7 (VP7). It also governs the G-type which often at times is used to determine and identify the strain. This glycoprotein in conjunction with VP4 takes part in developing resistance towards infection (Peña-Gil et al., 2021).

2.1.2. Non-Structural Viral Proteins (NSP)

The viral proteins work in conjunction with non-structural proteins whose aim is to establish a host- virus interaction that will ensure effective infection and replication. There are six viral non-structural proteins(Papa et al., 2021).

Non-structural Protein 1(NSP1) is RNA binding protein and a product of gene5. It inhibits the expression of interferon (IFN) and IFN stimulated gene products. This is done through the NSP1 binding on the IRF3.This results in proteasome facilitated breakdown of IFN regulatory factors (IRFs). Hence preventing interferon gene transcription of the infected cell and no immune response (Amimo et al., 2021).

The non-structural protein2 (NSP2) is part of the intermediates created during replication. It accrues in the viroplasms, areas of RNA genome replication and in the assembly sites of incomplete viral particles. Its activities are not specific but it has an affinity to single stranded RNA. It functions as a source of energy for the packing mRNA into replication intermediates from NTPase activity (Papa et

al., 2021).

During the initial infection the Rotavirus takes over the hosts translation activity to favour its replication. It does this by completely shutting off the cell's protein synthesis mechanism. The rotavirus mRNA lacks the poly(A) tail that is to be recognised by 3' end to be the poly(A) binding protein which is essential in translation. The rotavirus genome compensates this by having the 3' bound to non-structural protein 3(NSP3). In doing so it completely prevents translation of the cell's mRNA while ensuring effective translation of rotavirus mRNA (Yuan et al., 2021).

Non-structural protein 4(NSP4) also known as rotaviral Enterotoxin non-structural protein 4, is acknowledged to interrupt cellular Calcium ion (Ca^{+2}) regulation by transferring it to the endoplasmic reticulum. It targets the mitochondria by moving into it, causing the dissipation of the mitochondrial membrane. It plays the role of an intracellular receptor for the dual layered virions during the entry of subviral particles in the endoplasmic reticulum thus allowing its entry into them. It causes the destabilization of the endoplasmic reticulum membrane thus increasing the amount of Calcium ions(Ca^{+2}) in the cytoplasm resulting in apoptosis (Panda et al., 2021). NSP4 restricts the action of intestinal brush border disaccharidases in the cell membrane and thus stimulates the start and perseverance of diarrheal disease (Omatola & Olaniran, 2022).

Non-structural Protein 5 (NSP5) is situated on segment 11 of the rotavirus RNA strand. It works with NSP2 to activate cellular kinases for its own

phosphorylation. NSP2 and NSP5 interact to form structures that resemble the viroplasm (Papa et al., 2021).

Non-Structural Protein 6(NSP6) is also encoded by segment 11 of the rotavirus RNA strand. It is a sequence independent nucleic acid binding protein. It plays a regulatory role for NSP5 and has a similar affinity to ssRNA and dsRNA from an out-of-phase open reading frame (Garba et al., 2024).

2.2. Classification of Rotaviruses

Rotaviruses convey three vital antigenic specificities: group, subgroup, and serotype (Muyyarikkandy et al., 2024). This results in them being placed in groups which range from A to H founded on group specificity determined largely by VP6 (Boussettine et al., 2020). Group A rotaviruses are the chief source of most infections in man, infections caused by groups B and C in humans are rare. An emphasis has been put on developing vaccines solely on the basis of group A infection. Subgroup specificity controlled by VP6 is used as the basis for describing antigenic characteristics of several rotavirus variations in epidemiologic studies. A great number of human rotaviruses (HRVs) are either in subgroup I or subgroup II. VP4 and VP7 proteins which form the outermost layer control serotype specificities autonomously. VP4 is a spike protein, while VP7, is a glycoprotein which is found in large quantities, it forms a greater part of the outer layer (Asensio-Cob et al., 2023).

Rotavirus reassortants indicate that VP7 and VP4 mutually convey epitopes responsible for inducing neutralizing antibodies (Kwami, 2017). This led to the

double classification of rotaviruses using the specific reaction aimed at neutralizing the proteins on the outer capsid. This means that neutralizing antibodies in the hyper immune antiserum generated to combat rotavirus variants target the exterior glycoprotein VP7 (Linhares & Justino, 2023).

2.3. Replication of Rotaviruses

2.3.1 Attachment and Cell Entry

The rotavirus virion attaches itself onto the target host cell (Sadiq et al., 2018b). This process is made possible through VP4 which is the spike protein with specific host receptors that contain sialic acid and co-receptors (S. Caddy et al., 2021). The spike protein VP4 is cleaved into VP5 and VP8 by proteases that resemble trypsin(S. Caddy et al., 2021). Attachment is aided by VP8 which works together with the terminal sialic acid receptors. The resulting VP5 spike escalates the infectivity rate of the virion(S. Caddy et al., 2021). The virion then enters the cell cytoplasm via receptor mediated endocytosis resulting in a decline in the level of calcium which in turn causes the dissolving of the outer capsid protein thus making it a double layered particle. This is accompanied by the formation of viroplasms which take part in genome replication, encapsidation and capsid assembly(Sadiq et al., 2018a).

2.3.2 Transcription and Translation

The double layered particles move to the cytoplasm where they become active molecules that take part in transcription. The rotavirus transcriptional complex consists of VP1, VP3 including the 11 segments that make up the viral dsRNA of rotavirus. The plus sense single strand RNA transcripts are formed from the

negative sense genomic RNA within the rotavirus double layered particles found in the cytoplasm of the host cell. The newly formed plus sense RNA strands are moved outside the double layered particles into the cytosol. The plus sense RNA strands are used as a template for replication and translation of negative sense RNA and viral proteins (Sadiq et al., 2018a). Plus sense RNA strands ran from 5' to 3'.

Formation of mRNA that assists in protein synthesis from the minus strand is the initial stage that marks the beginning of cell infection by a virus. Some plus strands of rotavirus are added into new virus particles to commence the process of virus assembly. Rotavirus replication is entirely cytoplasmic. This is because it has its own polymerase (Tohmé & Delgui, 2021).

Positive-sense viral RNA is similar to mRNA and thus can be immediately translated by the host cell to proteins (Markiewicz et al., 2021). Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA polymerase before translation(Markiewicz et al., 2021).

2.3.3 Virion assembly and Release

Once synthesized the viral proteins (VP) move into viroplasms where the assembly of RNA and viral structural proteins occurs followed by the double layered particle (DPL) formation. These bud from the viroplasm through the endoplasmic reticulum with the mediation of NSP4 and VP6. DLP formation takes place simultaneously with the assembly of the outer capsid thus forming fully mature, triple layered rotavirus particles (TLPs). These are released from the

cell through lysis (Sadiq et al., 2018a).

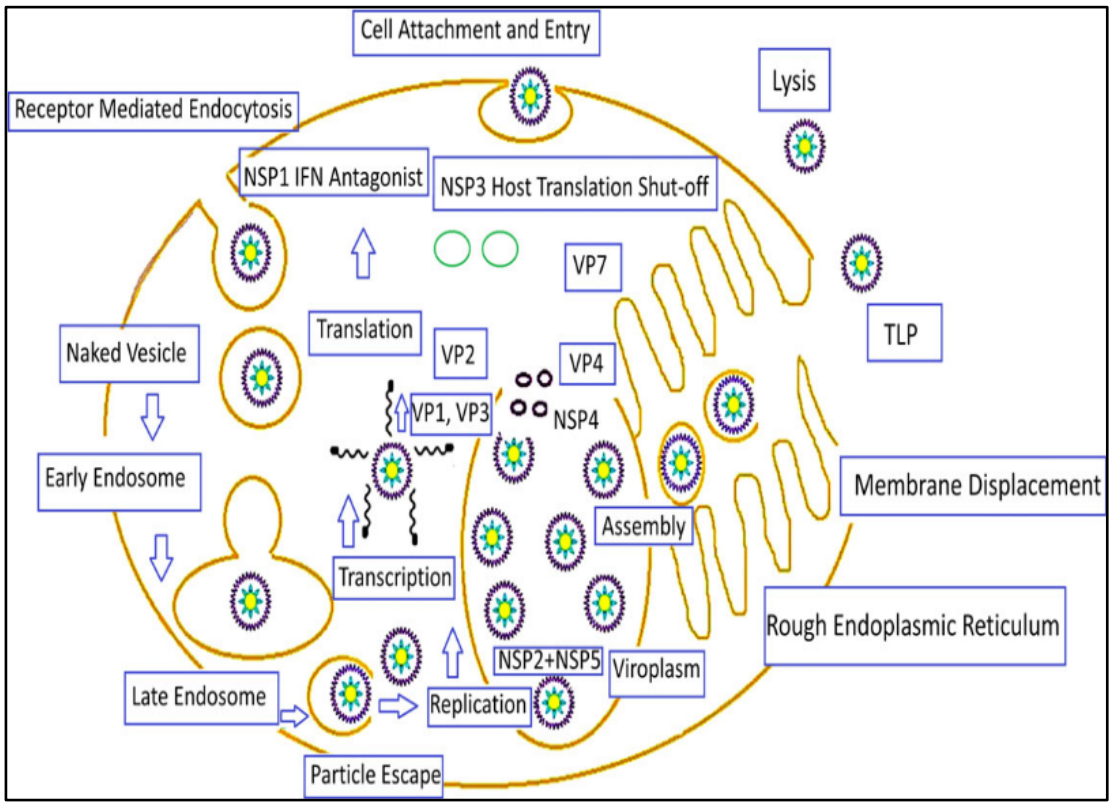

Figure 2.2-Entry,attachment , replication and release of a rotavirus virion, (Sadiq et al., 2018b).

2.4. Rotavirus Transmission and Epidemiology

Multiplication of the virus follows the introduction of rotavirus into the body via the oral – faecal route. This takes place in the epithelial layer of the ileum which normally results in reduced intestinal absorption of nutrients. The rotavirus infectious dose is between 100 to 1000 viral particles (Parashar et al., 2013).

Rotavirus has a segmented genome and due to this it undergoes recombination *in vivo* and *in vitro* as a mechanism of its evolution this results in novel viruses. Recombinants can arise by reassortment of genes during co-infection (Sadiq et

al., 2022). These unfamiliar strains are linked to 4.9% of the total infections globally and account for at least, twenty seven percent (27%) of the infections in Africa (Sadiq et al., 2018a).

Gastroenteritis is well-defined as the passing out of stools that are more loose than normal in a period of 24 hours or having more than two occurences of unexplained vomiting and diarrhoea as part of the symptoms described by explained by the guardian on arrival at the hospital (Demekong et al., 2024).Infections resulting from rotavirus are not readily detected from that caused by other infections. The main symptoms of rotavirus infection normally take about 24 to 72 hours to appear. The first symptoms are usually fever, vomiting followed by watery diarrhoea, abdominal pain, loss of appetite and dehydration which can last from 5 to7 days (Demekong et al., 2024).

Rotavirus is extremely transmittable and quickly spreads between individuals via tainted hands or items.Rotavirus being a viral infection, treatment using antibiotics and other drugs is ineffective. Mild cases of rotavirus infections are usually treated in a similar manner as other cases of diarrhoea through the administration of oral rehydration therapy. Severe cases normally result in dehydration and are often given fluids intravenously(Florez et al., 2020).

2.5. Pathogenesis

A rotavirus infection can either have symptoms or not. Its characteristics are affected by both the viral particles and the host (Omatola & Olaniran, 2022). In this case the most affected hosts that normally exhibit symptomatic infections

are children. Their protection against rotavirus is primarily through antibodies obtained through the placenta (maternal antibodies) at the neonatal stage (Otero et al., 2020).After birth, there is a reduction in maternal antibodies as age increases which in this case is termed as maximum age of susceptibility to severe rotavirus gastroenteritis. The virulence of the virus is determined by the proteins coded by the viral genome.

The main indication for a child infected with rotavirus is diarrhoea accompanied by a little inflammation in the intestines. Rotavirus chiefly affects the intestines, its pathogenesis occurs in many ways that normally result in malabsorptive diarrhoea due to the damage of enterocytes that are absorptive. This stems from the inhibition of the expression of enzymes that are absorptive and variations in the connections between enterocytes that lead to paracellular leakage (Liu et al., 2023).

2.6. Rotavirus Detection and Characterization Methods

2.6.1. Rotavirus Detection Methods

There are techniques for detecting the presence of in faecal samples obtained from infants having gastroenteritis. These techniques include: Electron microscopy, Antigen detection and Nucleic acid Detection (Babaei et al., 2022).

2.6.1.1. Electron Microscopy

It is a method that is highly precise but too demanding to be included as a routine step in detection for a huge number of stool samples (Zhang et al., 2020). The method requires costly equipment and highly skilled individuals. It is unreliable in

trying to identify the different strains of rotaviruses (Celma et al., 2020).

2.6.1.2. Antigen Detection

It is the commonly used technique in diagnosing rotavirus and depends on antigen identification of rotavirus particles in faecal samples. Antigen detection relys on the discovery of antigens of rotavirus particles in faecal samples. The most effective form of detection is Enzyme-Linked Immuno-Assay(ELISA).It utilizes rotavirus precise antibodies which causes specific antigens to adhere to the wells of plastic plates(Cassedy et al., 2021).

Antigen identification reaction remains a calorimetric process that happens through the addition of rotavirus-specific antibodies attached to an indicator enzyme (Cassedy et al., 2021). ELISA remains intense, precise and can be used on large sample volumes such as the 96-well plate format (Lothert et al., 2022). Optical Density (OD) outcomes are effortlessly documented using a standard plate reader thus allowing exploration of the outcomes with software.

A substitute to ELISA would be Latex Agglutination which uses latex particles enclosed in anti-rotavirus antibodies(Nafi, 2020).

The importance of rotaviruses in clinical sites has resulted in the monetising of numerous antigen identification techniques by companies in the pharmaceutical and biotechnology industry. The information on their sensitivity and precision is also available.

Organizers of investigation networks which includes Centres for Disease Control and Prevention (CDC) and the World Health Organization (WHO) favour the IDEIA kit by IDEIATM Rotavirus (Oxoid (Ely) Limited due to its lower price in comparison

to the rest of the kits (Giri et al., 2020).

A typical collection of rotavirus samples that are both positive and negative (Proficiency Panel) are acquired from any Rotavirus Regional Laboratory as part of quality regulating measures desired to confirm that the commercial assays are of high proficiency (Khan et al., 2020). A proficiency panel contains a collection of rotavirus positive and negative samples needed for any testing.

2.6.1.3. Nucleic acid Detection

Viral genome segments are seen directly after removal from virions in faecal samples belonging to rotavirus infected children. This is achieved through electrophoresis on acrylamide gels, and staining with silver nitrate (Geletu et al., 2021).

Subsequently, rotavirus Groups A to C exhibit unique arrangements of gene-segment movement and specific electropherotypes after electrophoresis. A distinct electropherotype pattern being observed is used as an indication for the occurrence of specific rotaviruses of Groups. Most samples with Group A rotavirus test positive on PAGE by exhibiting a specific array of rotavirus RNA fragments once electrophoresis and silver staining are done.

A variation of delicate reverse-transcription polymerase chain reaction (RT-PCR) systems were set up centred on primers specific to various diverse strains of rotavirus (Organization, 2009). RT-PCR remains handy in replicating RNA extracts comprising of whole rotavirus genome. Though, the process is fairly costly and demanding.RT-PCR perceives lower numbers of rotavirus RNA thus

inappropriate for repetitive rotavirus identification studies (Organization, 2009).

2.6.2. Rotavirus Molecular Characterization

Molecular characterization is a broad term that refers to using molecular markers, including DNA, RNA, and proteins, to determine the genetic characteristics of cells or tissues.

2.6.2.1.Serotyping and Subgrouping with Monoclonal Antibodies

A number of preferred Enzyme immunoassays permit identification of rotavirus VP6 and VP7 by means of serotype precise Monoclonal antibodies. Monoclonal antibodies are proteins made in a laboratory that bind to only one antigen. Serotypes are groups based on the types of antigens forud on the surface of the virion. Five serotypes of rotavirus G that are prevalent are G1, 2, 3, 4 and 9. The serotypes can be assigned a serotype straight from stools by means of more than a few ELISA set-ups integrating monoclonal antibodies` that bind in a serotype-specific way to VP7 protein. The binding specifities of VP6 MAbs can be used to allocate VP6 subgroupings I, II and non-I, II.

Studies using MAbs to form groups based on antigens have characteristically typed 60%-70% of variants found within communities. This technique is fast and cheap. It offers G serotype data together with appropriate reagents, which give data on genetic dissimilarities between strains of rotavirus with the same serotype (Organization, 2020).

VP7 serotyping. MAbs react to the VP7 (G) serotype antigen in a serotype precise way completing virus particles (Ward et al., 1991) Immediate serotyping

of rotaviruses in faecal samples is deemed successful due to the advancement of serotype specific ELISA methods. Studies involving rotaviruses in large samples indicated that serotypes G1-G4 were the major sources of gastroenteritis in children (Kumar et al., 2022). These antibodies are currently being utilized for studies in direct serotyping. In addition to the secondary results obtained through RT-PCR genotyping, the MAbs method also gives an antigenic degree of strain serotype that contrasts the genotype. Their use in serotyping ELISA allows identification of antigenic strains in the existing rotaviruses (Organization, 2009) .

The downside to MAb serotyping is that a good quantity of rotaviruses derived from faecal samples cannot be serotyped. This is because there is a very small amount of viral particles that are intact, antigenic disparity in shared serotypes makes them non-reactive with MAbs, there are also faecal inhibitors interfering with the attachment of the virus to the antibody (Tate et al., 2023).

This can be dealt with through increasing the size of the panels of MAbs comprising of antibodies of diverse antigenic variations of serotypes. Sample groups of rotavirus need to be big enough to be analysed by RT-PCR and identify the non-typable strains with MAbs (Organization, 2009). There is also a need for a continuous supply of MAbs and rotavirus hype immune antisera that must both be produced by animals which is a great set back due to the decline of laboratory animals use in investigation.

VP4 serotyping. Serotyping assays founded on MAbs specific for P1A[8], P1B[4], and P2A[6], serotypes which are the most common causes of infection in man

have been established (Mota-Hernández *et al.*, 2003). The assays have been vital in describing antigenic variations in serotypes. The occurences of numerous cross reactive epitopes in diverse P serotypes in rotavirus field isolates prevent using this assay for routine P typing practises (Omatola & Olaniran, 2022).

VP6 subgrouping. VP6 is the most common virion protein and its polyclonal antibodies are cross reactive in all rotaviruses (Caddy et al., 2020).Polyclonal antibodies (PABs) are a collection of immunoglobulin molecules that react against a specific antigen, each identifying a different epitope. They greatly outline the level of reactivity of rotaviruses. A set of antibodies responds VP6 protein of distinctive serotype G2 strains through small electropherotypes, fewer serotypes of G1, 3, 4, and 9 strains with extended electropherotypes. This reactivity pattern is referred to as Sub Group I (SGI) while those that react contrary to this are referred to as Sub Group II (SGII). These reactions are important indicators of epidemiology. In rare occurrences SGI and SGII MAbs may react with rotaviruses found in humans or others. Animal strains and some rare human rotaviruses have phenotype SGI specificity and an extended electropherotype (Martella et al., 2005).

2.6.2.2. Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS-PAGE)

Rotavirus genome is identified in specimens through extraction of viral RNA plus investigation using electrophoresis on a polyacrylamide gel accompanied with silver staining (Agbemabiese et al., 2023). During this process the negatively charged macromolecules are separated depending on sizes. Genome patterns

are seen in the gel through silver nitrate staining used to stain trace quantities of the genome in polyacrylamide gels. This gel can then dried and put in storage (Malla, 2023).

The dsRNA of rotaviruses of group A are categorized into four classes. Human and animal rotaviruses that belong to group A exhibit either long or short electropherotypes. It is these correlations in the RNA patterns and serotypes that have become useful in epidemiology (Organization, 2009).

SDS PAGE is tiresome and requires skill; it lacks uncertainty in the results. This means the genome arrangement of Group A rotavirus is readily notable from any other rotavirus gene arrangement. A given sample is categorized as Group A rotavirus positive when the 11 segments of dsRNA are detected while their arrangement is comparable to that of the control RNA of the same group which is 4-2-3-2 (Organization, 2009).

2.6.2.3. Rotavirus Detection by Cell Culture

Rotavirus strains grow with the assistance of proteolytic stimulation of enteric trypsin used by the virus for attachmentusing VP4 spike proteins (Amimo et al., 2021).The widely used cells in the development and classification of rotaviruses are continuous line cells derived from rhesus monkey kidneys together with those from primary monkey kidneys (Folorunso & Sebolai, 2020). To ensure that the virus is able to grow outside the host cell needs numerous sequences of passing it in primary cell. The newly formed stocks of rotavirus are refined through centrifugation followed by enumeration using plaque assay or

fluorescence focus assay (Zhu et al., 2022).

2.6.2.4. Propagation of Rotavirus in Cell Culture from Clinical Faecal Specimens

Growing rotavirus from faecal samples can be attained through use of primary African Green Monkey cells (AGMK cells) (Resch et al., 2020). Bacterial adulteration is lowered through centrifugation and retaining antibiotics in the culture all through the progression of the cells' infection. After passing the infected clinical sample in AGMK cells several times, the virus is made viable for replication in continuous cells(Resch et al., 2020).

2.6.2.5. Serological Methods

The Serological trapping technique of identifying rotaviruses includes covering the electron microscope grids with protein A and antiserum explicit to a given rotavirus strain. A coat of antibodies present on the grid rises the amount of particles of rotavirus to be identified. The procedure intensifies the amount of virions that will stick to the grids is and is widely utilized in viral identification and quantitative analysis (Nicolaieff et al., 1980).

2.6.2.6. Molecular Characterization of Rotavirus using RT-PCR

This helps determine the breeding conduct of species, singular procreative success and the presence of gene flow. It also helps determine the movement of alleles within and between populations of correlated species, followed by the ensuing consequences (Cowley et al., 2018).

2.7. Prevention and Control of Rotavirus Infections

Rotavirus is extremely transmittable and quickly spreads between individuals through tainted hands or items. The infection is caused by a virus therefore treatment using antibiotics is ineffective. Mild cases of rotavirus infections are usually treated in a similar manner as other cases of diarrhoea through the administration of oral rehydration therapy. Severe cases are often given fluids intravenously (Florez et al., 2020).

Initial exposure to rotavirus infections usually does not result in lasting immunity. Successive infections result in development of immunity against the virus. Repeated infections normally result in no symptoms or slight diarrhoea.

2.7.1 Vaccination

Vaccination is the best way of preventing rotavirus accompanied with improvements in sanitation , general hygiene of the households, improving the quality of water and the accomplishment of the available vaccination programs(Okechukwu et al., 2024). The assimilation of the rotavirus vaccine in most national vaccination programs has averted approximately over 28 000 fatalities among infants younger than 5 years, especially in Sub-Saharan Africa. This is nearly 20% of all mortalities as a result of diarrhoea amongst children of five years and below (Troeger et al., 2018).

On the advent of 2009 the World Health Organization had prequalified a total of four vaccines that is; ROTARIX (GlaxoSmithKline Biologicals) in 2009, RotaTeq

(Merck & Co., Inc.) in 2008, Rotavac (Bharat Biotech, Hyderabad, India) in 2018, and Rotasiil (Serum Institute of India PVT. LTD.,Pune) in 2018. By the end of 2018 a total of 74 countries had incorporated ROTARIX in their national immunization program this included Kenya. A total of fourteen countries were using RotaTeq while nine were using both (Burke et al., 2019).

Merck produced RotaTeq (RV5) a live pentavalent oral vaccine. Food and Drug Administration of the United States of America approved its use on February 2006 (Cortese & Parashar, 2009). It is made up of a combination of five viruses. Four of the bovine-human reassortant rotaviruses express human virus VP7 from serotypes G1, G2, G3, or G4,whereas the fifth reassortant virus contains VP4 (P[8]) from a human rotavirus strain (Parashar, 2016). These are then placed in a buffer solution made up of sucrose, sodium citrate, sodium phosphate monobasic monohydrate, sodium hydroxide, polysorbate 80 and tissue culture media. It might also contain minor quantities of foetal bovine serum and with no preservertives. It is given in three oral doses at the ages of 8weeks, 16 weeks and 24 weeks (Vetter et al., 2022).

GlaxoSmithKline produced a live oral vaccine ROTARIX (RV1). Approval of use was by Food and Drug Administration in the United States of America on April of 2008. It is specifically designated to prevent rotavirus infections resulting from G1, G3, G4 and G9 strains. It consists a single rotavirus variant of live weakened human 89-12 (type G1P1A [8]). The vaccine is a lyophilized powder made by dry freezing. It is this powder that is later restored to liquid form before administration. It consists of amino acids, dextran, Dulbecco's modified Eagle

medium, sorbitol and sucrose. Its diluting agent is made up of calcium carbonate, sterile water and xanthan. On no account are preservers added to it the vaccine (Yen *et al.*, 2011). It is given in two intervals of oral doses which is at 8 weeks and 16 weeks. The vaccine may also be started as early as when the child is 6 weeks old. The ideal age for the first dose is 14 weeks and 6 days. The peak age for all doses to be administered is 8 months. The shortest time period between doses is 4 weeks (Vetter et al., 2022)

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2.8. Theoretical Framework

As vaccination against rotavirus continues to increase, the importance of global investigation of the virus has developed into an important tool. Investigations on the impact of rotavirus vaccination help in finding out the different serotype placements in different countries. Several studies have been carried out on the efficacy of the vaccines approved by the World Health Organization. Surveillance of rotavirus produces valuable data on circulating rotavirus strains in a given population. This data is crucial in refining the vaccine, identifying emergent types and evaluating vaccine efficiency together with fluctuations in the strains present in a population. The results obtained from the international scrutiny program using mutual generic protocol indicate that thirty nine percent (39%) of the youngsters hospitalized for chronic diarrhoea in selected regions around the world test positive for rotavirus (Mwenda *et al.*, 2010).

In Brazil the vaccine was introduced earlier and ROTARIX was widely used as part of the national immunization program. It is during this phase that the strain G2P

[4] was discovered, this represented a gap in protection. The strain that was discovered was different from those of G and P and also its VP6 was somewhat different from other circulating human strains. The deficit in protection is seen by the strain constantly circulating in the population in Brazil (Folorunso & Sebolai, 2020).This has raised speculations that this heterotypic strain was favoured by immunization since it caused immunity against all strains but G2P4 (Leite *et al.*, 2008). This meant that the vaccine generated immunity for some of the strains but not all. This normally results in circulation of a strain that the population is not immunized against.

Africa and Asia experience the greatest variety due to mixed infections and frequent contact between man and domestic animals which also have the rotavirus (Bourdett-Stanziola et al., 2021). Many rotavirus strains can be found circulating at the same period and in the same region. This means that continuous surveillance is important (Bourdett-Stanziola et al., 2021).

A study in Indonesia stated that approximately half of the known variants found contained either a Glycoprotein or Protease sensitive antigen existing in the two vaccines. It also observed that less than a half of the strains found were neither catered for by the vaccines being used. It was noted that G12 strains were becoming predominant in Nepal and their emergence is now recognized as a global phenomenon (Buchy et al., 2021).

Seasonal occurences are also experienced in rotavirus infections in Kenya. Despite infections being experienced throughout the year, the frequency increases during the dry months. These are between January to March as well as

June to September (Gikonyo et al., 2020). This differs with other regions in Africa such as South Africa where infections occur during the fall and winter time. These seasons overlay with the seasons that lack rain.

Kenya has had its fair share of emerging strains that have become predominant .Data indicates that Kenya has all the main human rotavirus strains found in the world which are G1-4 and G9 while the major genotypes of P are P4,6 and 8. All these were detected during 1996- 1999 and 1999- 2002 studies. Between 1996 and 1999 P[8] was predominant, it then experienced a shift between 1999 and 2002 leaving the P[6] to be the predominant strain (Kiulia *et al.*, 2008).

2.9. Identification of Knowledge Gap

A large number of the rotavirus strains within the gene pool in Kenya between 1975 and 2002 were close to those found in other parts of the globe but emergent strains of VP7 were replacing them may be due to the vaccine selecting against them. This has resulted in the genotypes G8 and G9 substituting the previously common G1-G4 as seen between 2004 and 2005 (Rakau et al., 2021). Introduction of rotavirus vaccines may definitely cause a change in the strains found in the populace. The manifestation of divergent strains of rotavirus and mixed infections in Kenya may challenge the efficacy of the existing vaccines used to combat rotavirus.

The prevalence of rotavirus infections has not reduced considerably in Kenya despite the vaccine being part of the nationwide immunization program (Wandera et al., 2024). The low uptake of vaccine, great diversity in the strains

that have not been incorporated in the vaccine and also remerging types are some of the reasons that attribute to the low efficacy. Diversity in the strains and re-emerging types mostly affecting already vaccinated children. A study carried out on post vaccine rotavirus genotype distribution in Nairobi County showed an upsurge in the frequency of G2 strains and hence portraying a variation in the genetic diversity of rotavirus strains (Gikonyo et al., 2020). There was a decrease in the occurrence of G1P[8] post vaccination and an increase in G2P[4] by 12.2% and G9P[8] by 20.4% (Gikonyo et al., 2020). They also noticed the increase in the prevalence of G3[P4] by 6.1% and G2P[6] by 4.1% in a span of three years from January 2015- December 2017. This lays great emphasis on the need for long-term rotavirus surveillance (Agutu et al., 2017).

The study aims at providing more information regarding rotavirus prevalence post vaccine introduction, the age group most affected and effects of the vaccins on the strains existing especially in an agricultural based community in the year 2021-2022.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Site and Population

The study site was Kericho County Referral Hospital in Kericho County, South of Rift Valley. It is situated on Hospital Road, Kipchebor, Ainamoi constituency .The study population consists of children five years and below presenting with acute gastroenteritis at the hospital.

3.2. Study Design

The study design was a hospital based Cross Sectional Survey.

3.3. Sample Size Determination.

In prevalence studies, sample size formula for sample size determination is as below (Charan & Biswas, 2013).

$$n = \frac{Z^2 P(1-P)}{} = \frac{1.96^2 \times 0.145\ (0.855)}{} = 190.5$$

≈191 d^2 0.05^2

Where:

n= Minimum sample size

Z= Normal Standard deviation taken with a 95% Confidence Interval set at 1.96

P= Assessed prevalence for rotavirus infection amid children in Kenya (14.5%) (Muendo et al., 2018).

d= Precision of the estimate required = 0.05

3.4. Sampling

Patients for the research were selected using simple random sampling was used until the required sample size was reached. Vaccination against rotavirus was confirmed by the immunization booklet or word of mouth from parent/ guardian. If the guardian was unable to remember the vaccines received, the vaccination method was described (oral vaccination) and the age at which the child was given a particular vaccine (Muendo et al., 2018).

Stool samples were collected from patients presenting with symptoms of gastroenteritis. Each patient was requested to provide a stool sample only once during the study. Patients were given disinfected polypots with spoons in which they put the faecal samples during the hospital visit. The required amount of stool was half of the 10ml polypot containers. The specimens were frozen at -

20°C waiting for transportation in cooler boxes to KEMRI. Laboratory work was carried out with Biosafety Level 2 procedures in line with the WHO Biosafety manual.

3.5. Inclusion/Exclusion Criteria

3.5.1 Inclusion Criteria

Only children of age five years and below both hospitalised and non-hospitalised exhibiting severe non-bloody diarrhoea were enrolled into the study. The parent/guardian consented to have his/her child participate in the study.

3.5.2. Exclusion Criteria

Children five years and below hospitalised and non-hospitalized exhibiting severe bloody diarrhoea were excluded from the study.

3.6. Specimen Processing

The specimens collected included information about each sample as per the study protocol and the study objectives. Each sample had information on the age of the respective study participant, vaccination status and the gender. Upon receiving the samples at KEMRI (Nairobi) a small amount (roughly 10g) of loose faecal matter or 700µl of loose faecal aliquots were placed in cryovials and put in storage at -20°C for the purpose of cooling. According to the protocol in the WHO Rotavirus manual the faecal aliquots obtained from patients with symptoms of gastroenteritis were used to prepare 1ml of 10% (w/v) faecal suspension in phosphate-buffered saline (PBS), balanced salt solution (BSS) and 0.01M Tris solution (pH 7.5, 14.5mM NaCl, 10mM $CaCl_2$). This was vortexed and clarified

through centrifugation. The supernatant formed was collected and stored at -70^{o}C for the long term.

3.7. Laboratory Methods

3.7.1. Rotavirus Enzyme-linked Immunosorbent Assay (ELISA)

ELISA was carried out as following protocols outlined in the WHO manual for Rotavirus detection and characterization methods (Organization, 2009). The wells of plastic plates come already coated with rotavirus specific antibodies. The antigen was detected in a colorimetric reaction by means of a second rotavirus-specific antibody attached to a detector enzyme. Faecal suspensions that were prepared and well-preserved in PBS were further diluted in ProSpecT Rotavirus sample diluent to make 10% suspension of faeces. Two drops (100µl) of each diluted fecal suspension and at least one negative and positive control were added to separate micro wells. Then 2 drops of the conjugate were added to each micro well (Parija, 2023). The conjugate consists of rotavirus specific rabbit polyclonal antibodies conjugated to horse radish peroxidase. The plate was sealed and incubated at 30 ºC for one hour.The contents of the wells were aspirated and wells washed by filling each well with 400 µl diluted wash buffer. The wash buffer is 3, 3', 5, 5'-tetramethylbenzidine (TMB) in buffer. Fluid from each well was aspirated out 5 times. After the last wash, the contents of the wells were removed then the plate was struck on a clean paper towel. The automatic washers were used after being programmed to complete 5 wash cycles. After the last wash, the plate was overturned and tapped on absorbent paper to eliminate the last drops of wash buffer. Two (2 drops) of substrate were

added to each micro well. The plate was covered again and incubated at 30ºC for 10 minutes. Substrate reaction was stopped by adding 2 drops of Stop Solution (Sulphuric acid) to each micro well.This resulted in colour change. The fluid in the micro wells was thoroughly mixed before reading the results. The results were read using a spectrophotometer at 450nm. The Negative Control value was less than 0.150 absorbance units. The Positive Control value was greater than 0.500 absorbance units (Organization, 2009).

3.7.2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)

The samples that were found to be positive rotavirus underwent SDS-PAGE. Initial withdrawal of the rotavirus RNA from the faecal suspension through the use of Phenol-Chloroform followed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

3.7.2.1. Phenol-chloroform extraction of RNA from stool

The extraction was done while everything was on ice and in accordance with the protocol in the WHO Manual of rotavirus detection and characterization methods of 2009. A 450 µl of 10% stool suspension was put into an Eppendorf tube. 50 µl of pre-warmed solution of 1 M Sodium Acetate (NaAcetate) containing 1% Sodium Dodecyl Sulphate (SDS) was added into the Eppendorf tube. This mixture was vortexed for 10 seconds and incubated at 37^{o}C for 15 min in a water bath. Phenol-chloroform that was at a pH of 8.0 was then introduced at a ratio of 1:1 followed by one minute of vortexing and further incubation at 56^{o}C in a water bath for fifteen minutes. The Eppendorf tubes were opened and resealed immediately to reduce the pressure build up due to vortexing. Subsequent

vortexing followed for one minute. Centrifugation followed thereafter at 5000 rpm for five minutes. The upper aqueous section of the contents in the Eppendorf tube containing the dsRNA were poured into sterile Eppendorf tubes while making sure the interphase material that contained the viral caspids did not contaminate the extracted material in the aqueous form. Approximately 40µl of 3M Sodium Acetate pH 5.0 was added to the dsRNA solution obtained during the extraction together with 700 µl of ice-cold absolute ethanol thus filling the tubes. This was gently mixed by turning them upside down several times/ six times. Incubation of the mixture was then done at -20^{o}C for two hours followed by incubation at -70^{o}C for half an hour. The Sodium Acetate assisted in the precipitation of nucleic acids.The samples were then centrifuged at 12000rpm for 15 minutes at 4^{o}C.The ethanol was decanted immediately after and the tube inverted to place the dsRNA pellets onto a paper towel to dry for thirty minutes. The pellets obtained were then resuspended in 30 µl of PAGE Sample dye before loading it on to the PAGE Gel (Organization, 2009).

3.7.2.2 .Polyacrylamide gel electrophoresis (PAGE)

The glass plates were cleaned with soap and water, then wiped with 96% ethanol which was then left to evaporate. The glass plates were then assembled for gel casting as per the manufacture's instruction and the top level of the resolving gel was marked using a marker pen while some room was left for the stacking gel above the resolving gel.

The resolving gel was prepared by addition of 15.8ml of distilled water 10ml of acrylamide stock, 3.75ml of resolving gel buffer, 30 µl of

Tetramethylethylenediamine (TEMED) and 100µl of ammonium persulfate into a falcon tube. The acrylamide solution was immediately pipetted between the glass plates to the mark and the gel was overlaid with a layer of water – saturated iso-butanol so as to ensure formation of an even interface and exclusion of oxygen. This was left to polymerize for one hour. 3% gel spacer was then prepared through addition of 6.8ml of distilled water, 1.6ml of acrylamide stock, 1.25ml of spacer gel buffer, 10 µl of TEMED and 500 µl of ammonium persulfate into a new falcon tube. This spacer gel is loaded on top of the resolving gel and a comb was immediately inserted to form wells. The gel was allowed to set for one to two hours that is until the interface between the gel and the overlay was seen (Organization, 2009). The liquid on top of the resolving gel was poured off and the top of the gel washed three times with distilled water. Excess liquid was removed through inserting a piece of filter paper between the glass plates to absorb it. The gel apparatus was then placed upright as the stacking gel was prepared then loaded on top of the resolving gel while immediately positioning the comb. The gel was left to polymerize for one hour before loading the samples. The comb was then removed and the glass plates assembled in the electrophoresis apparatus (Organization, 2009). A running buffer was added to the bottom reservoir and glass plates inserted into the electrophoresis tank. The wells were filled with the electrophoresis buffer and air removed from under the gel bottom. Each dsRNA sample in PAGE buffer was loaded into the designated gel well. The electrophoresis was run at 15mA for 15 hours in Tris –glycine buffer at room temperature(Organization, 2009). Two

samples containing short and long electropherotype rotavirus strains were used as positive controls (on different gels) along with a sample known to be negative for rotavirus which was used as a negative control (Organization, 2009).

3.7.2.3. Silver staining of dsRNA in gels

The running buffer was poured out and the gel between the glass plates was removed. The bottom right corner of the gel orientation was cut and the stacking gel discarded. 200ml of fixing solution 1 (40% methanol) was added to each gel and rotated for 30 minutes in an orbital shaker at room temperature. The fixing solution 1 was aspirated and replaced with 200ml fixing solution 2 (30% ethanol). This was then followed by a 30 minute rotation at room temperature on the orbital shaker. The fixing solution 2 was then aspirated and 200ml of freshly prepared silver nitrate staining was added. This is followed by 30 minutes rotation at room temperature on an orbital shaker. The silver nitrate staining solution was aspirated and the gel is washed twice with distilled water for duration of two minutes each time. A developing solution (3% NaOH and 0.75% formaldehyde) was prepared by adding sodium hydroxide to previously prepared formaldehyde and water solution. 50ml of this developing solution was added to the rinsed gel followed by agitation by hand for 30 seconds to remove any black precipitate. The developing solution was then aspirated and more 200ml was added. This was followed by rotation for approximately five minutes at room temperature until the RNA bands were visible. The developing solution was then drained off and the stopping solution (5% glacial acetic acid) added so as to stop additional color development. Rotation for 10 minutes at room temperature was

done before rinsing the gel using distilled water. The gel was then dried in a standard vacuum gel drier. The procedure adhered to the WHO Manual of rotavirus detection and characterization (Organization, 2009).

3.7.3 Reverse Transcriptase –Polymerase Chain Reaction (RT-PCR) amplification

G and P strains of rotavirus were detected and determined by first obtaining viral RNA from stool samples. The viral RNA underwent examination using semi-nested RT-PCR with primers specific for genes coding G-type (VP7) or P-type (VP4) previously done by Mousavi-Nasab (2020).

Reverse Transcriptase-PCR of rotavirus consists of: denaturation of dsRNA, its reverse transcription, and amplification of cDNA that has been formed. PCR comprised of steps that included heating the DNA to isolate the two template strands, followed by adding of two primers complimentary to the section being replicated, incorporating a heat-stable DNA polymerase enzyme which catalyzes addition of the primers by means of the DNA strand being the template (Aycan & Yildiz, 2024). The process was recurrent with the freshly formed cDNA heat-denatured plus the enzymes lengthening the primers attached to the liberated single DNA strands (Barril & Nates, 2012) .

3.7.3.1. RNA Extraction using QIAGEN

The RNA from the stool samples that had been collected was extracted using the QUIAGEN kit. There was a need to obtain viral RNA for RT-PCR.

Previously prepared 10% stool suspensions were vortexed and left to settle for one hour at room temperature. 250 µl of the supernatants obtained was

transferred to sterile 1.5ml Eppendorf tubes The use of QIAGEN kit combined the selective binding properties of a silica-based membrane with the speed of microspin. This method was highly suited for simultaneous processing of multiple samples.

The samples were first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions were then adjusted to provide optimal binding of the RNA to the QIAamp membrane; samples were loaded onto the QIAamp Mini spin column. The RNA bound to the membrane and contaminants were efficiently washed away in 2 steps using 2 different wash buffers. High-quality RNA was eluted in a special RNase-free buffer, ready for immediate use or safe storage. The purified RNA was free of protein, nucleases, inhibitors and other contaminantsdue to the use of the wash buffers. The special QIAamp membrane guaranteed exceptionally high retrieval of pure, complete RNA in just 20 minutes without using phenol/chloroform extraction or alcohol precipitation. The buffering conditions of the lysate were adjusted to provide optimum binding conditions for the viral RNA before loading the samples onto the QIAamp Mini column. Viral RNA is adsorbed onto the QIAamp silica membrane during 2 brief centrifugation steps. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can hinder downstream enzymatic reactions, are not retained on the QIAamp membrane as described by Widen and Silbert(2016).

3.7.3.2. RT-PCR for G Genotying

The protocal was as described by the WHO Rotavirus Manual 2009 as provided

by the West African Regional Rotavirus Laboratory. Consensus primers sBeg 9 (GGCTTTAAAAGAGAGAATTTC) - position 1-21 and End 9 (GGTCACATCATACAATTCTAATCTAAG) – position 1062-1036 for rotaviruses that belong to group A (Gouvea et al., 1990) were used as seen in Table 3.1 below on oligonucleotide primers for G serotyping .Consensus primers sBeg and End 9 were used in the first cycles of RT-PCR amplification. The primers were at the laboratory and the database used was GenBank database.

Table 3.1

Oligonucleotide primers for G serotyping (Gouvea et al., 1990).

Primer	Sequence (5'-3')	Position(nt)	Strain (genotype)

SBeg	GGCTTTAAAAGAGAGAATTTC	1-21	Group A
Beg9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1-28	Group A
End9	GGTCACATCATACAATTCTAATCTAAG	1062-1036	Group A
EndA	ATAGTATAAAATACTTGCCACCA	922-944	Group A
aAT8	GTCACACCATTTGTAAATTCG	178-198	69M (G8)
aBT1	CAAGTACTCAAATCAATGATGG	314-335	Wa (G1)
aCT2	CAATGATATTAACACATTTTCTGTG	411-435	DS-1 (G2)
aDT4	CGTTTCTGGTGAGGAGTTG	480-498	ST-3 (G4)
aET3	CGTTTGAAGAAGTTGCAACAG	689-709	P (G3)
aFT9	CTAGATGTAACTACAACTAC	757-776	WI61 (G9)
G12	CCGATGGACGTAACGTTGTA	548-567	G12
RVG9	GGTCACATCATACAATTCT	1062-1044	Group A

Note. Reprinted from "Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimen," by V Gouvea, R I Glass, P Woods, K Taniguchi, H F Clark, B Forrester, Z Y Fang, 1990, *Journal of* Clinical Microbiology.

The RT master mix containing: 2.0µl of 5xAMV buffer; 0.25µl each of 10 mM dATP, dCTP, dGTP, dTTP and 0.2µl AMV RT was prepared. Each of the VP7 consensus primers of sBeg and End 9 were diluted into 20pmol followed by 1 µl

of each primer added to all tubes containing the samples. 5 µl of dsRNA was added to the primer. Double distilled water (ddH_2O) was added to a final volume of 10 µl, and mixed by pipetting. The samples were then denatured through heating for five minutes at 94ºC. The sample tubes were then immediately transferred to an ice-bath. Into each sample tube 3.2 µl of RT master mix was added within five minutes then thoroughly mixed. This was followed by a ten second spinning and incubation of the tubes at 42ºC for half hour. The PCR amplification master mix consisting of ; 1.0µl each of 10mM dATP, dCTP, dGTP, dTTP; 4µl 21 10xTaq buffer, 2.4 µl 25 mM $MgCl_2$, 29.3 µl deionised water was then prepared and placed in ice. 0.3 µl Taq polymerase was added immediately before the master mix was used. The sample tubes were centrifuged for ten seconds after their incubation at 42ºC then placed on ice in a sample rack. To each RT sample tube 36.8 µl of amplification master mix was added. This was mixed then placed on ice. The samples were put in the thermocycler and amplified for 30 cycles at 95^0C for half a minute, 42ºC for half a minute than 72^oC for one minute. The PCR fragments were analyzed on 1% TAE agarose gels to determine their sizes using 100 base pair (100bp) molecular weight markers compared to VP and cDNA products. Table3.2 below was used on the expected sizes of the VP7 genotyping PCR products (Gouvea et al., 1990).

Table 3.2

The expected sizes of the VP7 genotyping PCR products (Gouvea et al., 1990).

Primer	Genotype	expected product size (bp)
beg9 and end9	VP7 genotypes	1062
9FT9	G9	306
9ET3	G3	374
G12	G12	513
9DT4	G4	583
9CT1	G2	625
9BT1	G1	749
9AT8	G8	885

Note. Reprinted from "Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimen," by V Gouvea, R I Glass, P Woods, K Taniguchi, H F Clark, B Forrester, Z Y Fang, 1990, *Journal of* Clinical Microbiology.

The genotyping was carried out using RV G9 and a mixture of type specific primers which were; aBT1 (G1), aCT2 (G2), aDT4 (G4), aET3 (G3) determined G1-4 whereas aAT8 (G8), and aFT9 (G9) determined G8 and 9 rotavirus VP7 genotypes. Each genotyping and consensus end primer was diluted to the level where it contained 20pmol of the primers. The first-round RT-PCR product was placed into labelled tubes of 2 µl. The genotype master mix made up of; 1.0µl each of 10 mM dATP, dCTP, dGTP, and dTTP; 7 x 1.0µl 20pmol for each genotyping primer and conserved end primer; 4.0µl 10x Taq buffer; 2.4 µl 25mM $MgCl_2$; 0.3µl Taq polymerase was prepared in a 1.5ml Eppendorf tube on to this 21.3 µl deionized H20 was added. To the 2 µl of first-round RT-PCR product in

each labelled tube, 38 µl of master mix was added. Amplification followed with 30 cycles at 95^{o}C for half a minute, 55^{o}C for half a minute and 72^{o}C for 60 seconds. PCR fragments/bands were run on 2% TAE agarose at 80 -90 volts with suitable molecular weight markers used to define the genotype of the rotavirus strain as described in world health organisation manual for rotavirus (Organization, 2009). It should be noted that RT-PCR was used to make more copies of the obtained genomes and genotyping was used to find out the types of genomes or strains present.

3.7.3.3. RT-PCR FOR P Genotyping

In each amplification reaction, thin walled tubes of 0.5ml were selected and labelled at the top using sample numbers, primer used and the date. Positive and negative controls were included. On the first cycle of amplification for P genotyping (VP8 subunit of VP4 gene), the RT master mix which contained; 2.0µl 5xAMV buffer; 0.25µl each of 10mM dATP, dCTP, dGTP, dTTP and 0.2 µl Avian Myeloblastosis Virus Reverse Transcriptase (AMV RT) was prepared and put on ice. Each VP4 consensus primer (con2 and con3) as seen in Table 3.3 as designed by (Gentsch et al., 1992) was diluted to 20 pmol followed by addition of 1 µl of each primer to all sample tubes. 5 µl of dsRNA was added to the primers and deionised water mixed up to a final volume of 10 µl through pipetting. This was followed up by the denaturing of the samples for 5 minutes at 94ºC. All sample tubes were instantly transferred into an ice bath. To each sample tube 3.2 µl of RT master mix was added within 5 minutes, mixed well and spun for 10 seconds then incubated at 42ºC for half an hour. The PCR amplification master

mix made up of; 1.0 µl each 10mM dATP, dCTP, dGTP, and dTTP; 4.0 µl 10x Taq buffer; 2.4 µl 25 mM $MgCl_2$; 29.7µl deionised water was prepared. 0.3µl Taq polymerase was added to the sample tubes just before use. The sample tubes then underwent the centrifugation process for 10 seconds after incubation at 42ºC and were placed in a sample ice rack. Thirty cycles of 95^{o}C for a half a minute 42^{o}C for half a minute, and at 72^{o}C for one minute amplifications were carried out. The PCR fragments formed were examined on 1% TAE agarose gels to determine their sizes using 100bp molecular weight markers as seen in table 3.4 on expected sizes of VP4 genotyping PCR products(Gentsch et al., 1992) for VP4 full length VP4 cDNA products (Organization, 2009).

Table 3.3

Oligonucleotide primers for P serotype PCR (Gentsch et al., 1992).

Primer	Sequence (5'-3')	Position (nt)	Strain (genotype)
1T-1	ACTTGGATAACGTGC	339-356	KU [P8]
2T-1	CTATTGTTAGAGGTTAGAGTC	474-494	RV5 [P4]
3T-1	TGTTGATTAGTTGGATTCAA	259-278	1076 [P6]
4T-1	TGAGACATGCAATTGGAC	385-402	K8 [P9]
5T-1	ATCATAGTTAGTAGTCGG	575-594	69M [P10]
Con3	TGGCTTCGCCATTTTATAGACA	11-32	Group A
Con2	ATTTCGGACCATTTATAACC	868-887	Group A

Note. Reprinted from "Identification of group A rotavirus gene 4 types by polymerase chain reaction," by J R Gentsch, R I Glass, P Woods, V Gouvea, M Gorziglia, J Flores, B K Das, M K Bhan, 1992, *Journal of* Clinical Microbiology.

Viral Protein 4 genotype amplification was carried out using consensus primer

con3 and a mixture of specific primers 1T-1, 2T-1, 3T-1, 4T-1 and 5T-1 to determine rotavirus VP4 genotypes (Gentsch *et al,* 1992). Each genotyping and consensus end primer was diluted to contain 20pmol of the primer. To labelled tubes 2 µl of first round RT-PCR product was added. The genotype master mix consisting of; 1µl each of 10mM of dATP, dCTP, dGTP, dTTP, 4µl of 10x Taq buffer, 2.4µl of 25mM $MgCl_2$, 0.3µl Taq polymerase and 22.3µl deionised water was prepared in a 1.5ml Eppendorf tube. 39 µl of this master mix was then added to each labelled tube containing first round RT-PCR products and mixed well. The tubes were then placed in the thermocycler where they underwent 30 cycles of 95^{o}C for half a minute, 55^{o}C for half a minute and at 72^{o}C for one minute. The PC fragments formed were run on 2% TAE agarose gel at 80-90 volts with appropriate molecular weight marker to determine the genotype of the rotavirus strain and compared to table 3.4 (Gentsch et al., 1992) of expected sizes of VP4 genotyping PCR products (Organization, 2009).

Table 3.4.

The expected sizes of the VP4 genotyping PCR products (Gentsch et al., 1992).

Primer	Genotypes	Expected product size (bp)
Con2 and Con3	Group A	876
3T-1	P[6]	267
1T-1	P[8]	345
4T-1	P[9]	391
2T-1	P[4]	483
5T-1	P[10]	59

Note. Reprinted from “Identification of group A rotavirus gene 4 types by polymerase chain reaction,” by J R Gentsch, R I Glass, P Woods, V Gouvea, M Gorziglia, J Flores, B K Das, M K Bhan, 1992, *Journal of* Clinical Microbiology.

3.8. Data Management

Participants in the study were selected using random numbers. No names were required on the consent forms. The samples were examined by means of ELISA, polyacrylamide gel electrophoresis (PAGE) with multiplex RT-PCR as a way of investigating the G and P types of circulating rotaviruses. The procedure was done as per the WHO Manual of Clasification and discovery of Rotavirus (Organization, 2009).

Data acquired from each participant was noted and later documented in a spread sheet. The information was kept secure with passwords recognized and accessible by the principal investigator alone.

Information generated was exposed to exploratory data analysis. Final analysis was done for each objective in terms of the strains that were prevalent, the age group affected and the different strains existing in the population post inclusion of the rotavirus vaccine in Kenya.

3.9. Ethical Approval

Ethical approval was acquired from University of Kabianga- Institutional Ethics and Research Committee. Research permit license was obtained from National Council of Science Technology and Innovation. Research letters were obtained from the County Commissioner, County Director of Education and County Director of Health (Appendices I-VI). Only patients whose parents/guardians

signed the consent forms were subjected to the study (Appendix X). Target patients were determined by a clinician. Prescribed laboratory tests were used to confirm diagnosis. Stool specimens were collected and used in laboratory diagnosis. The participants did not incur any serious risks or inconveniences.

3.10. Applicability of Results

The data obtained in this study will be of benefit The County Government Health sector. This data is significant in that it is based on post vaccination era. The results of the data analysis will be availed to the county government health committee and dissemination of results will be done through peer reviewed journals for other stakeholders to benefit from it.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

During the study, a total of 200 faecal samples were collected from children exhibiting acute gastroenteritis. The samples were collected over a period of 12 months. The study was for one year.

4.2 Presentation of Results

4.2.1 Demographic characteristics of the study participants

The number of males with acute gastroenteritis was 106 which represented fifty three percent (53%) of the total sample whereas the number of female children below five years and exhibiting acute gastroenteritis was 94 which represented forty seven percent of the total sample (Table 4.1). The mean age of the study participants was 30 months. The average number of siblings the study participants had in each home was 3 siblings. Their vaccination status was recorded, where 84% were confirmed as vaccinated with proof of their vaccination, 2.5% were not vaccinated and 13.5% were uncertain since they did not have proof or could not remember the child being vaccinated. As for the guardians their average age was noted as 29.8 years.

Table 4.1
Demographics of the Study participants.

Characteristics		Number=n(%)	Positive cases
Gender			
	Male	n=106 (53%)	14
	Female	n=94(47%)	9
Mean Age of Study participants (months)		30.3	
Mean No. of siblings		3	4
Vaccination Status	Uncertain	n=27(13.5%)	1
	Vaccinated	N=168(84%)	22
	Not Vaccinated	n=5(2.5%)	
Guardian/Parent mean age (Years)		29.8	
Hospital Status	Inpatient		9
	Outpatient		14

4.2.2.1. Prevalence of rotavirus infections in children below 5 years with acute gastroenteritis

All the 200 samples were subjected to Enzyme Immuno-sorbent Assay (ELISA) for the detection of rotavirus. The kit used was the ProSpect Rotavirus Microplate Assay which is a qualitative enzyme immunoassay for the detection of Group A rotavirus in human faecal samples. The antibodies used in this kit were rotavirus specific rabbit polyclonal antibodies conjugated to horse radish peroxidase since the procedure was sandwich ELISA. Of the total samples, 23 were found to be positive of rotavirus The prevalence rate of rotavirus infection in Kericho County Referral Hospital was confirmed as 23/200 (11.5%) as shown in Figure 4.1.

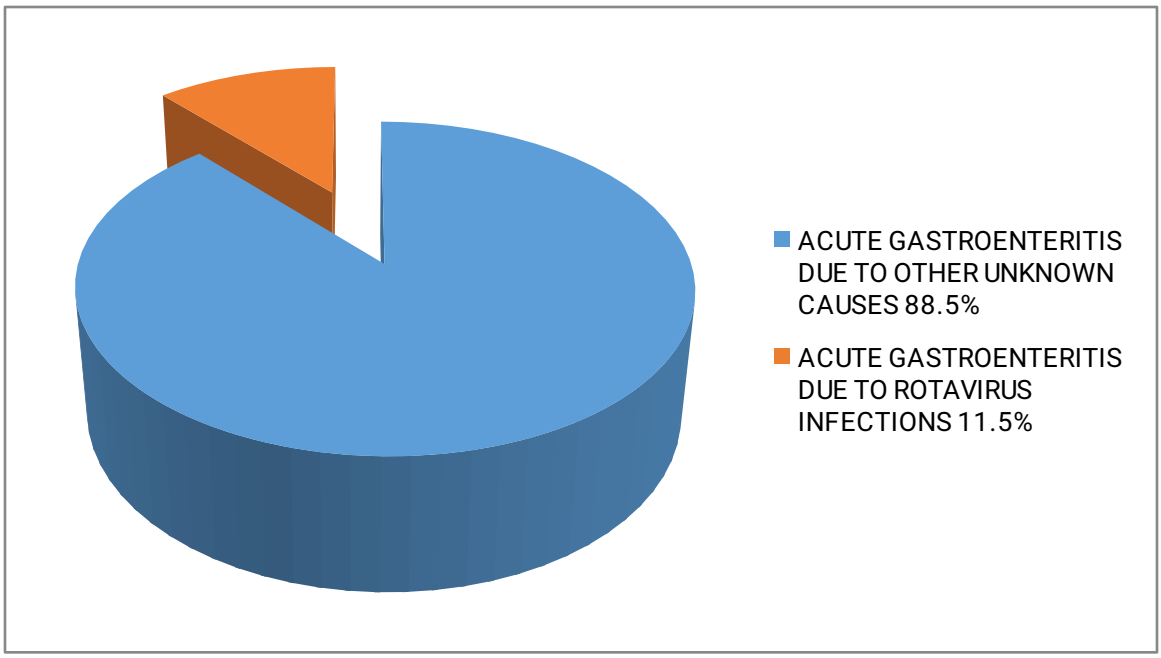

Figure 4.1-Prevalence of Group A rotavirus in children of five years and below at Kericho County Referral Hospital

4.2.2.2. Prevalence of rotavirus against gender

The percentage of male children who were positive for rotavirus is 14/23 (60.87%) whereas the female children positive for rotavirus was 9/23 (39.13%). Data showed that more male children were likely to experience a serious bout of diarrhoea as compared to their female counterparts. This also reflects on higher rotavirus infection positivity as shown in Figure 4.2.

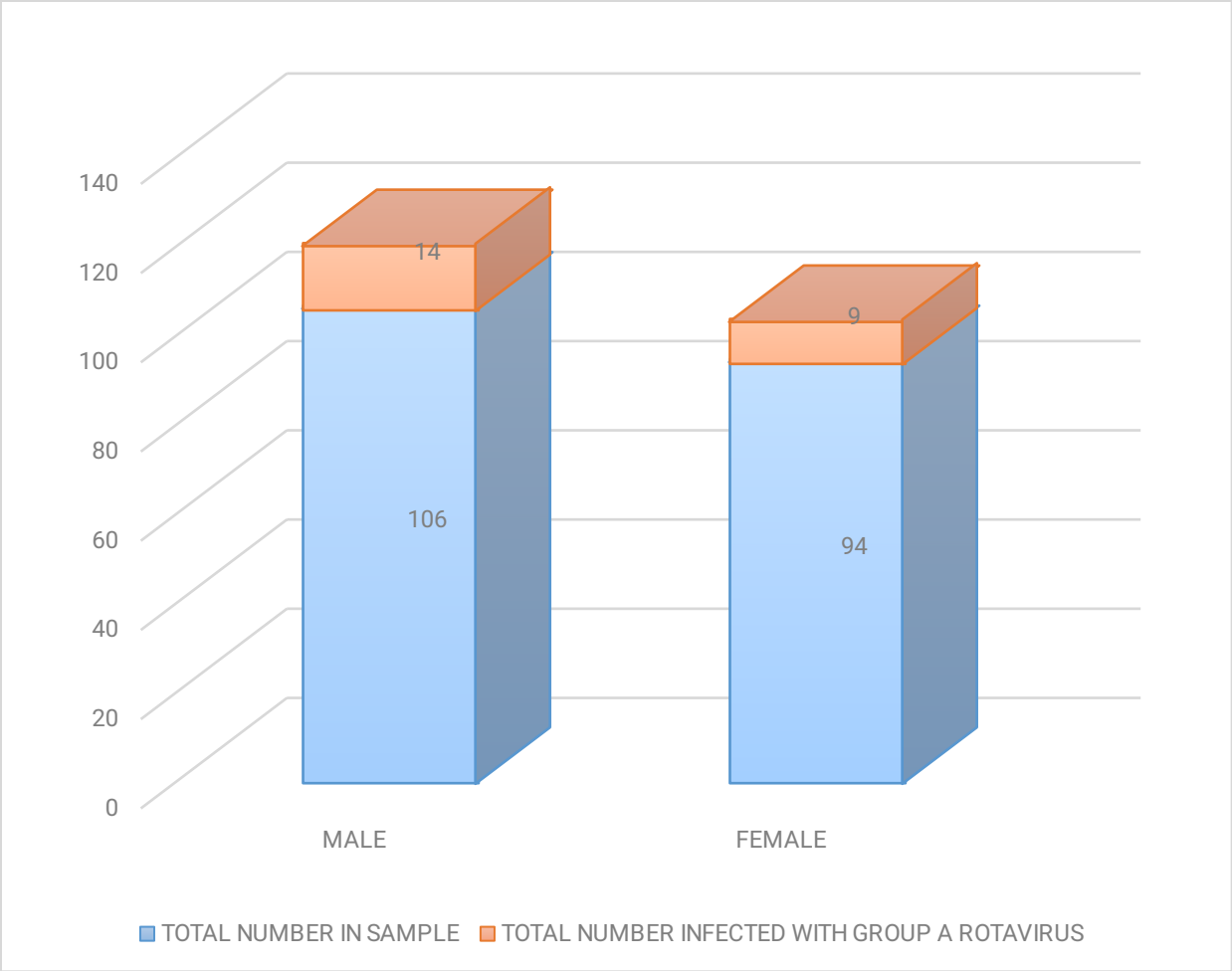

Figure 4.2: Number of infected individuals per gender in the sample group.

4.2.2.3. Age of patients with rotavirus diarrhoea

The rates of infection were as follows: Age of 0 to 10 months-3/23 (13%), 11 to 20 months -3/23 (13%), 21 to 30 months -6/23 (26%), 31 to 40 months 5/23 (22%),41 to 50 months 3/23 (13%) and finally 51 to 60 months 3/23 (13%). The highest percentage of rotavirus infections were within the 21-30 months age range followed by 31-40 months age range.

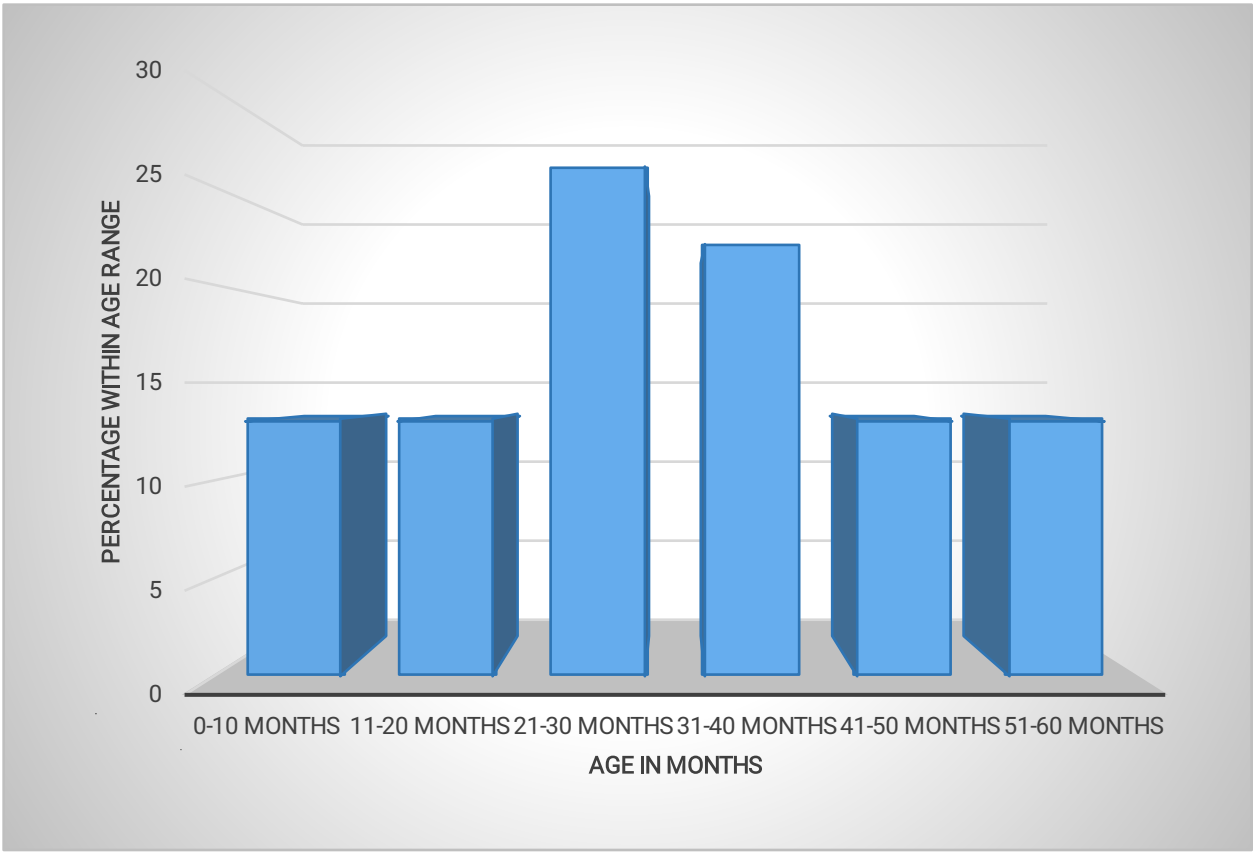

Figure 4.3- Percentage of infection in each age category

4.2.2 Percentage isolated rotavirus electropherotypes in children five years and below using PAGE.

The samples that tested positive for rotavirus infection in ELISA were subjected to PAGE testing. PAGE typing produces rotavirus RNA electrophoretic patterns showing 11 segments that are usually seen for rotavirus genomes as a result of gene molecules migrating through the gel medium as shown in Figure 4.4. This was what was used to distinguish long and short electropherotypes.

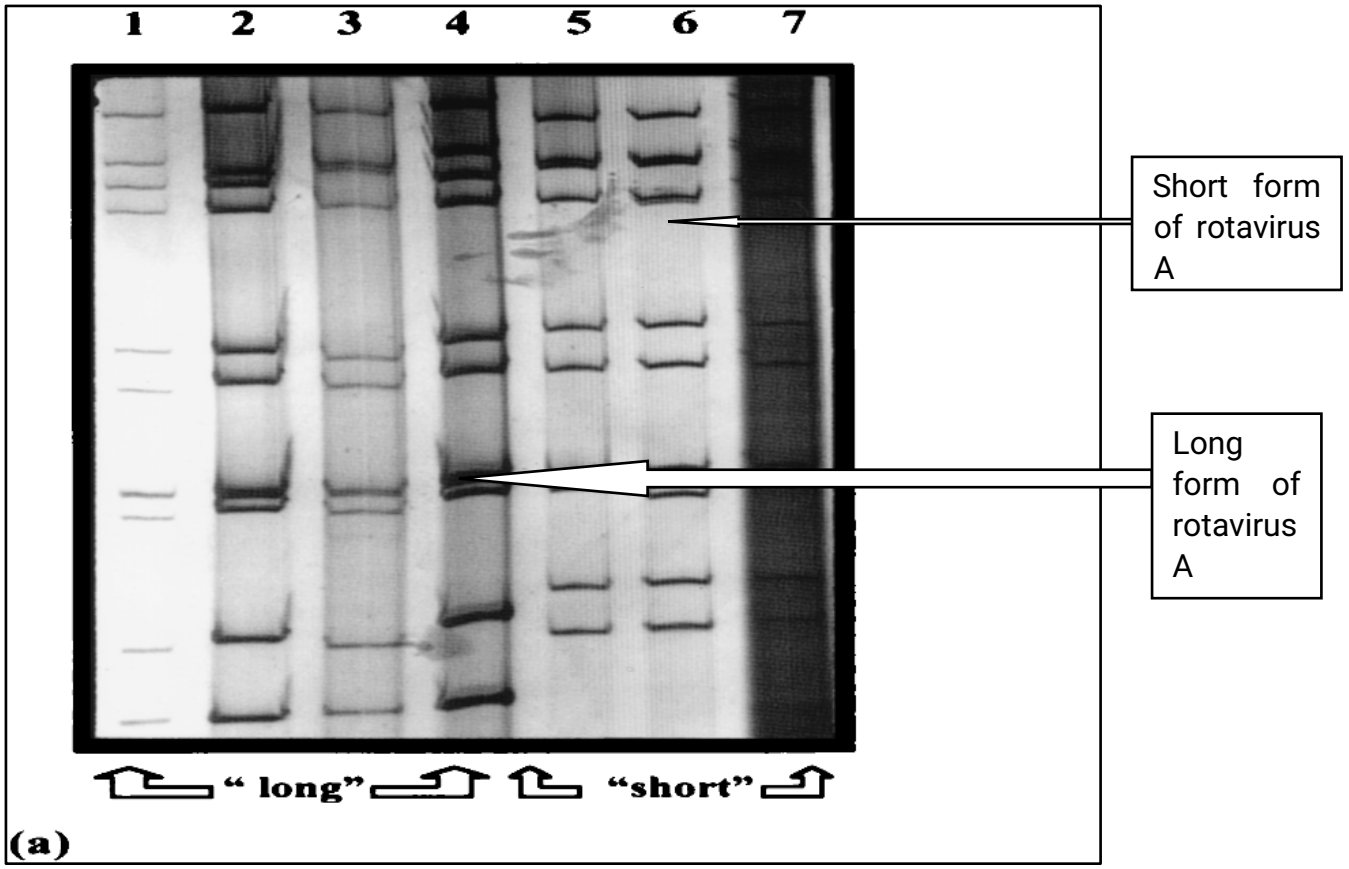

Figure 4.4 Representative electropherotype variants detected. Lanes: 1 through 4, long electropherotype patterns; 5 through 7, short electropherotype patterns of rotavirus Group A infections identified by polyacrylamide gel electrophoresis (O'Halloran et al., 2000).

Twenty two (96%) of the isolated strains were long variants electropherotypes except for one (4%) which did not show the patterns as expected. During the current study the long electropherotype was predominant. Long electropherotypes are often associated with G1, G3, G4 and G9 genotypes whereas the short electropherotypes are mostly associated with G2.The electropherotype patterns were suggestive but not confirmative of a given genotype.

4.2.3 Genotyping

Genotyping was aimed at finding out the type of strains existing in the positive samples using primers.

4.2.3.1. VP 7 Genotypes

Viral Protein (VP7) genotyping shows the distribution of group A rotavirus G and P genotypes. The G types (VP7 associated) were determined using consensus primers sBeg and End 9, and 16/23 (69.57%) were positive confirming G gentype. The G type was allocated after a PCR with G1-G4, G6, G8 and G9 specific primers (sBeg and End 9) was performed on the rotavirus cDNA template. The non-typeable positives for rotavirus were 2/23 (8.70%). The negative for G genotype were 5/23 (21.74%) as seen in Figure 4.5.

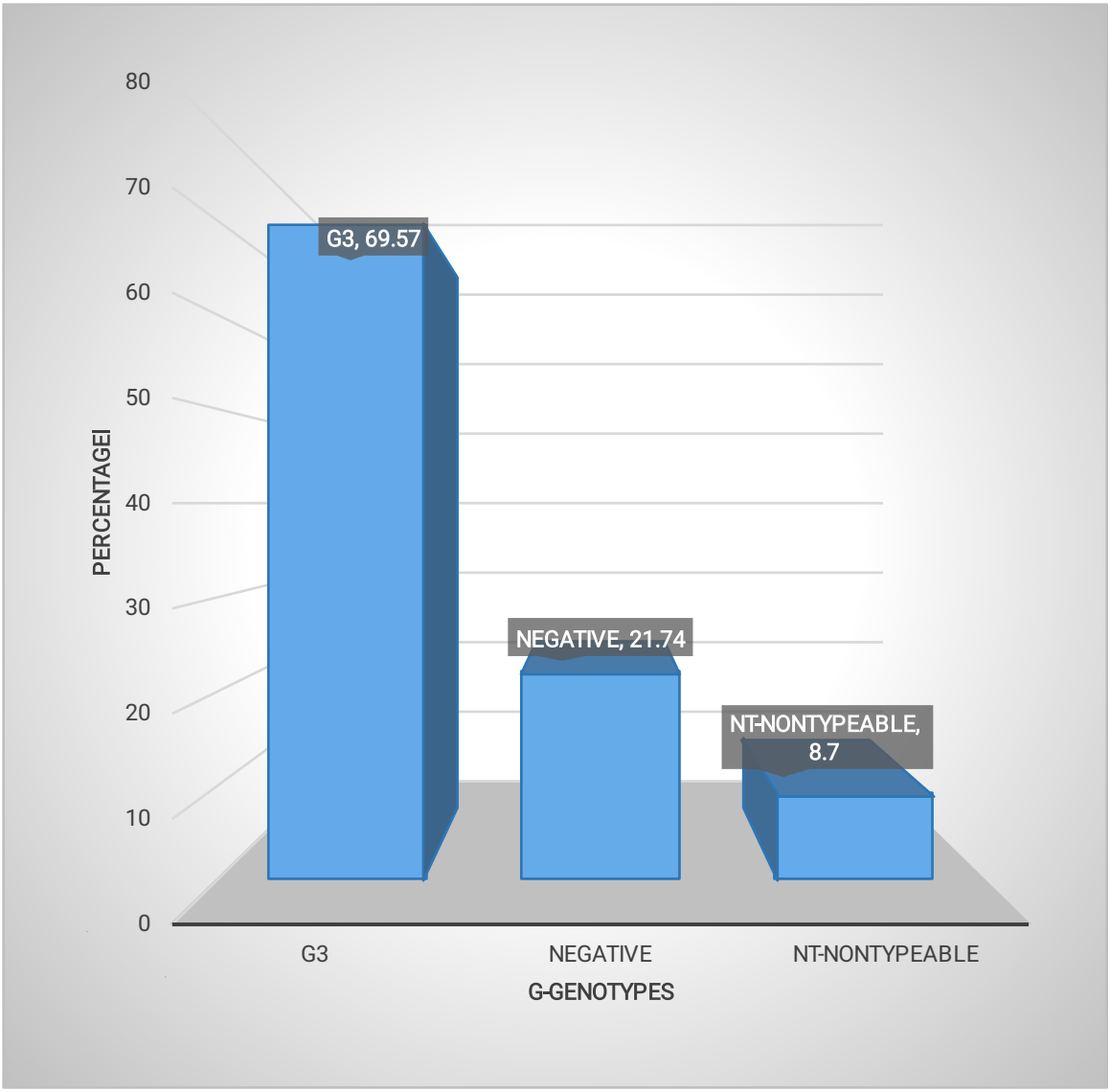

Figure 4.5 -Percentage of Group A Rotavirus, G-genotypes. 69.57 is the percentage of the G3 genotype positive samples, 21.74 is the percentage of the samples that tested negative and 8.7 is the percentage representing the non-typable samples.

Different strains of group A rotaviruses display different patterns when separated on Agarose gel. They also display unique bands depending on their genotype.

The figure 4.6 below shows the G type patterns on agarose gel through a transilluminator and what base pairs was to be expected for each G genotype molecular marker . L represents the molecular marker which is used to indicate the presence of different genes at specific genes depending on the basepair observed in each band. This was used to identify the G types found in the samples.

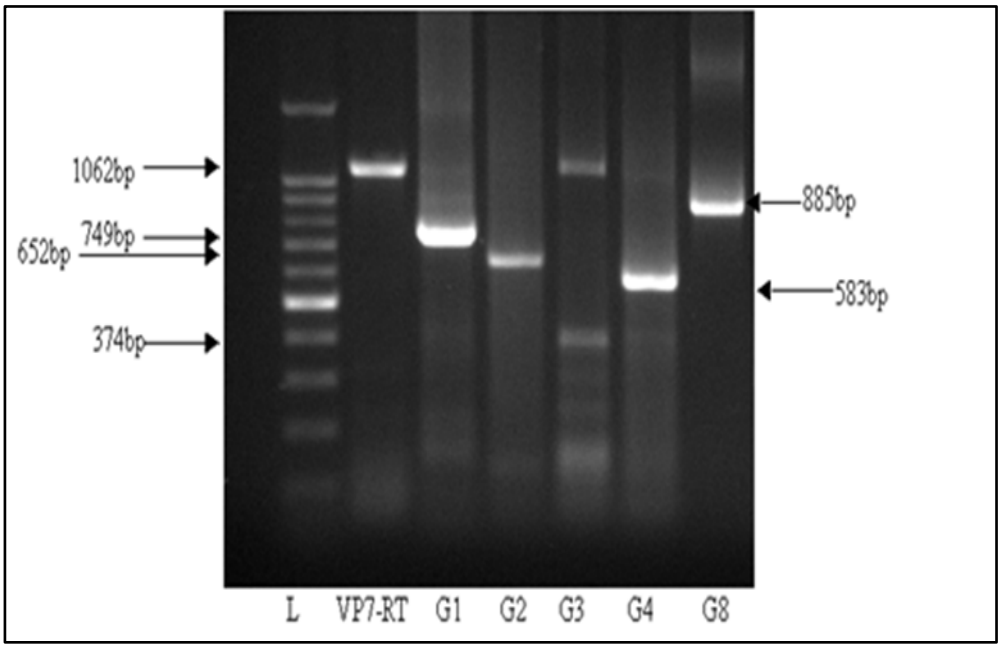

Figure 4.6- Representative G types defined by RT-PCR and identified by conventional 2% agarose gel electrophoresis. Lanes: L 100-bp molecular size marker ladder VP7- RT :represents the full gene of group A rotavirus, G1: is the molecular marker for G1 genotype at 749 base pairs, G2 is the molecular marker

for G2 genotype at 653 base pairs, G3:is the molecular marker for G3 at 374 basepairs, G4 represents genotype G4 with 583 basepairs and G8 represents genotype G4 with 885 basepairs

4.2.3.2. VP4 Genotypes

P genotypes associated with VP4 were effectively seen in 15/23 (65.22%) of the positive samples. The P type was allocated after a PCR with P[4], P[6], P[8], P[9], and P[10] specific primers (consensus primer con3 and a mixture of specific primers 1T-1, 2T-1, 3T-1, 4T-1 and 5T-1) was carried out on rotavirus cDNA templates. P[6]-1/23 (4.35%), Mixed P[6]P[8]-1/23 (4.35%),P[8]-8/23 (34.78%), Non-typeable positive for P genotype 5/23 (21.74%) and those negative for P genotype are8/23 (34.78%) as shown in figure 4.7 .

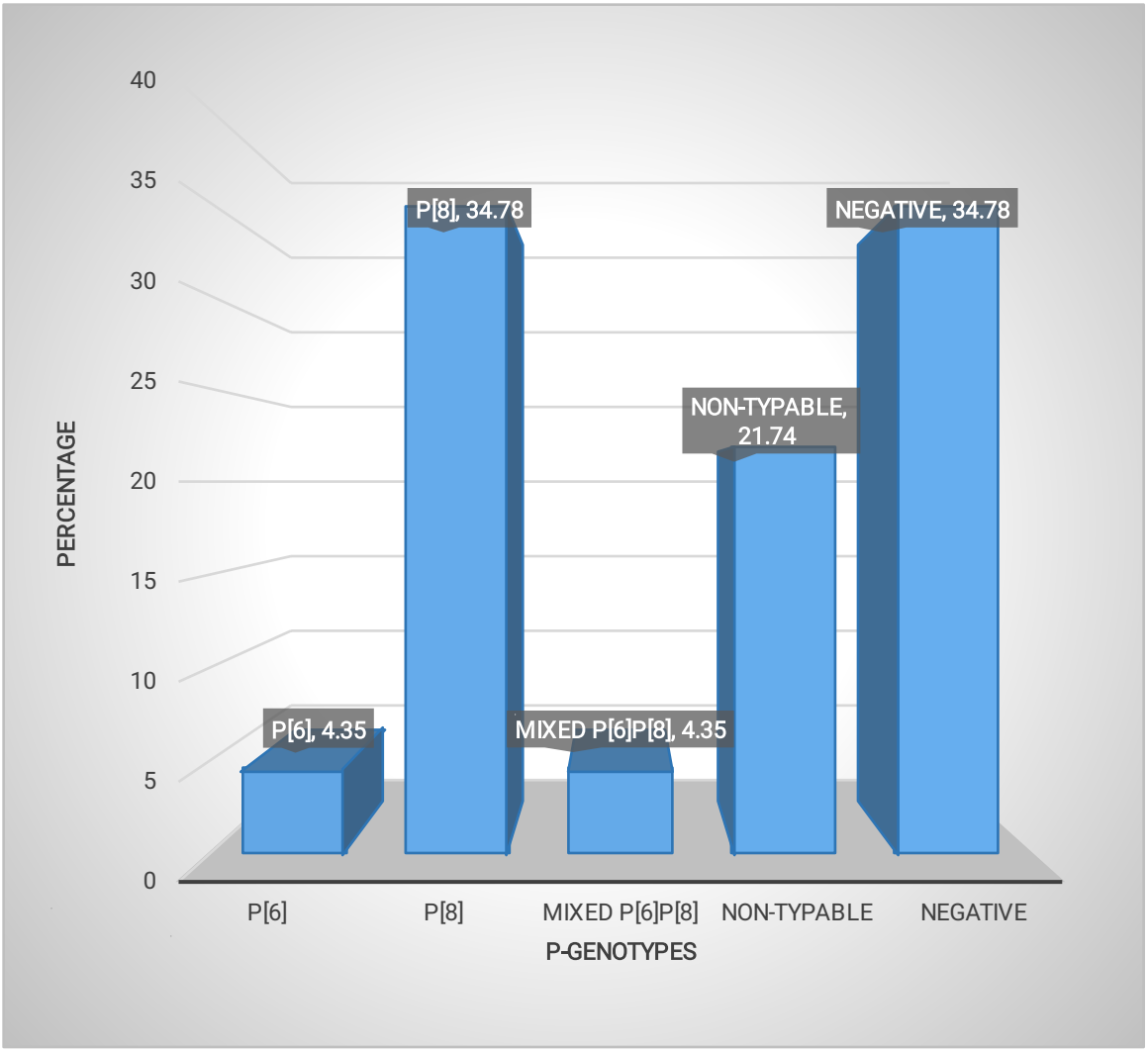

Figure 4.7 – Percentage of Group A Rotavirus, P-Genotypes

The figure below displays the different P types in agarose gel displayed in a transilluminator. L represents the molecular weight marker used to indicate the presence of different genes depending on the basepairs observed in each band. This was used to identiy the P types found in the sample. Figure 4.8 indicates what was to be observed from different groups A rotavirus P genotypes.Results obtained showed that 4.35% displayed a band similar to P[6] genotype,34.78%

displayed a band similar to P[8] 4.35% displayed bands a both P[8]and P[6] simultaneously thus indicating a mixed infection whereas 34.75% did not display any bands.

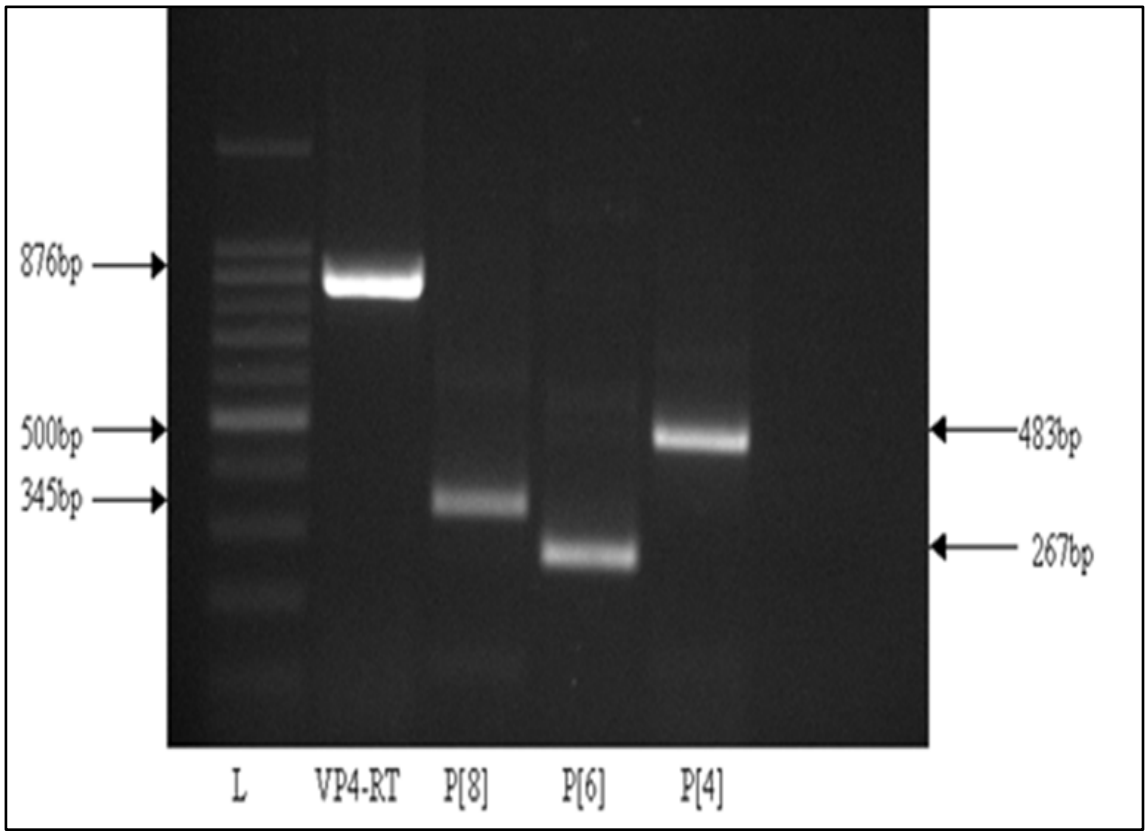

Figure 4.8- Representative P types defined by RT-PCR and identified by conventional 2% agarose gel electrophoresis. Lanes: L 100-bp molecular size marker ladder VP4- RT :represents the full gene of group A rotavirus, P[8]: is the molecular marker for P[8] genotype at 345 base pairs, P[6]: is the molecular marker for P[6] genotype at 267 base pairs, P[4]:is the molecular marker for P[4] at 483 basepairs.

4.2.3.3. Combination of G and P types

During the typing of assays it was observed that a specific G type could always be correlated to a P type; namely, G3, would coexist with P[8] that is G3P[8] which

occurred in 8/23 (34.78%) and only once did a mixed infection occur of G3P[6]P[8] -1/23 (4.35%) as shown in table 4.3 below.

Table 4.2
G and P type combinations detected in children of five years and below.

G/P combination type	Children (N=23)(%)
Common types	
G3P[8]	8/23 (34.79%)
Uncommon types	
G3P[6]P[8]	1/23 (4.35%)
Non typeable	
GntPpos	3/23 (12.00%)
GposPnt	7/23 (30.43%)
GntPnt	4/23 (17.39%)

GntPpos- G non-typeable but with identified P type

GposPnt- P non-typeable but with identified G type

GntPnt- both G and P non-type able

4.3 Discussion of Results

A total of 200 children were sampled with clinical manifestation of acute gastroenteritis. Twenty three (23) of the samples collected tested positive for rotavirus resulting in a prevalence rate of 11.5% (Figure 4.1).

The study also discovered that more boys were affected by rotavirus gastroenteritis. This might be because boys have more freedom with their

environment or surroundings and less attention from their guardians as compared to their female counterparts. Girls also tend to at times to be careful of their hygiene, less trusting with ingesting items that might be contaminated with rotavirus as compared to boys to add on to the close attention they receive from their guardians.

Rotavirus innfections were recorded in children with the age ranges of 21-30 months followed by the age ranges of 31-40 months. The lowest rotavirus infections were recorded at 0-10 months and this could be attributed to passive immunity associated with early life development. The rotavirus vaccine is often given at 8 weeks.The second booster dose is given at the age of 12 weeks. Acquired immunity normally lasts for 2 years thereafter. It is therefore true to attribute the increased rate of infection in older age brackets to the reduced active immunity and decreased passive immunity. This study confirmed an age shift in rotavirus infections to 21-40 months and this phenomenon is often witnessed with programmatic vaccine introduction in a country.

The impact of programmatic vaccine introduction was positive as confirmed by a low in prevalence rate compared to published data done before massive vaccination was done in the country. One such study was that of Agutu *et al* 2017 where the prevalence was found to be 31.5% .in a cross sectional study carried out Gertrude's children's Hospital in 2012. A comparison between the two studies is important because the current study findings represent post vaccination data and the latter represents pre-vaccination data. To confirm the validity of the effect of vaccine introduction on the frequency of rotavirus

infections, other researchers found consistent results in Kiambu County Hospital (29.6%) and Mbita District Hospital (11.2%) (Shah et al., 2017).

All samples presented 11 segments of dsRNA pattern similar to the positive Group A rotavirus controls that consisted of long and short strain samples. This was with the exception of one which despite being positive in ELISA which did not give any result. The characteristic grouping of rotavirus RNA segments of 4-2 -3-2 was observed. This showed that the samples were positive for Group A.

There were 23 (11.5%) samples that were positive for rotavirus when subjected to PAGE and silver staining. Positive Group A ELISA samples display a unique pattern when subjected to PAGE and Silver staining. Viral Protein (VP7) genotyping indicated the distribution of group A rotavirus genotypes G and P genotypes. Overally 69.7% of the positive samples were of G-types (VP7 associates).

The G types (VP7 associated) were determined using consensus primers sBeg and End 9. The G type was determined using PCR with G1-G4, G6, G8 and G9 specific primers (sBeg and End 9) which were performed on rotavirus cDNA template. The non-typeable positives were 2/23 (8.70%) while negative samples for G genotype were 5/23 (21.74%).

In Kenya, the predominant strains between 1999 to 2000 G3 and G1 (Nyangao et al., 2010). Arising from these previous studies, it is evident that there has been dynamism in circulating strains being G3P[8] compared to those of pre-vaccination era.

Seheri (2014), having explored circulating strains over a five year period, reported predominance of G9[8] and G8P[4] being predominant strains in Africa between 2007 to 2011. This indicated the dynamic change in the types of strains in circulation in a population even in pre-vaccination era. Around the same period, a study done in Eastern Kenya indicated that recurring serotype replacement of genotypes in Eastern Kenya were G9,G1 and G8 between 2009 and 2011. G12 strains was also observed for the first time in this (Kiulia et al., 2014).

There is a strong evidence that the G3 found to be predominant currently was not common in pre-vaccination era. In a review looking at both pre vaccination (January 2010- June 2014) and post vaccination (July 2014 – December 2018) eras across Kenya., it was observed that predominant strains during the former era were G1P [8] , G8P [4] (15.8%), G9P [8] (13.2%), and the least dominant were G2P [4] and G3P [6] (Mwanga et al., 2020) .During the post vaccination era the most predominant strains were G1P [8] , G2P [4] and G3P [8] respectively (Mwanga et al., 2020). Another study carried out that indicated G9P[8] as the predominant strain in 2016 (Gikonyo et al., 2020). Before the introduction of rotavirus vaccine, the most prevalent strain was G1P[8] which was then replaced by G2P[4] after vaccine introduction. This indicated a serotype replacement and impact of the vaccine to the existing strains.

Different rotavirus strains were observed in different areas in Kenya and that there has been remarkable change in strain types in circulation before and after introduction of ROTARIX vaccine in Kenya. It is unclear whether the existing ROTARIX vaccine with G1 serotype provides cross protection against these

emerging strains found in the children at Kericho County Referral Hospital.Kenya. It might also be that the ROTARIX vaccine is only targeting the strain used in its composition alone.

The findings from this study confirm reduction of rotavirus related hospitalization as evidenced by reduced prevalence. The prevalence calculated was obtained by dividing the number of positive saples by the sample size and finding the percentage. The percentage was compared to prevalences calculated from similar studies done in other hospitals. A comparative study was carried out on the impact of rotavirus vaccination on rotavirus hospitalisation rates among a resource-limited rural population in Mbita. There was a comparison on rotavirus associated hospitalisations and strain distribution in a period 2 years post vaccination introduction compared to the existing 3 year pre-vaccine baseline.

4.4 Limitations of the Study

The study had several limitations:

1. This scope was also limited due to absence of published data on rotavirus infections to be used as baseline at Kericho County Referral Hospital.
2. More over only patients at the hospital setting were recruited in this study. Any child who met the selection criteria but did not have an oppourtunity to seek treatment in a hospital was excluded.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Introduction

The main focus of this study was the prevalence of rotavirus post vaccine introduction from July 2021- July 2022. The study was also checking on the effects of the vaccine on the rotacvirus strains that existed at the time of the research and determine if there was a change in the strains after vaccine introduction. The information obtained during the study was compared to similar studies carried out in other counties in Kenya. There was a shift in the age that was regularly infected, from ages 0 – 12 weeks pre-vaccine introduction to 21-40 months post- vaccine introduction.

5.2 Summary of the Findings

The prevalence rate of group A rotavirus in this study was 11.5% which was lower compared the studies carried out in Kilifi County, Kenyatta Refferal Hospital and Nairobi County during which the prevalences observed were 13.8%, 14.5% and 15.2% respectively (J. Gikonyo et al., 2019; Lambisia et al., 2020; Muendo et al., 2018) . This was a reduction in the prevalence compared to pre-vaccination data.

During this study it was observed that the predominant G genotype was G3 at 16/23 (69.57%) of the infections. This was different compared to strains that existed during the pre vaccination era which had G9, G1 and G8 as the predominant strains (Kiulia et al.,2014). The P genotype makes up 15/23 that is 65.22% of the infections, 5/23 (21.74%) were non type able for P genotype.

The most predominant combination of strains was G3P[8] which was present in 34.79% of the positive samples.This was a contrast compared to the strains observed between 2010 to 2011 in Kenya where the predominant strains were G9[8] (Seheri et al.,2014).This indicated genotypic shift, which will be of key significance for upcoming studies done in Kericho County.

The discovery of rotavirus genotypes for the first time in children at Kericho County Referral Hospital is a hint that the occurrence of specific genotypes may change with time in the area.

As seen in the positive samples, rotaviruses do undergo reassortment which is one way in which rotaviruses evolve to survive (Tanaka *et al.*, 1988). The identification of combination infections of G and P in the sample collected emphasizes this (Table 4.3). This further places a need of continuous surveillance to access whether the current vaccines will succeed in the prevention of gastroenteritis caused by rotavirus infections. Genotyping is used in finding out the diversity of the existing strains and combination of G and P genotypes of rotavirus. This is normally very significant in monitoring emergence of new strains (Wu et al., 1994).PCR which is more sensitive and has been used in rotavirus determining rotavirus genotypes (Ushijima et al., 1994).

The main vaccine administered by the government through public health facilities is ROTARIX which is a monovalent vaccine. It contains a single rotavirus strain which was meant to provide protection against G1, G3, G4 and G9 strains. It was observed that despite the use of ROTARIX as a vaccine the children still got

infected with rotavirus thus underscoring the ability of rotavirus to undergo reassortment and serotype placement.

5.3. Conclusions

Gastroenteritis due to Rotavirus is one of the major causes of severe dehydrating diarrhoea in children prevalent at Kericho County Referral Hospital.The lower prevalence rate of infection observed during the study confirms the positive impact of a programmatic vaccination.

The study observed that children above 20 months of age are the ones mostly affected as compared previously to children below this age. Considering the observation of a shift of infection to older age groups, inclusion of vaccination into older age groups might be advisable or a booster vaccine at older ages.

The fact that the infected children had already received vaccination against rotavirus infections was also noticed which were also a surprise and an indicator on a necessary review of the vaccine being administered.

The combinations and the strains that existed during the pre-vaccination period were not the ones that were found during the research. Despite vaccination the children still got rotavirus infections.This indicated that maybe the strains that were targeted while the vaccine was being prepared may not be the ones circulating in the population.This undermined the effectiveness of the vaccine.This may be dealt with by introducing other vaccines formed with other strains to increase protection.

The strains observed during the study do varied greatly compared to similar studies carried almost at the same time but in different counties. Thus no two

areas even in the same country are the same considering the fact that the climatic conditions, social economic factors and even infrastructure between Nairobi and Kericho differ greatly.

5.4. Recommendations

1. The noticeable change of the occurrence of rotavirus infection fron 0-12 months to the older ages of 21-40 months. The health sector or it stakeholders may consider the introduction of a booster vaccine at the older age ranges. This will prevent infections in older children.
2. The prevalence of rotavirus was reduces due to the vaccine introduction but infections still occur. There should be sensitisation by the health sector on the importance of the rotavirus vaccine and how to prevent rotavirus infections through proper hygiene in households.
3. Rotavirus vaccine affected the exisitng of strains in the population. It was noted that the strain in the vaccine was not among the circulating strains. Hence the vaccine might be eliminating the one strain and favouring the other strains. It might be time to include the other vaccines that have other strains in their make up such as ROTASIL and RotaTeq. This will ensure that many strains have been protected against.
4. There were also other non-typeable strains in the sample population. This meant that new strains that have not been documented were also available. More studies on the different rotavirus strains, their genotyping classification is advised. This will ensure the availability of their primers for research and

the development of new vaccines that will cater for their infections.

5.5. Suggestions for further Research

1. The existence of non-typeable strains in the study dictates for more research to be done on the genotyping of the new emerging strains of Rotavirus with a focus on creating new primers for RT-PCR.

2. The fact that all the infants that were infected had already been vaccinated is an emphasis for research in vaccine development. This would assist in creating vaccines that would provide protecction from the existing and emerging strains. The favoured vaccine would be multi-valent containing various attenuated strains if possible.

3. The infections occurred in older children. This brings to light the importance of research on ways of mitigating reinfection at later ages in children especially the consideration of booster vaccines.

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APPENDICES

APPENDIX I: CLEARANCE FOR FIELDWORK

UNIVERSITY OF KABIANGA

ISO 9001:2015 CERTIFIED

OFFICE OF THE DIRECTOR, BOARD OF GRADUATE STUDIES

REF: PG/C/MIC/0005/16 Date: 29th July, 2019

Beth Khayeli Nyamanga.
Department of Biological Sciences.
University of Kabianga.
P.O Box 2030- 20200.
KERICHO.

Dear Ms. Nyamanga.

RE: CLEARANCE TO COMMENCE FIELD WORK

I am glad to inform you the Board of Graduate Studies during its meeting on 8th May, 2019 approved your research proposal entitled **"Prevalence and Genetic Diversity of Rotaviruses in Children with Acute Gastroenteritis at Kericho County Referral Hospital".**

I am also acknowledging receipt of two copies of your corrected Proposal.

You are now free to commence your field work on condition that you obtain a research permit from NACOSTI.

Please note that, you are expected to publish at least one (1) paper in a peer reviewed journal before final examination (oral defence) of your Masters thesis.

Thank you.

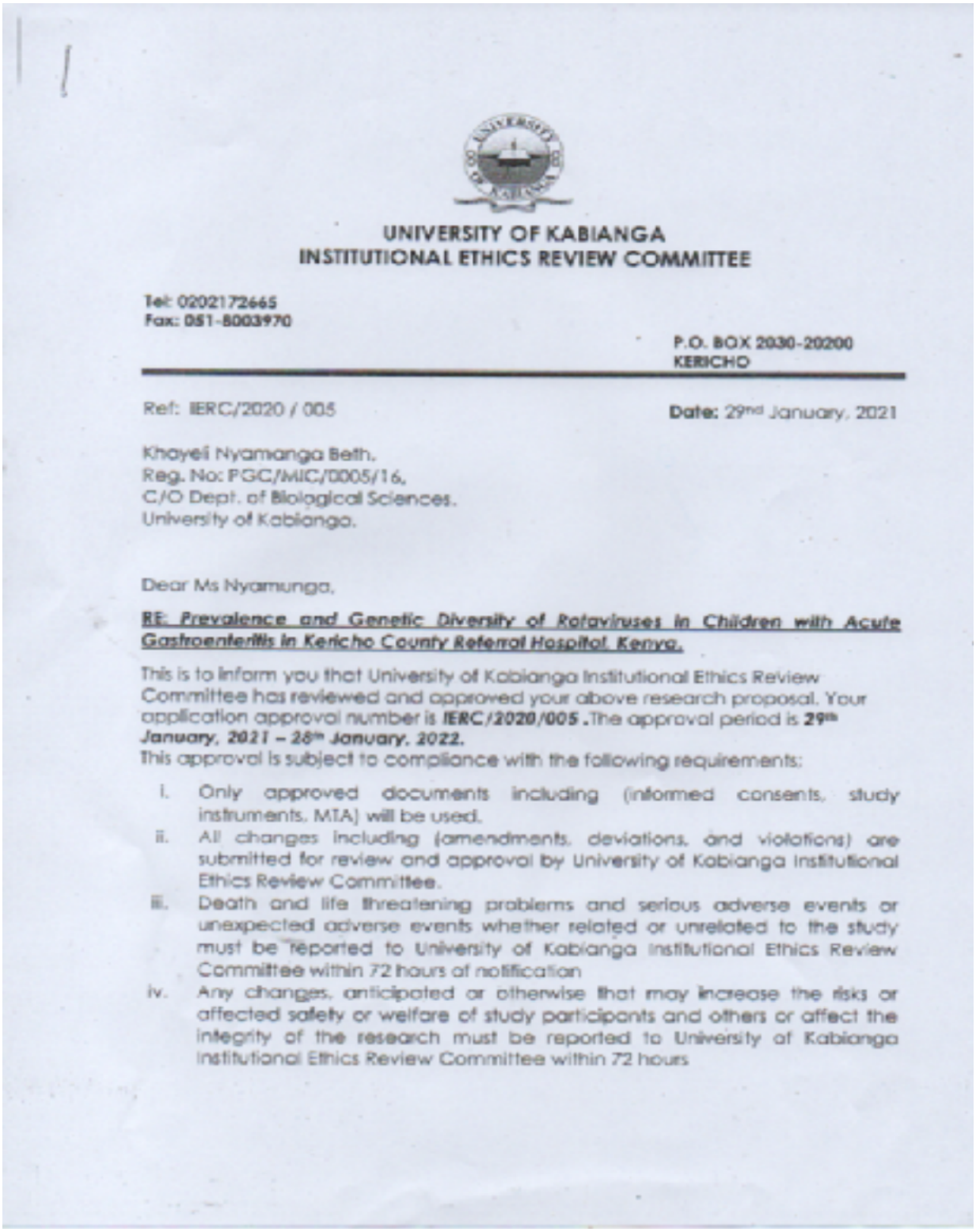
Yours Sincerely,

UNIVERSITY OF KABIANGA
26 JUL 2019

Prof. J. K. Ribolt
DIRECTOR, BOARD OF GRADUATE STUDIES

cc 1. Dean, SST
2. HOD, Biological Sciences Department
3. Supervisors

APPENDIX II: ETHICAL CLEARANCE FROM INSTITUTIONAL ETHICS REVIEW COMMITTEE



UNIVERSITY OF KABIANGA
INSTITUTIONAL ETHICS REVIEW COMMITTEE

Tel: 0202172665
Fax: 051-8003970

P.O. BOX 2030-20200
KERICHO

Ref: IERC/2020 / 005

Date: 29nd January, 2021

Khayeli Nyamanga Beth,
Reg. No: PGC/MIC/0005/16,
C/O Dept. of Biological Sciences.
University of Kabianga.

Dear Ms Nyamunga,

RE: *Prevalence and Genetic Diversity of Rotaviruses in Children with Acute Gastroenteritis in Kericho County Referral Hospital, Kenya.*

This is to inform you that University of Kabianga Institutional Ethics Review Committee has reviewed and approved your above research proposal. Your application approval number is ***IERC/2020/005*** .The approval period is ***29th January, 2021 – 28th January, 2022.***
This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by University of Kabianga Institutional Ethics Review Committee.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to University of Kabianga Institutional Ethics Review Committee within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to University of Kabianga Institutional Ethics Review Committee within 72 hours

v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. 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.
vii. Submission of an executive summary report within 90 days upon completion of the study to University of Kabianga Institutional Ethics Review Committee.

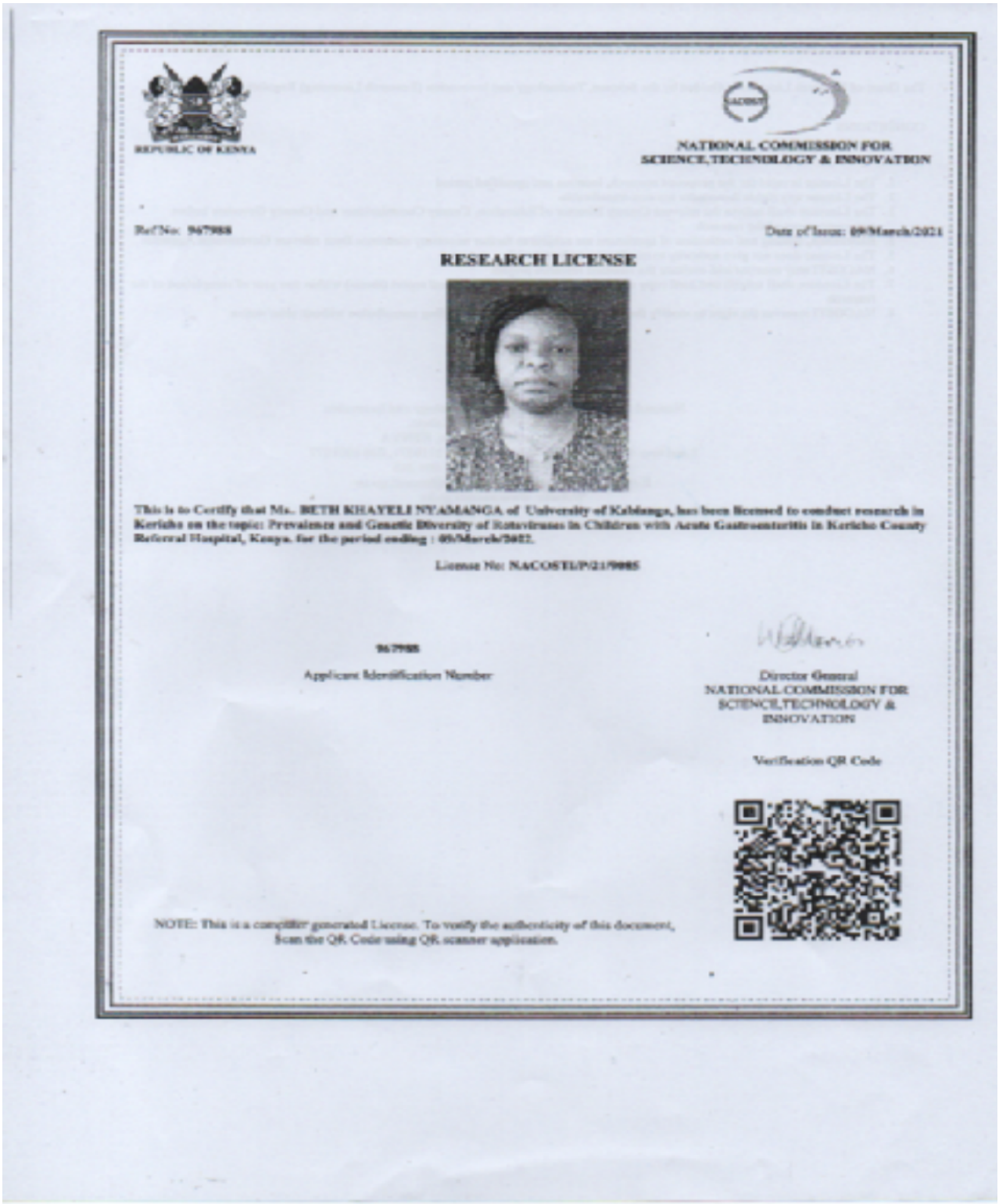
Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Dr. Erick Mibei, PhD.
CHAIRMAN, INSTITUTIONAL ETHICS REVIEW COMMITTEE.

cc: DVC (PR&D)
Director (Research, Extension and Linkages)

APPENDIX III: RESEARCH LICENSE FROM NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 967988

Date of Issue: 09/March/2021

RESEARCH LICENSE

This is to Certify that Ms.. BETH KHAYELI NYAMANGA of University of Kabianga, has been licensed to conduct research in Kericho on the topic: Prevalence and Genetic Diversity of Rotaviruses in Children with Acute Gastroenteritis in Kericho County Referral Hospital, Kenya. for the period ending : 09/March/2022.

License No: NACOSTI/P/21/9985

967988

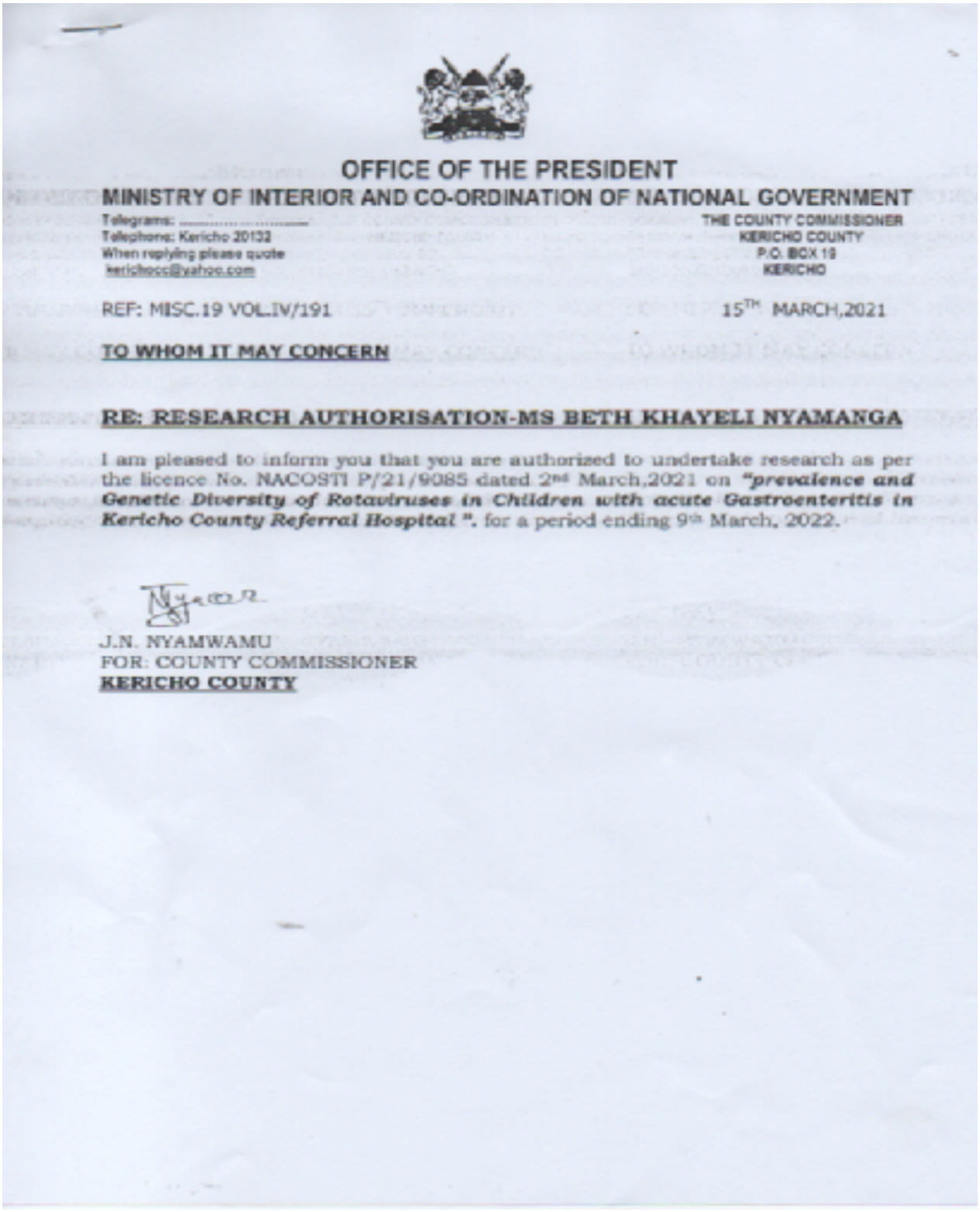
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

APPENDIX IV: RESEARCH PERMIT LETTER FROM KERICHO COUNTY COMMISSIONER



OFFICE OF THE PRESIDENT

MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT

Telegrams: .....
Telephone: Kericho 20132
When replying please quote
kerichocc@yahoo.com

THE COUNTY COMMISSIONER
KERICHO COUNTY
P.O. BOX 18
KERICHO

REF: MISC.19 VOL.IV/191

15TH MARCH,2021

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORISATION-MS BETH KHAYELI NYAMANGA

I am pleased to inform you that you are authorized to undertake research as per the licence No. NACOSTI P/21/9085 dated 2nd March,2021 on ***"prevalence and Genetic Diversity of Rotaviruses in Children with acute Gastroenteritis in Kericho County Referral Hospital "***. for a period ending 9th March, 2022.

J.N. NYAMWAMU
FOR: COUNTY COMMISSIONER
KERICHO COUNTY

APPENDIX V: RESEARCH PERMIT LETTER FROM KERICHO COUNTY DIRECTOR OF EDUCATION

MINISTRY OF EDUCATION

STATE DEPARTMENT OF EARLY LEARNING AND BASIC EDUCATION

Email:cdekerichocounty@gmail.com
When Replying Please Quote:

County Education Office
P.O BOX 149
KERICHO

Ref: No. KER/C/ED/GC/VOL.11/89

15th March 2021

TO WHOM IT MAY CONCERN.

RE: RESEARCH AUTHORIZATION: BETH KYAYELI NYAMANGA LICENCE NO.NACOSTI/P/21/9085

I refer to the Director NACOSTI Letter Ref: No.967988 dated 9th March 2021 granting the student above authority to conduct research at Kericho County Referral Hospital Her area of study is titled ***PREVALENCE AND GENETIC DIVERSITY OF ROTAVIRUSES IN CHILDREN WITH ACUTE GASTROENTERITIS*** for the period ending 9th March 2022.

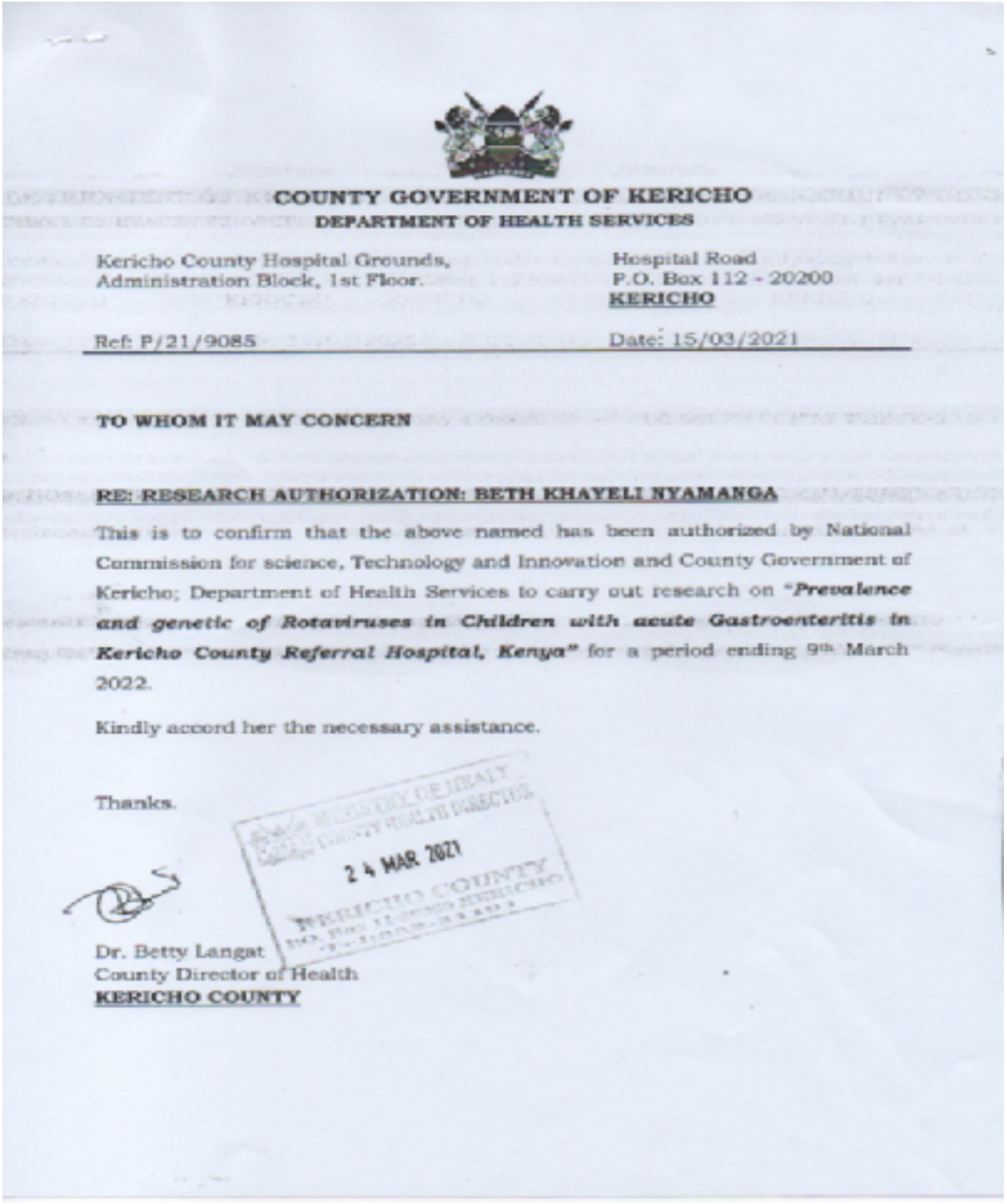
This is to request your office to accord her the necessary support during the research period.

Thank you.

16 MAR 2021

ROSE K SAGARA
COUNTY DIRECTOR OF EDUCATION
KERICHO COUNTY.

APPENDIX VI: RESEARCH PERMIT LETTER FROM KERICHO COUNTY DIRECTOR OF HEALTH



COUNTY GOVERNMENT OF KERICHO
DEPARTMENT OF HEALTH SERVICES

Kericho County Hospital Grounds,
Administration Block, 1st Floor.

Hospital Road
P.O. Box 112 - 20200
KERICHO

Ref: P/21/9085

Date: 15/03/2021

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORIZATION: BETH KHAYELI NYAMANGA

This is to confirm that the above named has been authorized by National Commission for science, Technology and Innovation and County Government of Kericho; Department of Health Services to carry out research on ***"Prevalence and genetic of Rotaviruses in Children with acute Gastroenteritis in Kericho County Referral Hospital, Kenya"*** for a period ending 9th March 2022.

Kindly accord her the necessary assistance.

Thanks.

24 MAR 2021

Dr. Betty Langat
County Director of Health
KERICHO COUNTY

APPENDIX IX - DATA COLLECTION INSTRUMENTS

CAREGIVER/GUARDIAN DETAILS FORM

Age: ……
Education level: …….
Parity: …….
Residence/distance from health facility: ……..

Employment status: ……..

How much earned per month: ……..

INFANT/CHILD DATA

1. Age : ……..months Date of birth: ……/……/……..
2. Gender: Female ☐ Male: ☐
3. Department: Inpatient Outpatient ☐
4. Rotavirus vaccination status: Vaccinated Not ☐ vaccinated ☐ Unascertained
5. Nutritional status: Malnourished Yes No ☐
6. Frequency of diarrhea: ……/day
7. Duration of the diarrhea:…….days Diarrhea is defined, as the incidence of three or more, loose, liquid, or watery stools within 24 hour period
8. Stool Consistency : Watery Mucoid Bloody Other ☐
9. Name of the person collecting the information and the stool specimen: ……

10. Date: ……/……/……. Time: …….

APPENDIX X - ADULTS CONSENT FORM (PARENT/GUARDIAN)

Title: PREVALENCE AND GENETIC DIVERSITY OF ROTAVIRUSES IN CHILDREN WITH ACUTE GASTROENTERITIS AT KERICHO COUNTY REFERRAL HOSPITAL

INVESTIGATOR

PRINCIPAL INVESTIGATOR

1. Nyamanga Beth Khayeli

INTRODUCTION

I would like to acquaint you with this research embarked on by Nyamanga Beth Khayeli, a Masters student at University of Kabianga. I would be obliged if you aid in this study.

REASON FOR THE STUDY

The research focuses on a diarrhea causing microbe called 'Rotavirus' in infants of 5 years and below. The study is based in Kericho County Referral Hospital. It will assist in providing information on the various strains of the virus found in the region and the importance of the virus.

HOW MANY PEOPLE PARTICIPATE IN THE STUDY?

191 individuals are to take part in the research.

WHAT IS INVOLVED IN THE STUDY?

This research comprises of unsystematic collection of stool samples from children attending Kericho County Referral Hospital. The stool samples will be collected from children whose guardians will allow them to participate in the study. A disinfected vessel will be provided

to in which to collect the stool sample from your child during the clinic visit. The gathered samples will be delivered to KEMRI to undergo sequencing and typing.

APPROVAL FOR STORING AND DELIVERY OF SAMPLES FROM KERICHO COUNTY REFFERAL HOSPITAL TO CENTRE FOR VIRUS RESEARCH KEMRI

YES ☐ NO ☐

KINDLY TICK ONE BOX

DURATION OF SAMPLE COLLECTION

Sample collection is to take 2 months

RISKS OF THE STUDY

The study is not intrusive. The stools will be collected in the containers through the normal defecation process. Hence no pain inflicted.

BENEFITS TO TAKING PART IN THE STUDY

There are no direct benefits from the study. Instead, its outcomes will be shared with the concerned authorities that will then inform the community, centered on the outcomes, and take the essential courses of action.

DISCRETION

All the data acquired will be strictly private and encrypted. Access to the date will only be by the Principal investigator, supervisors, relevant officials at Kericho County Referral Hospital and on need basis, a selected expert from the Institutional Ethical Review Committee. Identification throughout the study will only be by the

Number that you will be given. Healthcare givers will only gain access of the attained outcomes with permission from you.

No payment is required to allow your child to take part in the research.

SUMMARY OF THE RIGHTS OF THE CHILD PARTICIPATING IN THE RESEARCH

The research is completely charitable. Hence thus allowed to withdraw from it at any time and no penalties are going to be imposed. The child is not surrendering any legal rights if you sign the document giving permission.

CONTACT INFORMATION

For any questions related to the study or remarks please contact:

Principal Investigator: Nyamanga Beth Khayeli
Telephone: 0725635159.
Email: bkhayeli@gmail.com

YOUR CONSENT

I have read the Consent Form and conditions of this project. Every inquiry has been responded to and in aiding _____, I offer my charitable permission.

PERMISSION FOR FAECAL SAMPLE COLLECTION GIVEN: YES ☐ NO

KINDLY TICK ONE BOX

SIGNATURE OF PARTICIPANT _____ **DATE:**

PRINTED NAME OF PARTICIPANT: _____

Name of the witness_____ **SIGN** _____

* Only in case of the subject being illiterate that a thumb is used.
*Excluding the person collecting specimen

SIGNATURE OF PERSON OBTAINING CONSENT: _____

DATE:_____

PRINTED NAME OF INDIVIDUAL PROCURING PERMISSION: _____
(Must be the principal investigator or individual designated in the checklist to obtain consent)

SIGNATUREOF PRINCIPAL INVESTIGATOR: _____

DATE: _____

CONSENT FORM IN KISWAHILI

FORMU YA KUFAHAMISHA IDHINI (MZAZI/MSIMAMIZI)

KICHWA: Uchunguzi wa virusi vya aina ya rota vinaleta kuhara miongoni mwa watoto wa miaka tano na chini katika hospitali ya Rufaa ya Kaunti ya Kericho.

WACHUNGUZI:

MCHUNGUZI MKUU: Nyamanga Beth Khayeli

UTANGULIZI

Ninakukaribisha ushiriki katika utafiti huu unaotimizwa na Nyamanga Beth Khayeli, anayesomea chuo kikuu cha Kabianga. Madhumuni ya utafiti huu ni kuzingatia virusi vya aina ya Rota vinavyokasidi kuendesha ,kati ya wana katika Kaunti ya Kericho.Matukio ya utafiti huu ni kuarifu aina ya virusi hivi katika Kaunti ya Kericho. Ukinipa ruhusa yako na kushiriki katika utafiti huu nitashukuru sana .

MADHUMUNI

Utafiti huu unajihusisha na uchunguzi wa virus iwa aina ya rota, vinavyosababisha kuhara miongoni mwa watoto katika Kaunti ya Kericho.Matokeo yatakayopatikana yatawasilishwa kwa wadhamini wa sekta ya afya ya kenya illi waweze kuielemisha jamii kuhusu ugonjwa huu na jinsi ya kuuzuia

JUMLA YA WASHIRIKI

Utafiti huu utafanyiwa wahusika 191 ambao ni wana wa miaka mitano na chini.

KANUNI ZA UTAFITI

Utafiti huu unafanyiwa wagonjwa wanaotibiwa kwenye Hospitali ya Rufaa ya Kericho.Unahusisha ukusanyaji wa choo kutoka kwa wagonjwa wanaoonyesha ishara za kuwa na kuhara kutokana na rota. Wagonjwa hao watapewa chupa watakazoweka kinyesi na kumpa mchuguzi mkuu. Choo zenye zitakuwa na virusi hivi baadaye zitasafirishwa hadi KEMRI, kufanyiwa utafiti wa faida.

RUHUSA YA KUCHUKUA NA KUSAFIRISHA SAMPULI KUTOKA HOPITALI YA RUFAA YA KAUNTI YA KERICHO HADI MAABARA YA UCHUNGUZI KEMRI

NDIO LA □

MUDA WA MRADI

Utafiti huu utadumu kwa wa miezi miwili.

MADHARA

Madhara yeyote hayatasababishwa na mradi huu. Mhusika atahitajika kutia kinyesi chupani anapoenda choo kwa haja kubwa.

MANUFAA

Mhusika hatapata manufaa yoyote ya kibinafsi . Lengo kuu ni kwamba matukio yatakayopatikana baada ya kupata ruhusa ya mshiriki yatatumiwa kuielimisha jamii juu ya virusi vya rota katika Kaunti ya Kericho.

SIRI YA HALI YAKO

Kitakachopatikana kwenya mradi huu kitakuwa ni siri. Mchunguzi mkuu , wasaidizi wa uchunguzi na maofisa watakaohusika katika Hospitali ya Rufaa ya Kericho ndio pekee watakaokua na ruhusa ya kuyafikia. Jina lolote halitachapishwa hata baada ya kukamilika kwa uchunguzi.Utajulikana tu kwa nambari yako utakayopewa baada ya wewe kupeana idhini.

Hakutakuwa na kodi utakayotozwa wewe au mtoto kwa kushiriki kwenye utafiti.

MUHKTASARI JUU YA HAKI ZAKO KAMA MSHIRIKI KATIKA MRADI

Kushiriki ni kwa ridhaa yako na waweza jiondoa wakati wowote unaotaka pasipo kupata mapato. Hauta hujumiwa kwasababu ya msimamo wako dhidi ya mradi huu.

TAARIFA/ RIPOTI/UJUMBE

Maswali yanayohusu mradi huu yatakamilishwa na:

Mchunguzi mkuu: Nyamanga Beth Khayeli

Simu: 0725635159

Barua Pepe: bkhayeli@gmail.com

IDHINI YA MUHUSIKA

Nimeipitia fomu hii na amri za mradi huu. Maswali yangu yamejibiwa hivyo basi kwa niaba ya_____ nitapeana ruhusa ya sampuli ya mtoto wangu kuchukuliwa kwa utafiti huu.

RUHUSA YA KUCHUKUA KINYESI NDIO LA ☐

CHAGUA MOJA

Jina la mshiriki:_____Tarehe:_____Sahihi:_____

Jina la shahidi : _____Sahihi :_____Tarehe: _____

*Kwa washiriki wasiojua kusoma na kuandika tumia alama ya kidole *Yahitaajika awe mtu huru

Sahihiya anayechukua idhini :_____Tarehe:_____

Jina_____Tarehe_____

(Lazima awe mtafiti ama mtu aliyepewa jukumu la kupewa idhini)

Sahihi ya mchunguzi mkuu :_____Tarehe :_____

(Anayethibitisha kuwa mshiriki anafaa kushiriki na kwamba karatasi ya idhini imepatikana)

APPENDIX XI-PUBLICATION