# Prevalence of Group A Rotavirus and Electrolyte profiles in children presenting with Acute Diarrhoea at Kenyatta National Hospital.

**Bernard Wamiti Gatinu (M.B.chB)**

**A Thesis Submitted in Part Fulfillment for the Degree of Master of Medicine (Paediatrics) In the University of Nairobi.**

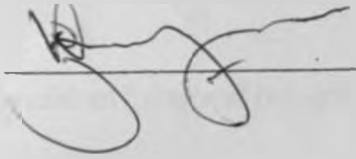


**2007**

**Declaration**

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

BERNARD WAMITI GATINU

Signature  Date 15-15 Nov 2007

This thesis has been submitted to the University of Nairobi with our approval.

**Supervisors**

**Dr. Grace Irimu,**
Lecturer,
Department of Paediatrics,
College of Health Sciences,
University of Nairobi.

Signature _____ Date 26th Nov 2007

**Professor W. Macharia,**
Associate Professor,
Department of Paediatrics,
College of Health Sciences,
University of Nairobi.

Signature _____ Date 27th Nov. 2007

**J.O. Nyangao, Msc**
Research Scientist,
Center for Rotavirus Research
Kenya Medical Research Institute.

Signature _____ Date 26th Nov 2007

## Dedication

This book is dedicated to my wife Faith for her unwavering support throughout the course and our two children Natasha and Kevin.

To all the children living and brought up in difficult circumstances.

## Acknowledgements

Many people have been involved either directly or indirectly in making the completion of this dissertation a success.

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## Abbreviations

ELISA: Enzyme Linked Immunosorbent Assay

EM: Electron Microscope

ENS: Enteric Nervous Syndrome

G: Glyco-protein

HIV: Human Immunodeficiency Virus

HRV: Human Rotavirus

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IVF: Intravenous fluid

KNH: Kenyatta National Hospital

KEMRI : Kenya Medical Research Institute

LPA: Latex Particle Agglutination

NSP: Non-Structural Protein

ORS: Oral Rehydration Solution

P: Protease

PAGE: Polyacrylmide gel electrophoresis

PFC: Pediatric Filter Clinic

RNA: RiboNucleicAcid

VP: Viral Protein

WHO: World Health Organization

EPEC: Enteropathogenic Escherichia Coli

## List of Tables and Figures

## Study Definitions

Diarrhoea- Passage of three or more liquid or semi-solid stools in the preceding twenty-four hours.

Acute diarrhoea - Diarrhoea that lasted less than fourteen days since onset to the time of presentation at KNH paediatrics' filter clinic (PFC).

Rotavirus diarrhoea – Diarrhoea where rotavirus antigen was detected in the stool specimen.

Hypernatremia - A serum sodium level above 145 mmoles/litre (mmols/l)
Hyponatremia - A serum sodium level below 135mmol/l.

Hyperkalemia - A serum potassium level above 5.5mmol/l.
Hypokalemia - A serum potassium level below 3.5mmol/l.

Urea – Normal reference value up to 7.5 mmols/l

Creatinine – Normal reference value up to 80 micromoles/l (µmols/l)

Fever - Axillary temperature above $37.7^0$ Celsius (C).
Hypothermia - Axillary temperature below $35.5^0$ C.

**Summary**

**Introduction**

Diarrhoeal disease is a major cause of morbidity and mortality in children less than five years of age worldwide. It accounts for 21% of all deaths in this age group with rotavirus infection being responsible for a quarter of all the deaths. In Kenya, diarrhoea is the third leading cause of all deaths in children. About 20-30 children present daily with acute diarrhoea at Kenyatta National Hospital (KNH). The admission rate to the paediatrics general wards is 30%. Group A rotavirus is the most important aetiological agent in diarrhoea. The main cause of morbidity and mortality is severe dehydration complicated by electrolytes imbalance and metabolic acidosis.

**Objectives:** The objectives of the study were to determine the prevalence of group A rotavirus infection in children aged 59 months and below presenting with acute diarrhoea at KNH, to compare the serum sodium, potassium, urea and creatinine in the severe forms of dehydration in the Human Rotavirus Positive (HRV +ve) and the HRV negative (–ve) children and to compare their clinical and social-demographic characteristics.

**Study setting:** The study was conducted in KNH Paediatrics Filter Clinic (PFC) and the general paediatrics wards.

**Study population:** The study population consisted of children aged 59 months and below who presented with acute diarrhoea at the PFC in KNH.

**Study design:** This was a hospital based cross sectional survey.

**Methods:** Samples of stool were collected from all recruited children who met the inclusion criteria. Rotavirus antigen testing was done using the Enzyme Linked Immuno-Sorbent Assay (ELISA) technique.

Light microscopy and bacterial cultures on the stool samples were performed. Blood samples were drawn to determine sodium, potassium, urea and creatinine levels in all the children with severe dehydration and those in hypovolemic shock.

**Results:** one hundred and ninety two children were recruited into the study. Rotavirus was positive in 103 of all children with acute diarrhoea thus giving an overall prevalence of 53.4% (95% C.I. 46.3% - 60.9%). Two peaks of rotavirus infection were observed at the fifth and the ninth month of age. Children with only one prior episode of diarrhoea were more likely to have rotavirus diarrhoea than those who had more prior episodes (OR 2.4, 95% CI 1.0-5.6). The duration of rotavirus diarrhoea was significantly shorter with a mean of 3.5 (1-10) days as compared to those negative for rotavirus (p value 0.0001). Children who had severe vomiting of five times and above per day were more than two times likely to have rotavirus diarrhoea (OR 2.6, 95% CI 1.3-5.3). Vomiting was reported to have lasted significantly longer ($\geq$ 3 days) at presentation to KNH in the rotavirus group than in those who tested negative for rotavirus (p value 0.01).

There were 162 stool samples cultured for bacteria infection, 16.6% of the culture tests were positive for bacteria. The organisms grown included Enteropathogenic *Escherichia Coli* (48%), Shigella (33.3%) and Salmonella (7.4%). Nine children had bacterial co-infection with rotavirus.

Electrolyte profiles, urea and creatinine levels were analyzed in 92 children who had the severe forms of dehydration. The derangement profiles were not significantly different between the group positive and that negative of group A rotavirus.

The admission rate to the general paediatrics' wards was 41%. The children admitted had severe dehydration, not adequately managed at PFC or were in hypovolemic shock; rotavirus was positive in 59.4% of those admitted.

Nineteen children with acute diarrhoea died during the study period, 63% were rotavirus (+ve). The overall case fatality rate for rotavirus-associated diarrhoea was 11.6%.

**Conclusion:** Group A rotavirus is a major cause of diarrhoea in children presenting with acute diarrhoea at KNH. Morbidity and mortality associated with rotavirus diarrhoea is very high. Electrolytes, urea and creatinine derangements are common in acute diarrhoea though they are not significantly worse with rotavirus infection. Infants are more likely to be have rotavirus diarrhoea, with a single prior episode of diarrhoea disease being associated with a higher likelihood of being rotavirus positive

**Recommendation:** Prevalence of rotavirus infection in diarrhoea is high and calls for appropriate measures to reduce its associated mortality and morbidity.

## Introduction

Diarrhoea disease is a major cause of morbidity and mortality worldwide. More than one billion diarrhea episodes occur every year in children below the age of five years. In the developing countries, it causes 2-2.5 million deaths annually accounting for 17% of all deaths in children less than five years of age [1]. The annual incidence of diarrhoea is 3.5-4.6 episodes/child/year in Kenya making it the third leading cause of morbidity and mortality among children [2]. Rotavirus is the most common cause of acute severe diarrhoea in children. Severe outcomes associated with acute diarrhoea show a divergent pattern in areas with different levels of economic development with 85% of global diarrhoea deaths occurring in the developing countries [3]. Eighty two percent of all rotavirus related deaths occur in children from the poorest nations of the world [4].

The leading causes of hospitalization and deaths among children with diarrhoea diseases are dehydration and electrolyte derangement [5].

Rotavirus diarrhoea is significantly more severe than non-rotavirus diarrhoea [6], with group A rotavirus established as the most important etiological agent of dehydrating gastroenteritis in infants and young children world wide [7].

### Structure of rotavirus

Bishop in Australia described rotavirus as a pathogen in 1973 from a duodenal biopsy. It was initially called Duovirus [8]; later it was renamed rotavirus due to its wheel like structure.

Rotavirus is a member of the Reoviridae [9]. It is approximately 75nm in diameter and has a non-enveloped, complex, triple layered capsid structure that surrounds a genome composed of eleven segments of double stranded RNA. Each segment encodes one or more polypeptides.

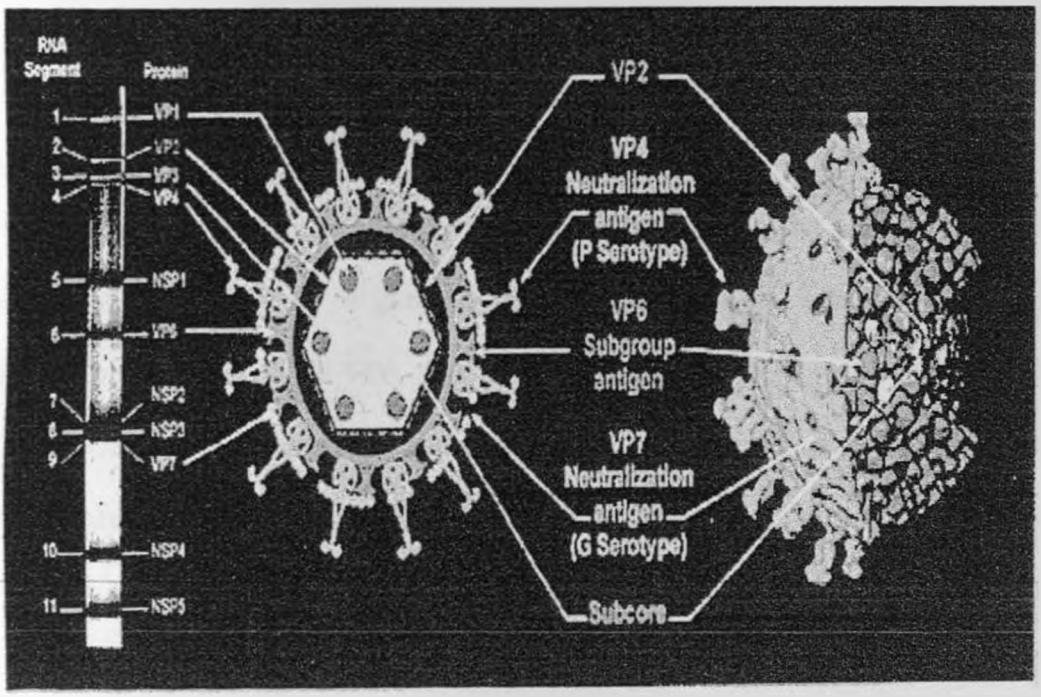


Figure 1. Gene coding assignments and three-dimensional structure of rotavirus particles. Double-stranded RNA segments separated on polyacrylamide gel (left) code for individual proteins, which are localized in the schematic of virus particle (center). Digital reconstruction of the rotavirus structure illustrating the outer capsid, inner VP6 shell and the core (right), by image processing of electron micrographs of particles embedded in vitreous ice (Estes 1996).

There are six structural (viral) proteins (VP) and six non-structural proteins (NSP), each encoded in a unique genome segment except NSP5 and NSP6 which are encoded in overlapping reading frames of a single segment as shown in figure 1 above.

Viral proteins (VP) 1,2 and 3 form the core of the virion and are involved in genome replication and packaging. VP4, present as spikes on the outer capsid, is cleaved by intestinal proteolytic enzymes to VP5 and VP8 subunits to initiate the process of viral penetration into the host cells. VP6 forms the intermediate capsid and is most abundant viral protein. VP7 is an outer capsid glycoprotein and may be involved in viral attachment as well as inducing antibody response[9] .

**Classification of rotavirus**

Rotaviruses are classified into groups, subgroups, serotypes and on the basis of electrophoretic migration of gene segments. The group and subgroup specificity are present on the inner capsid VP6, thus far only groups A, B and C have been identified as human pathogens, however the same groups are found in animals. Groups D, E, F, G have only been identified in animals and birds. Majority of infections are caused by group A rotaviruses. Group B rotavirus infections are uncommon but have recently been associated with outbreaks in China and India [23]. Group C rotavirus has been isolated in Kenyan children though its prevalence is not well determined [11].

The classification of rotaviruses according to serotypes is based on both VP4 (protease sensitive) and VP7 (glycoprotein) antigenic specificity. Both VP7 and VP4 proteins induce neutralizing antibodies, they are involved in protective immunity, and as such are important targets for vaccine development. There are twenty **P serotypes**; Serotypes P4, P6, P8 are most frequently associated with human infections [12].

**G serotypes** are fourteen, G-types 1, 2, 3 and 4, accounts for 80% of human infections. G8 and G9 are emerging as common serotypes with G9 described in Kenya [13].

Gene reassortment similar to antigenic shifts seen in influenza A virus may occur when two different rotavirus serotypes infect a child at the same time. This may bring about mutational changes over time leading to new serotypes.

**Electrophoretic mobility** defines the rotavirus electropherotypes. The RNA segments fall into four size classes based on contour length as measured by electron microscopy and RNA migration profiles after electrophoresis through polyacrylamide gels. There are two types of electropherotypes, long and short which gives rise to the concept of genogroups.

**Epidemiology of rotavirus**

Human rotavirus (HRV) diarrhoea is a public health problem throughout the world. It is the commonest cause of severe dehydrating diarrhoea in young children. It is found with a similar frequency in both developing and the developed world. It takes its greatest toll on infants and young children less than 24 months of age. Its peak is on $4^{th}$ month to $2^{nd}$ year of life, by two years most children are infected [14].

The prevalence of HRV in hospital based studies is 25-65% [15,16] of all children presenting with severe dehydration requiring hospital admission and 5-40% in community based studies [11] of children with varying levels of diarrhoea illness.

The median age of children hospitalized with rotavirus gastroenteritis in Africa is six months, whereas in developed countries the median age for hospitalization is greater than eleven months [17]

In developed countries, hospitalization rates of severe diarrhoea are between 2.2-5/1000 in children under five years, whereas it is as high as 30/1000 in developing countries [18].

In temperate countries infections peak in the winter while in tropical countries, infection is prevalent throughout the year with a slight increase in the dry seasons [19]. In Kenyatta National Hospital, a study done about in 1983 showed rotavirus had a prevalence of 25.3% as a single causative agent of acute diarrhea in children and 12% as co-infection with bacteria [20]. Further studies carried out during the period 1991-1994 showed prevalence of group A rotavirus was 28.4% in the urban hospitals (Nairobi) and a range of 22.5% (Nanyuki) to 13.7% (Kitui) [21] in rural settings in children with diarrhoea. A prospective study (2001-2002) done on children presenting with acute diarrhoea at clinics in Nairobi and its suburbs showed a rotavirus prevalence of 11% [11]. More recent studies have shown the prevalence of G-serotypes-1 in Kenya to be 60% whereas G9 was 15% [13,22].

**Transmission of rotavirus**

Large amounts of rotavirus particles are excreted during acute infection. ( about $10^{12}$ virions/gram of faeces). Infective dose is low; 100 to 1000 virus particles are enough to cause infection and disease. The transmission routes are faeco-oral and aerosol spread. Water borne outbreaks [23] have been documented. However the level of personal hygiene does not appear to have an effect on transmission [24].

Children infected with rotavirus continue to shed the virus for up to two weeks after cessation of diarrhoea. Excretion for up to 57 days has been observed [25]. This prolonged shedding of virus and repeated asymptomatic infection of older children and adults may explain the maintenance of infective virus reservoir.

**Pathogenesis of rotavirus**

Human rotavirus infects the mature villous enterocytes of the upper small intestines; it does not infect immature crypt cells or colonic enterocytes. VP4 seems to be the major ligand binding HRV to the cells (enterocytes), HRV receptors on the enterocytes are unclear. Once attached, HRV is endocytosed inside the cell or enters directly [26].

**Mechanism of rotavirus diarrhoea**

After infection, in the first 12-24 hours, enterocytes are intact but brush border disaccharidases (sucrase, maltase, lactase) are reduced due to interference of enzyme transport to the brush border, thus no hydrolysis of carbohydrates to monosaccharides leading to non-absorption of disaccharides and consequent osmotic diarrhoea.

NSP4 opens enterocytes calcium channels leading to efflux of sodium and water, thus resulting in secretory diarrhoea [27]. Increased intracellular (enterocytes) calcium cause enterocytes death leading to malabsorption.

Secreted NSP4, or other effector molecules released from infected cells, may also stimulate the enteric nervous system (ENS) [28]. Indeed, experiments with agents that block function of the ENS showed that rotavirus infection induced secretion via stimulation of the ENS [29].

This may explain the mechanism(s) by which relatively few infected cells, causing little visible damage to the mucosa, can elicit a diarrhoeal response. Infection resolves as HRV runs out of susceptible cells and the generation of the specific humoral response [30].

**Clinical features of rotavirus infection**

Clinical features range from asymptomatic infection to mild short-lived watery diarrhoea to severe gastroenteritis with dehydration. The stools are pale and watery, with a milky odor. Vomiting forms part of the disease entity and may precede diarrhoea.

Respiratory signs are often found during HRV gastroenteritis. Other extra intestinal manifestations include acute myositis, polio like paralysis and encephalitis [31]. Neurological involvement is suggested by several reports of children with concomitant convulsions and rotavirus diarrhoea which is only attributable to rotavirus infection when all the other causes of convulsions have been ruled out [32]. Hepatic involvement in rotavirus infection is suggested by the associated elevation of the liver enzymes and biliary atresia seen more in group C rotavirus infections [33]. Rotavirus has also been demonstrated to replicate in the liver and kidneys of an immunodeficient child [31]. Rotavirus antigens have been detected in myocardium of patients who died unexpectedly without any other detected pathology [34]. In vitro studies demonstrate the ability of rotavirus to replicate in primary islet cells; an observation that correlates with the temporal association of infection with development of pancreatic islet cells autoantibodies [35].

In hospitalized patients, the duration of diarrhoea is usually 2-23 days with a median of 6 days. Rotavirus infection is usually self-limiting in children with a normal, healthy immune system and an adequate level of nutrition [36]. Severe disease and death are seen more often in malnourished children [37].

**Water and electrolytes imbalance in rotavirus infection**

Rotavirus disease leads to loss of fluids and electrolytes through diarrhoea and vomiting giving rise to dehydration and electrolyte derangements. There is associated metabolic acidosis due to decreased renal perfusion thus reduced regeneration of bicarbonates as well as reduced tissue perfusion leading to ketosis. Severe dehydration, electrolytes derangements and associated metabolic acidosis are the usual causes of morbidity and mortality. Electrolytes imbalance complicates care especially if there is no laboratory support [38]. In a study by Wasunna on acute diarrhea in children in KNH hyponatremia was found in 48% while 51% of the patients studied had hypokalemia.

Hypokalemia and hyponatremia were observed more frequently in infections with rotavirus, enteropathogenic *Escherichia coli* and campylobacter jejuni [20].

**Immunity to rotavirus infection**

Infection occurs throughout life, however only the first infection causes severe disease, subsequent infections cause progressively milder symptoms even when infection is with different serotypes or new mutational serotypes. Infection with HRV confers immunity as neutralizing antibodies increase with age and with repeated viral exposure [25] thus reducing the severity of consequent infections. Relative risk of experiencing a further attack of HRV diarrhoea is reduced to as less as 0.08 after a third HRV disease [25].

Some rotavirus infections are asymptomatic which suggests both viral and host factors can affect disease severity such as the different alleles of VP4.

Host factors such as malnutrition have been shown to increase severity by delaying recovery of small intestines and modification of intestinal inflammatory response after rotavirus infection [37].

Rotavirus disease may be related to age dependent protease expression, as viral infectivity requires protease cleavage of VP4. Newborns have low levels of protease in the gut and rotavirus infections are therefore mainly asymptomatic at this age. Protection in neonatal period is also conferred via transplacental maternal antibodies and by antibodies and other factors such as lactoadherin transferred through breast-feeding [39]

Infection with HRV elicits both cellular and humoral response. There is both jejunal mucosal (IgA) and serum (IgG) antibody response. Serum IgG correlates with protection from infection while IgA jejunal neutralizing antibodies are associated with protection from clinical disease. Heterologous rotavirus antibodies are cross protective against infection (especially for severe disease) due to other serotypes.

Cellular immunity mainly cytotoxic T cell appears important in resolution of HRV infection and offer cross protection against the different G serotypes. However, HRV does not appear as an opportunistic infection in human immunodeficiency virus (HIV) disease and indeed HIV infected children are able to clear and recover from HRV as adequately as their immuno competent counterparts [40].

Co-infection with other intestinal pathogens does not appear to increase severity of HRV disease

**Diagnosis of rotavirus infection**

Antigen detection tests are the most practical and cost effective methods of rotavirus diagnosis. The principal of enzyme linked immunosorbent assay (ELISA) and latex particle agglutination (LPA) are the techniques commonly applied. Both the specificity and sensitivity of these tests are 90-95% [13]. LPA is useful when few stool samples are being tested while ELISA is appropriate for testing a large number of stool samples. The antigen kits are specific for group A rotavirus.

Polyacrylamide gel electrophoresis (PAGE) entails the electrophoresis of stool viral genomic RNA on polyacrylamide gel extract. It has a specificity of 100% and a sensitivity of 80-90% [13]. The electrophoretic pattern of the 11dsRNA segments varies between the different HRV groups such as A, B, C.

Electron Microscopy of faeces is the gold standard in diagnosis of rotavirus infection. It is very specific and almost 100% sensitive but unfortunately time consuming and expensive to install and thus not routinely used. Other diagnostic modalities include rotavirus reverse transcriptase polymerase chain reaction tests, rotavirus tissue cultures and rotavirus antibody (IgA, IgG) detection.

**Prevention of rotavirus infection**

There is no specific medical treatment for rotavirus infection. The prevalence of rotavirus infection is similar in both the developed and the developing world and improvement in hygiene and environmental sanitation does not result in reduction of rotavirus infection prevalence [23,24]. The above measures have had an incomplete role in prevention probably due to other modes of transmission not amenable to basic personal hygiene such as aerosol and surface transmission [24]. Therefore, prophylactic vaccination against rotavirus may offer an alternative strategy of preventing severe disease [41, 42].

The strategy of preventing rotavirus through vaccination derives from studies demonstrating that wild type rotavirus infection induces immunity against subsequent severe rotavirus gastroenteritis and the attenuation of the rotavirus following progressive sub-culturing in cells [43]. "Rotashield" vaccine, introduced in 1998 showed an efficacy of 95% against severe rotavirus gastroenteritis and an efficacy of 73% against rotavirus gastroenteritis of any severity in those immunized [43,44]. The vaccine was withdrawn the following year due to a probable association with intestinal intussusception in children vaccinated [45]. Several studies done after the withdrawal have however demonstrated the risk of intestinal intussusception associated with rotavirus vaccination to be similar to that in placebo recipients [46]. Newer vaccines namely "Rotarix" and "Rotateq" have shown a similar efficacy as the earlier vaccines with no associated side effects and are currently in use though not extensively in the prophylaxis against rotavirus infection [17, 46].

## Study Justification and Utility

Rotavirus is responsible for about a quarter of all diarrhoeal related deaths; most of which occur in the developing countries [47]. There is a high burden of human rotavirus infection leading to gastro-enteritis, which has been poorly controlled, as measures such as good hygiene and environmental cleanliness have not been shown to reduce disease prevalence. The prevalence of rotavirus infection is similar in both the developed and the developing world and improvement in hygiene and environmental sanitation does not result in reduction of rotavirus infection prevalence [23,24]. Vaccines are now available and promise to reduce incidence of severe diarrhoea and hospitalization and consequently mortality. There is thus need to collect data for baseline estimation of rotavirus disease burden in our local hospital before the introduction of the vaccines. The data will be useful in the justification of vaccine use and eventually influence assessment if vaccine is widely introduced.

Very few studies assessing urea, creatinine and electrolyte profiles in children with rotavirus diarrhoea especially those with severe dehydration have been undertaken. There is thus a knowledge gap in the electrolyte derangements in children with rotavirus diarrhoea. The study sought to quantify morbidity and consequences of rotavirus gastro-enteritis.

**Main objective**

1) To determine the prevalence of group-A rotavirus in children aged 59 months and below presenting with acute diarrhoea in Kenyatta National Hospital (KNH).

**Specific Objectives**

1) To compare sodium, potassium, urea and creatinine profiles in children with severe forms of dehydration between rotavirus positive and rotavirus negative children presenting with acute diarrhoea in KNH.
2) To compare social-demographic and clinical characteristics between children presenting with acute rotavirus positive and rotavirus negative diarrhoea in KNH.

## Materials and Methods

### Study site

The study was carried out at Kenyatta National Hospital (KNH) in the Pediatric Filter Clinic (PFC) and the general pediatric wards. KNH is the national referral hospital as well as a primary care hospital for patients within its catchment area. PFC is the first point of contact between the sick child and the clinician attending. The hospital has four general pediatric wards, which in total admit approximately 1200 children per month of whom 22% have a diagnosis of gastro-enteritis. On average, 20-30 children present with diarrhoea disease at PFC daily, of these 6-10 children are admitted to the pediatrics wards with severe dehydration secondary to diarrhoea. The case fatality rate of severe diarrhoea is 12-25% (Source: Records Department KNH).

### Study population

The children included in the study were aged 59 months and below who presented to the KNH pediatric filter clinic with acute diarrhoea.

### Study Design

This was a cross- sectional descriptive hospital survey.

The **Sample size** was determined using the formula by Henderson and Sundaresan, 1982.

$n = pqz^2/d^2$

n= Sample size;

p= Assumed prevalence of rotavirus in acute diarrhoea of 11% [11]

q= (1-p);

d = Margin of error (½ confidence interval)of 0.1;

z= Standard normal deviate (1.96);

$n= (0.11 \times 0.89 \times 1.96^2)/ 0.05^2 = 150$

The minimal sample size 150; we recruited 192 children in our study.

**Sampling procedure**

All the children presenting on the weekdays from 8:00am to 4:00pm with acute diarrhoea were assessed at the emergency triage area by the principal investigator. The children who met the inclusion criteria were consequently sampled. The principal investigator explained the purpose of the study and procedures involved to the caretakers of eligible children before obtaining a written consent (Appendix A).

**Inclusion criteria**

Children aged 59 months and below presenting to the pediatric filter clinic with acute diarrhoea whose parents/guardians consented to the study.

**Exclusion Criteria**

Children who presented with diarrhoea of greater than fourteen days duration.

Children who were unable to provide a stool sample within 12 hours of presentation to the PFC.

**Definitions**

Diarrhoea was defined as the passing of three or more liquid or semisolid stools in the preceding twenty-four hours. Acute diarrhoea was taken as duration of diarrhoea of less than fourteen days since onset. In all rotavirus positive specimen samples, rotavirus was taken as the causative organism of diarrhoea.

**Clinical methods**

Clinical history and physical examination was undertaken by the principal investigator and the details entered in the study questionnaire. The eligible child's caretakers were interviewed using a predesigned questionnaire (Appendix B).

The degree of dehydration was assessed using clinical signs such as skin turgor, sunken eyeballs, capillary refill, heart rate and peripheral pulses character, respiration rate and pattern, and level of mental consciousness. The level of dehydration was classified using the World Health Organization (WHO)/ Emergency Triage and Treatment (ETAT) guidelines as "severe dehydration in shock", "severe dehydration", "some dehydration" and "no dehydration" (Appendix C).

All the children recruited had their nutrition status assessed by taking their body weights to the nearest 100g, length for children aged up to 18 months and height for those above 18 months was taken in centimeters. Every child recruited was examined for features of visible wasting and oedema of malnutrition. The National Center for Health Statistics z-score of weight for height (wasting) was used to classify the level of malnutrition as per WHO guidelines [50].

The clinical outcome of all the recruited children was documented.

**Samples Collection**

The investigator obtained two stool samples in plastic polypots, approximately 3mls each, from every child recruited in to the study. Five milliliters of blood was obtained for electrolytes, urea, and creatinine from the child's peripheral vein, using sterile methods.

**Laboratory methods**

One set of stool samples was stored in a refrigerator at 2-8$^0$C . The stool samples were transported to Kenya Medical Research Institute (KEMRI) Nairobi weekly and analyzed by trained laboratory personnel, using an Enzyme linked immunosorbent assay (ELISA) stool antigen test for rotavirus.

The rotavirus antigen tests were performed as per the kits' manufacturers' protocol. The sensitivity of this antigen test was 95% with a specificity of 90%. The other set of stool samples was submitted to the University of Nairobi, Department of Pediatrics laboratories within one hour of collection for culture and fresh light microscopy. Salmonella, *Escherichia Coli* and Shigella were cultured using standard methods by trained laboratory personnel at the department of pediatrics laboratories.

The blood samples for electrolytes were analyzed at the KNH biochemistry laboratories using Olympus AU640. The reference values of electrolytes, urea and creatinine were as given in the KNH biochemistry laboratories.

**Statistical analysis**

Data was collected using a predesigned questionnaire. (Appendix B)

Data was double entered in the computer on collection, cleaned and analyzed using Epi-Info 3.2.2. Atlanta Georgia. Descriptive statistics including percentages were determined. Differences between the two groups were assessed using the Chi-square tests and odds ratio and p values determined. In case the value for a cell was less than 5, Fisher's exact test was used, a p value equal or less than 0.05 was considered significant. The variables with a p value less than or equal to 0.1 were entered in an unconditional logistic regression model to assess if they were independently statistically significant.

Data results are presented in tables, pie charts and graphs.

**Ethical Consideration**

Ethical approval was granted by the Kenyatta National Hospital ethical and Research Committee.

Patients who were in shock were resuscitated as a priority.

Informed consent was granted by the primary caretaker for each child recruited.

All the blood and stool results obtained were made available to the primary caregiver managing the child as soon as they were ready.

The investigator established an intravenous access while obtaining the blood sample, which was used for intravenous rehydration and other therapies.

**Results**

The study was carried out in the months of June to August 2006.

During the three subsequent months study period, 192 children with acute diarrhoea were consequently recruited. There were 101 (53%) males and 91 (47%) females forming the study population. Age range in the study population was one to 59 months with mean age being 10.9 months. Three children were aged less than two months. The age distribution was as shown in figure 2 below.

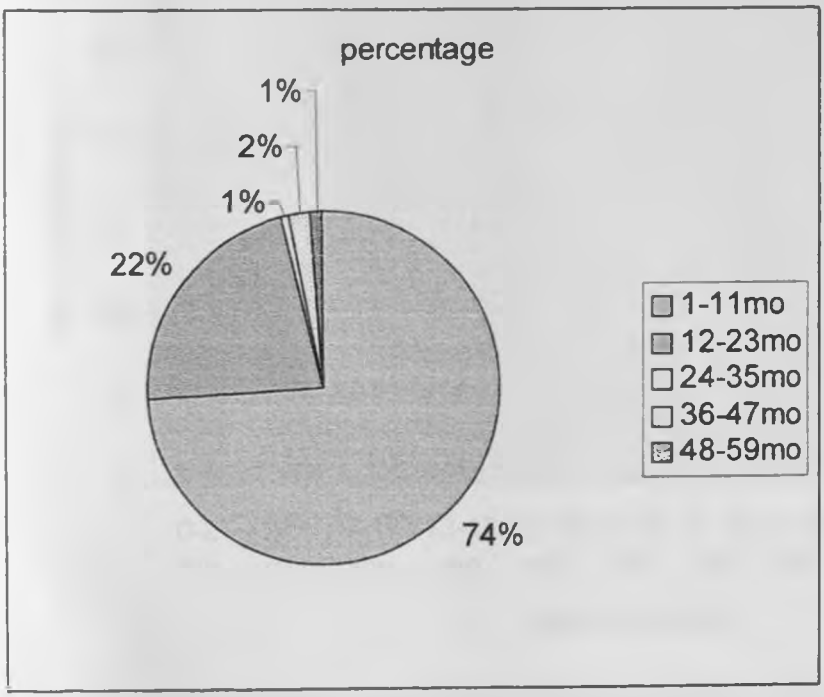


Figure.2. Age distribution of children presenting with acute diarrhoea KNH

The children forming the study group composed of 20 (10.4%) who were not dehydrated; 80 (41.6%) who had some dehydration, 69 (36%) with severe dehydration and 23 (12%) who were in hypovolemic shock.

One hundred and three children were positive for group-A rotavirus antigen in their stool thus giving a prevalence of 53% (95% C.I. 46.3%-60.9%). There were 56 (54.3%) males and 47 (45.7%) female whose stool specimens were rotavirus positive giving a male to female ratio for rotavirus infection as 1.2: 1 (p value 0.7).

The age distribution of rotavirus infection was as shown in figure 3 below.

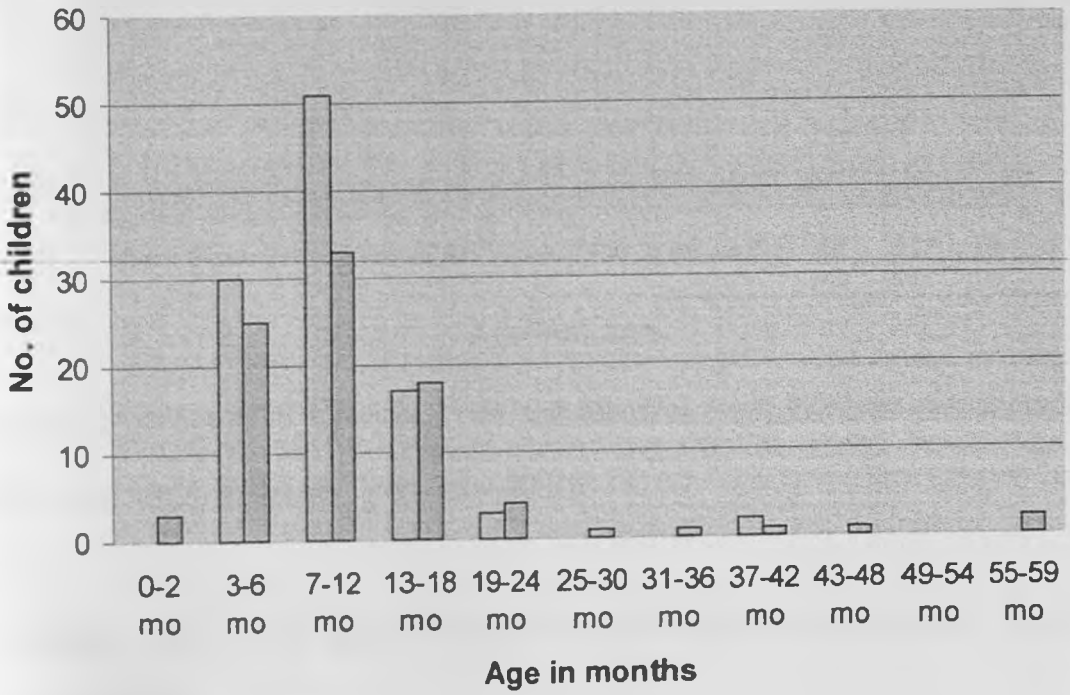


Figure 3. Age distribution of rotavirus (+ve) and rotavirus (-ve) children presenting with acute diarrhoea in KNH

Infants formed the majority of the children studied who tested positive for rotavirus in their stools 79 (76.6%) being rotavirus positive as shown in figure 3.

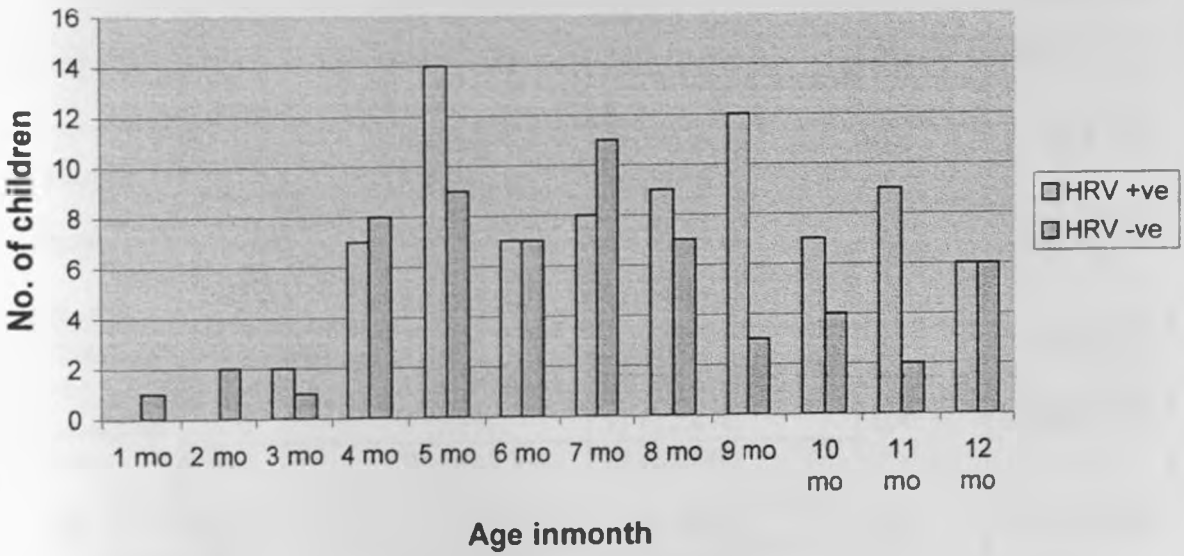


Figure 4. Distribution of rotavirus (+ve) and rotavirus (–ve) diarrhoea in infancy in children presenting with acute diarrhoea in KNH

As shown in figure 4, there appeared to be two peaks of rotavirus infection at 5$^{th}$ and the 9$^{th}$ months of life.

Table 1. Comparison of clinico-demographic characteristics of children presenting with acute HRV (+ve) and HRV (-ve) diarrhoea in KNH.

| Characteristic | | HRV +ve (103) | HRV -ve (89) | P-value | Odds ratio (95% C.I) |
|---|---|---|---|---|---|
| Sex | Male | 56 (54.4%) | 45 (50.6%) | 0.7 | 1.2 (0.6-2.1) |
| | Female | 47 (45.6%) | 44 (49.4%) | | |
| Age in months | Mean | 11 (3-42) | 10.1 (1-59) | 0.1 | N/A |
| | Median | 9 | 8 | | |
| Age bracket | ≤ 12 mo | 79 (76.7%) | 63 (70.8%) | 0.4 | 1.4 (0.7-2.7) |
| | > 12 mo. | 24 (23.3%) | 26 (29.2%) | | |
| **Nutritional status (WHz-score)** | **≥ -2 SD** | **93 (90.2%)** | **68(76.4%)** | **0.02** | **2.9(1.2-7.0)** |
| | **< -2 SD** | **10 (9.8%)** | **21(23.6%)** | | |
| Parents/guardian education | Non-formal | 1 (1%) | 3 (3.4%) | 0.3 | 0.3 (0.1-3.1) |
| | Formal | 102 (99%) | 86 (96.6%) | | |
| Parents/guardian age in years | < 20 | 8 (7.8%) | 11 (12.4%) | 0.4 | 0.6 (0.2-1.7) |
| | >20 | 95 (92.3 %) | 78 (87.6%) | | |
| Household contact with diarrhoea | Yes | 18 (17.5%) | 21 (23.6%) | 0.4 | 0.7 (0.3-1.5) |
| | No | 85 (82.5%) | 68 (76.4%) | | |
| Outside contact with diarrhoea | Yes | 5 (4.9%) | 10(11.2%) | 0.2 | 0.4 (0.1-1.4) |
| | No | 98 (95.1%) | 79 (88.2%) | | |
| Prior diarrhoeal episodes | Yes | 60 (58.3%) | 47(52.8%) | 0.5 | 1.2 (0.7-2.2) |
| | No | 43 (41.7%) | 42 (47.2%) | | |
| **No. of prior diarrhoeal episodes** | **One episode** | **35 (59%)** | **18 (37.5%)** | **0.05** | **2.4 (1.0-5.6)** |
| | **> One episode** | **25 (41%)** | **29 (62.5%)** | | |

In analysis of clinical and demographic characteristics as shown in table 1, the sex of the child does not appear as a correlate of human rotavirus infection with prevalence of rotavirus being 54.4% and 45.6 % in males and females respectively (p value 0.7).

Children aged three to eighteen months formed the majority (95%) of rotavirus infection with the median being nine months. There was no significant difference in the mean age of those positive for rotavirus and those without the antigen in their stools (p value 0.1).

Using the National Center for Health Statistics as the reference, the weight for height Z-scores of the study population were determined. Children who were severely wasted (z-score equal to or less than –3SD) were 13 with rotavirus present in 4 children.

Eighteen children were moderately wasted (z-score greater than –3SD to –2SD), six had rotavirus antigen in their stools. The children with a z-score greater than –2SD formed 82.8% with rotavirus being positive in 93 (90.2%). Thus the children with a z-score greater than -2 were more affected with the rotavirus infection (p value 0.02)

Contact with a person with diarrhoea either within or outside the household did not appear to influence the presence of rotavirus infection. There were 39 (20.3%) who had contact with a person with diarrhoea within the household while 15(7.8%) had contact outside household. The p value in both types of contact was 0.4 and 0.2 respectively, thus statistically not significant

When the number of prior diarrhoea episodes was considered, those who had only one episode of diarrhoea were more likely to be positive for human rotavirus with a p value of 0.05.

Nutritional status, number of prior diarrhoea episodes, age bracket and history of prior contact were subjected to multi-variate analysis to determine if they independently influenced the outcome. The presence of a single prior episode of diarrhoea remained independently significant (OR 2.8, 95% C.I 1.4-3.4).

Table 2. Comparison of Clinical characteristics of children presenting with acute HRV(+ve) and HRV(-ve) diarrhoea in KNH

| Characteristics | | Rotavirus +ve (103) | Rotavirus –ve (89) | P-value | Odds ratio (95% C.I) |
|---|---|---|---|---|---|
| Vomiting in preceding 24hrs | Yes | 95 (92%) | 75 (84%) | 0.1 | 0.5 (0.2-1.2) |
| | No | 8 (8%) | 14 (16%) | | |
| **Frequency of vomiting/24hr** | **Non severe ( ≤ 4)** | **62 (60%)** | **71(80%)** | **0.005** | **2.6 (1.3-5.3)*** |
| | **Severe ( ≥ 5)** | **41 (40%)** | **18 (20%)** | | |
| **Duration of vomiting in days** | **< 3 days** | **27 (28%)** | **33 (44%)** | **0.01** | **2.2 (1.2-4.1)** |
| | **≥ 3 days** | **68 (72%)** | **42 (56%)** | | |
| Frequency of diarrhoea/24hrs | Non-severe ( ≤ 5) | 27(26.2%) | 19 (21.3%) | 0.5 | 0.8 (0.4-1.6)* |
| | Severe ( ≥ 6) | 76 (73.8%) | 70 (78.7%) | | |
| **Duration of diarrhoea days** | **Mean** | **3.5(1-10)** | **4.9(2-14)** | **0.0001** | **N/A** |
| Blood in stool | Yes | 8 (7.8%) | 9 (10.1%) | 0.8 | 0.8 (0.3-1.2) |
| | No | 105 (92.8%) | 80 (89.9%) | | |
| Respiratory distress | Yes | 32 (31.4%) | 26 (29.2%) | 0.9 | 1.1 (0.6-2.1) |
| | No | 71 (68.6%) | 63 (70.8) | | |
| Convulsions | Yes | 6 (5.8%) | 7 (7.9%) | 0.8 | 0.7 (0.2-2.2) |
| | No | 97 (94.2%) | 82 (82.1%) | | |
| Abdominal distention | Present | 1(1%) | 4(4.5%) | 0.1 | 0.2 (0.02-1.9) |
| | Absent | 102 (99%) | 85 (95.5%) | | |
| Axillary temperature | Afebrile | 44 (42.7%) | 38 (42.7%) | 0.8 | 1 (0.5-1.9) |
| | Febrile | 59 (57.3%) | 51 (57.3%) | | |
| Dehydration status | Severe | 53 (51.5%) | 39 (43.8%) | 0.4 | 1.4 (0.7-2.5) |
| | Non-severe | 50 (48.5%) | 50 (56.2%) | | |

* Grading of severity was as used in the Vesikari scoring system [54]

As shown in table 2, the children who had five episodes of vomiting and above (classified as severe vomiting using the Vesikari score [54]) were 2.6 (95% C.I; 1.3 - 5.3) times more likely to have rotavirus infection as compared to those who were rotavirus negative

(p value 0.005). Those with equal to or greater than five episodes of vomiting in the preceding 24 hours formed 30.7% of the children studied with 41(69.5%) of them being rotavirus positive, 18(30.5%) of the children were negative for rotavirus.

Only eight percent of children with rotavirus diarrhoea gave no history of vomiting in the preceding 24 hours as opposed to 16 % in those negative for rotavirus. The duration of vomiting appeared to be associated with being rotavirus positive. The children with rotavirus infection were 2.2 (95% C.I; 1.2-4.1) times more likely to have a history of vomiting for more than three days as compared to those who were rotavirus negative (p value 0.01). Eighty-three children who had rotavirus in their stool presented with a history of vomiting before onset of diarrhoea or a simultaneous onset of both diarrhoea and vomiting, compared to 48 children who were rotavirus negative. This gives a positive predictive value of early onset of vomiting as a sign of rotavirus infection of 63.4% (sensitivity 80.5%, specificity 46.1%)

Diarrhoea in rotavirus infection lasted fewer days than in the rotavirus negative children with mean duration at presentation to the PFC being 3.5 days in the rotavirus positive group and mean of 4.9 days for those who were rotavirus negative. This was statistically significant with a p-value of 0.0001.

The children forming the study group composed of 20 (10.4%) who were not dehydrated; 80 (41.6%) who had some dehydration, 69 (36%) with severe dehydration and 23 (12%) who were in hypovolemic shock. In the children with no dehydration; eight (40%) had rotavirus infection while 12 had non-rotavirus diarrhoea. In those with some dehydration, 42 (52.5%) were rotavirus positive while 38 were rotavirus negative. Among the children who presented with severe dehydration, 40 (57.9%) were rotavirus positive while 29 were

negative for rotavirus. The children who presented in hypovolemic shock were 23, 13 (56.5%) of them had rotavirus diarrhoea while ten were negative for rotavirus. The degree of severity of dehydration was not statistically different in the children who were rotavirus positive and those negative for rotavirus ( p value 0.4).

The prevalence of rotavirus among children with fever was 57.3% (59), while it was 39.8 % in those without fever. Overall, there was no significant association observed for those with fever and being rotavirus positive (p value 0.8).

Overall, 17(8.9%) children in the study population had macroscopic presence of blood in their stool samples; eight of this children were positive for rotavirus. The p value for being rotavirus positive was 0.8; this was not statistically significant. Respiratory distress, convulsions and abdominal distention were observed in 32 (31.4%), six (5.8%) and one (1%) respectively in children who were rotavirus positive. The p values of each of this clinical characteristics being attributed to rotavirus positivity were statistically not significant.

Table 3. Comparison of mean serum urea, electrolytes and creatinine in children with acute HRV (+ve) and HRV (–ve) diarrhoea presenting with the severe forms of dehydration in KNH

| Electrolytes | | HRV +ve Mean ( ± 1 SD) | HRV –ve Mean ( ± 1 SD) | P-value |
|---|---|---|---|---|
| Sodium (Na) (mmols/l) | Mean | 130 (105-159) | 133 (105-152) | 0.5 |
| Potassium (K) (mmols/l) | Mean | 3.94 (1.7-6.9) | 3.6 (1.7-6.9) | 0.2 |
| Chloride (mmols/l) | Mean | 99 (61-128) | 104 (61-130) | 0.08 |
| Urea (mmols/l) | Mean | 9.4 (1-19) | 9.7 (1-19) | 0.8 |
| Creatinine (μmols/l) | Mean | 82 (40-200) | 81 (20-155) | 0.8 |

Blood samples were taken in all the 92 children who had the severe forms of dehydration for electrolytes, urea and creatinine evaluation. Hyponatremia was noted in 50 (54.3%) children, with 28 (30.4%) being normal and 14 (15.2%) being hypernatremic.

Among the HRV positive, as seen in table 3, the mean sodium was 130 mmols/l (105-159mmols/l), with a median of 132 mmols/l, whereas in the HRV negative, the mean was 133mmols/l (105-152mmols/l) with a median 0f 131 mmols/l (p-value was 0.5). Hypokalemia was found in 35 (38.5%) children, 44 (48.4%) had normal serum potassium levels while 12 (13.2%) had hyperkalemia.

The mean potassium level in the rotavirus positive was 3.94 mmols/l while the mean for those who were rotavirus negative was 3.6 mmols/l (The P-value was 0.2).

The urea levels were generally elevated. The mean urea levels for the HRV positive being 9.4 mmol. (range 1-19mmols/l) with a median of 9mmols/l. In the group that was

rotavirus negative, the mean was 9.7 mmol. (range 1-19mmols/l) with a median of 9mmols/l (p-value was 0.8). The mean creatinine level was 82 µmol/l (range 40-200µmols/l) with a median of 80 µmol/l in the HRV positive group, while mean in the HRV negative group was 81 µmol/l (range 20-155µmols/l) with a median of 86 µmols/l (p-value was 0.8).

Table 4.Comparison of frequency of Urea, electrolytes and creatinine derangements in children presenting with acute HRV (+ve) and HRV (-ve) diarrhoea in the severe forms of dehydration in KNH.

| Electrolyte/ urea/creatinine | | | HRV (+ve) | HRV (–ve) | P value | OR (95% C.I.) |
|---|---|---|---|---|---|---|
| Sodium mmols/l | Normal (135-145) | | 13 (29%) | 14 (30.4%) | 0.9 | 1.0 (0.4-2.6) |
| | Deranged | Low | 25 (55.5%) | 27 (58.6%) | | |
| | | High | 7 (15.5%) | 5 (11%) | | |
| Potassium mmols/l | Normal (3.5-5-5) | | 22 (49%) | 22 (48%) | 0.9 | 0.9 (0.4-2.2) |
| | Deranged | Low | 15 (33.3%) | 20 (43.5%) | | |
| | | High | 8 (17.7%) | 4 (8.5%) | | |
| Urea mmols/l | Normal (< 7.5) | | 19 (42%) | 21 (46%) | 0.9 | 1.1 (0.5-2.6) |
| | Elevated | | 26 (58%) | 25 (54%) | | |
| Creatinine µmols/l | Normal ( <80) | | 27 (60%) | 22 (48%) | 0.3 | 0.6 (0.2-1.4) |
| | Elevated | | 18 (40%) | 24 (52%) | | |

As seen in table 4, there was an overall evident urea, electrolytes and creatinine derangement which was however not significantly different in both the rotavirus positive and the non-rotavirus diarrhoea groups.

Table 5.Bacteria association with rotavirus diarrhoea in children presenting with acute diarrhoea in KNH.

| **Bacteria pathogen** | | HRV +ve (89) | HRV –ve (73) | P-value | Odds ratio for being HRV +ve (95% C.I |
|---|---|---|---|---|---|
| Shigella | 9 | 4 ( 4.5%) | 5 (6.8%) | 0.7 | 0.6(0.1-2.9) |
| Salmonella | 2 | 0 | 2 (2.7%) | 0.2 | 0.0(0.0-3.4) |
| E-coli | 13 | 5 (5.6%) | 8 (11%) | 0.001 | 0.2(0.1-0.6) |
| Mixed | 4 | 0 | 4 (5.5%) | 0.06 | 0.0 (0.0-1.2) |

We took 162 stool samples for fresh light microscopy and cultures for Shigella, *Escherichia Coli* and Salmonella.

Thirty stool samples could not be included for analysis; this was due to reasons beyond our control. On light microscopy, no parasites such as Entamoeba Histolytica, Giardia Lamblia, Helminthes, ova, cysts nor trophozoites were identified.

Bacteria pathogens were isolated in 28 (17.3%) of the stool samples Bacterial enteropathogens Shigella, Salmonella, and *Escherichia Coli* were detected in 9(6%), 2(1%) and 13 (8%) respectively. Four stool cultures yielded mixed growth of organisms. Fifty-six cases (35%) remained aetiologically unresolved.

Shigella species infection was observed in 4.5% of those who were rotavirus positive while it was seen in 6.8% in those in the rotavirus negative group, the p value for its association with rotavirus was 0.7, and this was not statistically significant.

Salmonella infection was not found in those positive for rotavirus while it was observed in 2.7 % in those with non-rotavirus diarrhoea, the p value for this association was 0.2, and this was statistically significant. *E-Coli* was present in 5.6% of the children with rotavirus infection while it was culture in 11% of those negative for rotavirus (p value for 0.001).

Among the bacteria pathogens, cultured *E-coli* was less likely to be associated with rotavirus (p value 0.001;OR 0.2). None of those with rotavirus had mixed bacteria infection in their stool.

**Outcome**

All the children (10.4%) who had no dehydration and no other co-morbidity were treated as out patients. The children with some dehydration (41.6%) were successively rehydrated orally, and after evaluation were discharged home.

The children managed and discharged at PFC were 113, (58.9%) of the study population; the rotavirus positive children in this group were 56 (49.6%). This group included 13 children who had severe dehydration who were successively given intravenous fluids and after evaluation were discharged home on oral fluids. The children admitted to the pediatrics general wards had either severe dehydration (which had not been adequately managed at PFC) or were in hypovolemic shock. There were 79 (41%) children admitted in the study population.

In this group, 47 (59.4%) were positive for group A rotavirus while 32 (40.5%) were rotavirus negative (p-value was 0.2, OR 1.5) .In the group admitted, 19 children died, 12 (63 %) were rotavirus positive, while 7 (37%) were negative for rotavirus (p value was 0.5, OR 1.5). The overall acute diarrhoea case fatality rate was 9.8 %. The case fatality rate for rotavirus diarrhoea was 11.6% while that of those negative for rotavirus was 7.8% (p value 0.53, OR 1.54)

The overall mean duration of hospitalization was 4.6 days (range 1-15) days, with a median of 4 days. Among those who died, mean duration of admission was 2.5 days (range 1-10) with a median of 2 days (P-value 0.0003). This was different from those who recovered indicating majority of deaths occurred within 48 hours of a admission. The mean duration for those positive for rotavirus was 3.8 days (range 1-11), median 4 days, while in the HRV negative mean duration was 5.3 days (range 1-15), median 4 days (P value was 0.0343). Among the deceased, the duration of admission to the time of death was shorter among the rotavirus positive than in the non-rotavirus group with a mean duration of 1.8 days and 3.5 days respectively; the difference was not statistically significant (p value 0.1).

**Discussion**

The findings of this study show that rotavirus is the leading associated cause of acute diarrhoea with a prevalence of 53.6% in children with acute gastro-enteritis presenting to the KNH Paediatrics Filter Clinic. This prevalence is higher than that observed of 11% by Mwenda J M, et al in 2003 on children with diarrhoea from clinics around Nairobi and its suburbs [11], though this study was done in the peripheral health facilities in the KNH catchment area and thus severity of acute diarrhoea was likely to be less. The Mwenda et al study was carried out in an outpatient setting and thus children likely to require hospitalization were not included. A study by Wasunna A O in KNH in 1983 found a prevalence of 37.3%[20], the study though only included children who were dehydrated (earlier classified as mild, moderate or severe dehydration), thus children presenting with diarrhoea with no dehydration were not evaluated for rotavirus infection. An important factor for the discrepancy between our study and that conducted by Wasunna is the possibility that the overall rate of non-rotavirus diarrhoea has declined over the past two decades [1,14,38]. The proportion of diarrhoea attributable to rotavirus may not have changed given the ease of transmission and the difficulties to control it through improvements in hygiene and sanitation. Our study was also carried out during the cool dry months of June to August, which is one of the two seasonal peaks of rotavirus diarrhoea [16,19], as observed by the Mutanda et al study, while the other studies conducted were distributed throughout most of the year.

The children in the study population were aged one to 59 months. The median age for rotavirus infection was nine months. The majority (76.7%) of the children with rotavirus infection were infants.

Two peaks of rotavirus infection were observed in the fifth and ninth months of live. This observation is attributable to the waning immunity after the first wild rotavirus infection and consequent development of more permanent immunity with continued rotavirus exposure thus the declining prevalence after infancy. This was similar to other studies carried out elsewhere in Africa [15,20,40]. Binka et al in Ghana in 1998 found a rotavirus prevalence of 39% with peak of infection at ninth to eleventh months. There is a difference in the age distributions of rotavirus infections in the developing and the developed countries. In the developing countries the highest rates occur during the first year of life [7,14,19,20], while in the developed the peak rates occur in the second year of life [47].

Rotavirus diarrhoea was not observed in children aged below two months, this could be explained by the pathogenesis of rotavirus infection [26,48] as infectivity requires the presence of age dependant proteases, which cleaves viral protein (VP) 4 to enable enterocytes intracellular penetration. The rate of infection also declined after 18 months of age possibly due to humoral and cellular immunity conferred by prior rotavirus infections [25] and thus children above this age group were less likely to develop severe diarrhoea to warrant seeking medical care in hospital. In a study by Kakai et al on children with diarrhoea at KNH, the children with specific Immunoglobulin-A to rotavirus were protected from rotavirus infection [55].

In this study, the dehydration status in the children with rotavirus infection did not differ significantly with those negative for rotavirus. In hospital based studies in Bangladesh, it was reported that children infected with rotavirus had less severe dehydration than those infected with other diarrhoegenic enteropathogens [41], conversely some studies reported

that rotavirus diarrhoea was particularly more severe compared with non-rotavirus diarrhoea [15,44,46].

Malnutrition did not appear to be an important factor in rotavirus infection. Our study observed significantly more rotavirus infection in the well-nourished children than in the malnourished. Binka et al [15] reported increased prevalence in the malnourished. In our study, only 17% of the children had a weight for height z score of equal to or less than minus 2SD and thus not much conclusion could be drawn from this whereas the Binka study involved more children and was spread over a whole year. The malnourished children were at an increased risk of having diarrhoea caused by other diarrhoegenic agents as compared to the children with normal nutrition and indeed enteropathogenic *E-coli* diarrhoea was significantly associated with malnutrition in our study.

In this study, children with rotavirus associated diarrhoea presented to hospital significantly earlier, with a mean duration of 3.5 days compared to 4.9 days in the non-rotavirus group. Vomiting was noted to start earlier in rotavirus infection and was seen as a major clinical observation in rotavirus infection thus could serve as an entry point for purposes of active surveillance in suspected rotavirus infection [19,53]. The frequency of vomiting per day was also significantly more in rotavirus infection. The sudden onset of rotavirus disease, the prevalence of vomiting at the initial stages of disease which usually precedes loose stools (sensitivity 80.5%, specificity 46.1%) in combination with the other components of the Vesikari scoring system [54], could be useful information for pediatricians and other health care workers in counseling the caretaker regarding possible aetiology of diarrhoea and vomiting.

In this study, we had 47.9% of the children presenting with severe dehydration and in hypovolemic shock. These children were analyzed for urea electrolytes and creatinine derangements. A low sodium level observed in 50 (54.3%) while 14 (15.2%) children were hypernatremic. The mean serum sodium level in both rotavirus positive and those negative for rotavirus was not statistically different. All the children, however had some form of fluid management prior to presenting to KNH with either plain water, home made solutions, oral rehydration salts at home or intravenous fluids for those referred from other hospitals, thus the sodium derangements might not be wholly attributable to the gastroenteritis. A similar study in KNH [20] showed hyponatremia to be present in 48% of the children with acute diarrhoea studied. Hypokalemia was observed in 38.9% of the children. The mean serum potassium levels in both groups of rotavirus positive and those with non-rotavirus diarrhoea was not statistically significant, despite the increased severity of vomiting and its duration in rotavirus diarrhoea. This observation could be explained by the expected metabolic acidosis in rotavirus diarrhoea thus the total body potassium could have been low despite what was apparently seen in the serum due to compensatory shift of potassium from the intracellular space in the presence of metabolic acidosis. The high level of urea in both groups is an indicator of the level of dehydration with the mean and the median above eight mmols/l in both groups. The high levels of creatinine with a mean level above 80 µmol/l in both groups could be an indicator of presence of pre-renal acute renal failure in acute gastroenteritis and underscores the need for prompt appropriate fluid treatment to improve circulation and restore renal perfusion.

In this study, only 17% of the stool specimens cultured were positive for the major diarrhoegenic bacterial pathogens, there was rotavirus co-infection with bacteria (Shigella

and *E-coli*) in 10%. A big percentage (32%) of the diarrhoeal disease remained aetiologically unresolved suggesting other agents such as adenovirus might be playing a significant role in diarrhoea causation. The Wasunna studied detected a co- infection rate of 12 %[20]. In practice, the co-infections could cause difficulties in diagnosis, treatment and prophylaxis of diarrhoea in these children. More studies would be recommended to further evaluate this aspect.

In this study, one hundred and nineteen (59%)children with acute diarrhoea were managed as outpatients; those positive for rotavirus were 49.6%. The admission rate to the general paediatrics' wards was 41%. The children admitted had severe dehydration, not adequately managed at PFC with appropriate intravenous fluids or were in hypovolemic shock; rotavirus was positive in 59.4%. Nineteen children with acute diarrhoea died during the study period, 63% were rotavirus (+ve). The overall case fatality rate for rotavirus-associated diarrhoea was 11.6%. Majority of the rotavirus deaths occurred within 48 hours of admission with a mean duration of 1.8 days. Those who recovered had a mean duration of admission of 3.8 days. The reported mortality in hospitalized children with acute rotavirus diarrhoea ranges from 1.6 % to 20% in the developed and developing countries respectively [4, 18,20].

The principal investigators in this study were not directly responsible for the management of the children and only the outcome was documented. The presence of co-factors, which could influence mortality such as the presence of Human Immuno-deficiency (HIV) infection, hypoglycemia, severe anemia and other concurrent infections were not evaluated in this study.

## CONCLUSIONS

1. Group A rotavirus is a major cause of diarrhoea in children presenting with acute diarrhoea at KNH with a prevalence of 53%.

2. Electrolytes, urea and creatinine derangements are common in acute diarrhoea though they are not significantly worse with rotavirus infection.

3. Majority of children with rotavirus-associated diarrhoea are infants.

4. A single prior episode of diarrhoea is associated with a higher likelihood of being rotavirus positive.

5. Vomiting in rotavirus infection is more severe and lasts longer than in non-rotavirus gastro-enteritis.

**Recommendation:**

1. The burden of rotavirus infection in diarrhoea in KNH is high, which calls for appropriate measures, such as an effective vaccine, to reduce infection and disease especially in infancy.
2. Vomiting preceding diarrhoea should be included in the definition of suspected rotavirus diarrhoea in rotavirus surveillance.
3. The high mortality in diarrhoea recommends a mortality audit and strategies to improve care of inpatients with rotavirus diarrhoea.

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**Appendix A**

**Consent Form**

Dear parent/guardian,

We are conducting a research study investigating the prevalence of rotavirus and electrolytes profiles in children with acute diarrhoea at KNH. We would like to include your child as a participant. This will require that we administer to you a questionnaire, examine and take stool and blood samples from your child. Participation in this study is voluntary and the decision on whether to participate or not, will not prejudice your child's care in any way. There is no risk your child will be exposed to by participating in this study. Phlebotomy will involve a venepuncture, a minimally invasive procedure. The amount of blood drawn is too minimal (about 3mls) to affect your child in any adverse way. Only one sample of blood will be obtained from your child. The blood sample will only be used to analyze the urea, creatinine and electrolytes status of your child, no other tests will be carried out and strict confidentiality will be observed at all times.

In all the instances, the child's primary care giver will be informed of all the results in view of more stringent follow up and added therapy.

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

Thank you for your co-operation.

**Consent**

Having read/explained to, the above, and understanding the purpose of this study and in the knowledge that it is voluntary and I can withdraw from it at any time during its course

**I**———————————— **of**————————————————

Do hereby accept to participate in the study.

———————————— ————————————

Parent's/Guardian's signature Investigator's signature

Date.————————

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

**APPENDIX B**

**CASE ASSESSMENT ON ROTA VIRUS GASTRO-ENTERITIS**

*Serial number* ---------- *Date* ---------- *Ward admitted*----------.

1. **Case**

Age of child/infant in months ☐

- Sex Male=1 Female=2 ☐
- Weight in gms ☐ Length in cms ☐
- Axillary temperature ☐
- Is the child breast feeding Yes =1 No=2 ☐
- Period of exclusive breast feeding in months ☐

2. **Mother / guardian**

i) Age in years ☐

ii) Education status ☐

None =1

Primary=2

Secondary=3

Tertiary=4

iii) Number of household members with gastro-enteritis ☐

iv) Contact with persons outside household with G.E

Yes =1 No =2 Don't know=3 ☐

v) Has case child had diarrhoea before?

Yes =1 No =2 ☐ If yes, how many times ☐

vi) Types of fluids given at home in last 24hrs (Code Yes=1 No=2)

ORS ☐

Plain water ☐

Milk/uji ☐

Home made solutions ☐

3. **Prior care sought before the child was brought to KNH** ( Code Yes=1 No=2)

- Herbalist / traditional healer ☐
- Religious healers ☐
- Bought over the counter medication ☐

4. **Referring health facility**

- Private practice=1
- Council clinic/dispensary=2
- District Hospital=3
- Self-referral=4

5. **Assessment of diarrhoea**

- Frequency of diarrhoea/day ☐
- Frequency of vomiting /day ☐
- Duration of diarrhoea ☐ (days)
- Duration of vomiting ☐ (days)
- Blood in stool Yes=1, No=2 ☐

6. **Clinical assessment of severity of dehydration**

Respiration Rate/min ☐ Pattern ; Deep Acidotic ☐ Gasping ☐

Cold extremities Yes=1, No=2 ☐

Capillary refill in secs ☐ Heart rate/min ☐

Pulses ; Normal strength =1 Weak =2 Impalpable ☐

Level of consciousness ; Alert=1 Verbal =2 Pain=3 Unconscious=4 ☐

Eye balls: Not sunken=1 Sunken=2 ☐

Skin pinch : Slightly decreased=1 Decreased=2 Markedly decreased (Longer than 2 secs) =3 ☐

**Hydration status** ☐

No dehydration = 1, Some dehydration = 2, Severe dehydration not in shock=3 and Severe dehydration in shock=4

7. **Other associated problems** (code Yes =1 No= 2)

Distended abdomen ☐

Convulsions ☐

Respiratory distress ☐

8. **Nutrition status** ( code Yes = 1 No = 2 )

Normal ☐ Visible severe wasting ☐ Bilateral pedal oedema ☐

**9. Venous blood analysis; urea, electrolytes and creatinine**

- $Na^-$ (mmol/l) ☐
- $K^+$ (mmol/l) ☐
- Chloride (mmol/l) ☐
- Urea (mmol/l) ☐
- Creatinine (µmol/l)

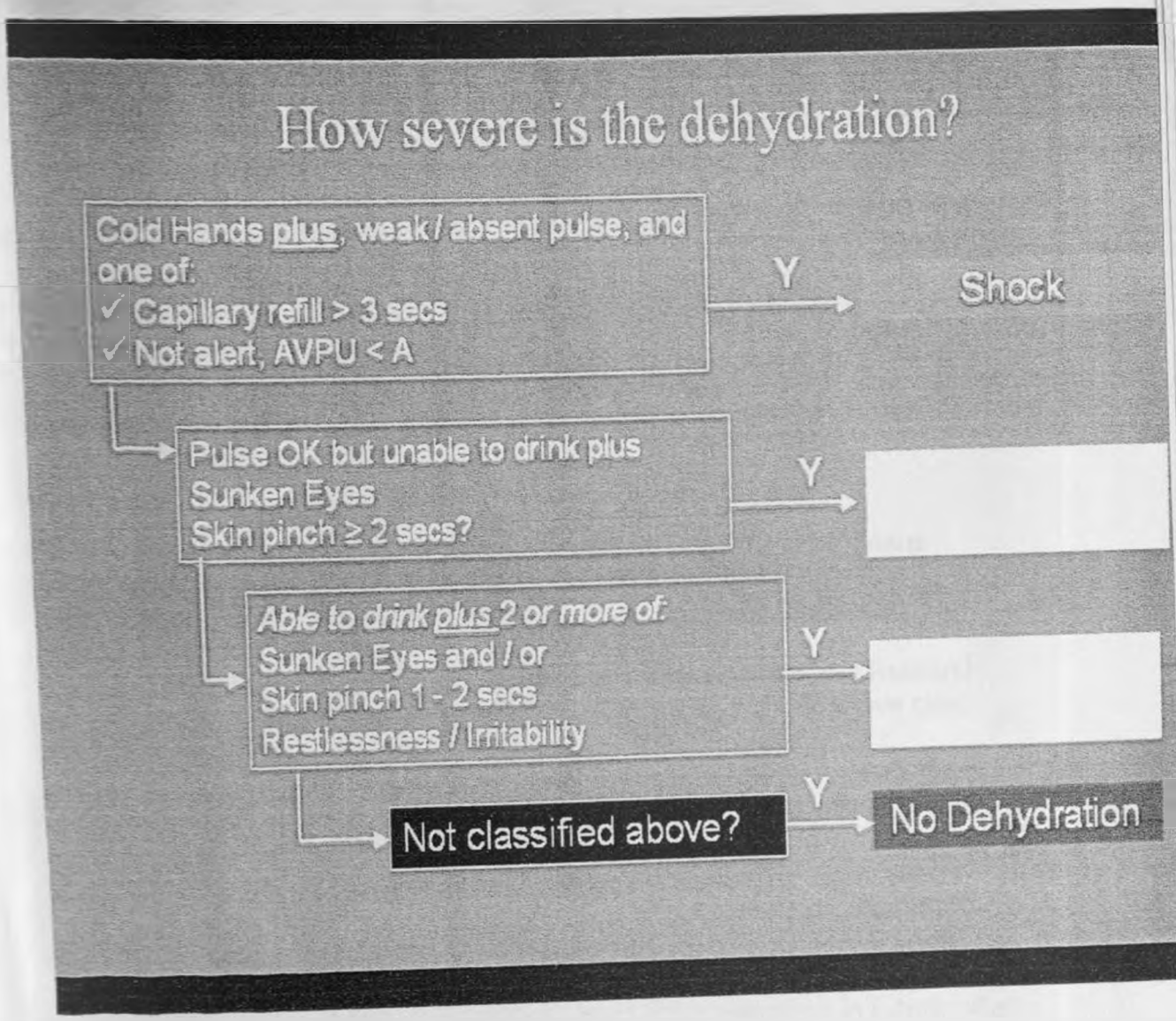
**10. Outcome**

- Rehydrated at PFC and discharged=1 Admitted to the wards=2 ☐
- Duration of admission in days ☐
- Outcome of hospitalization: Discharge =1 Dead =2 ☐

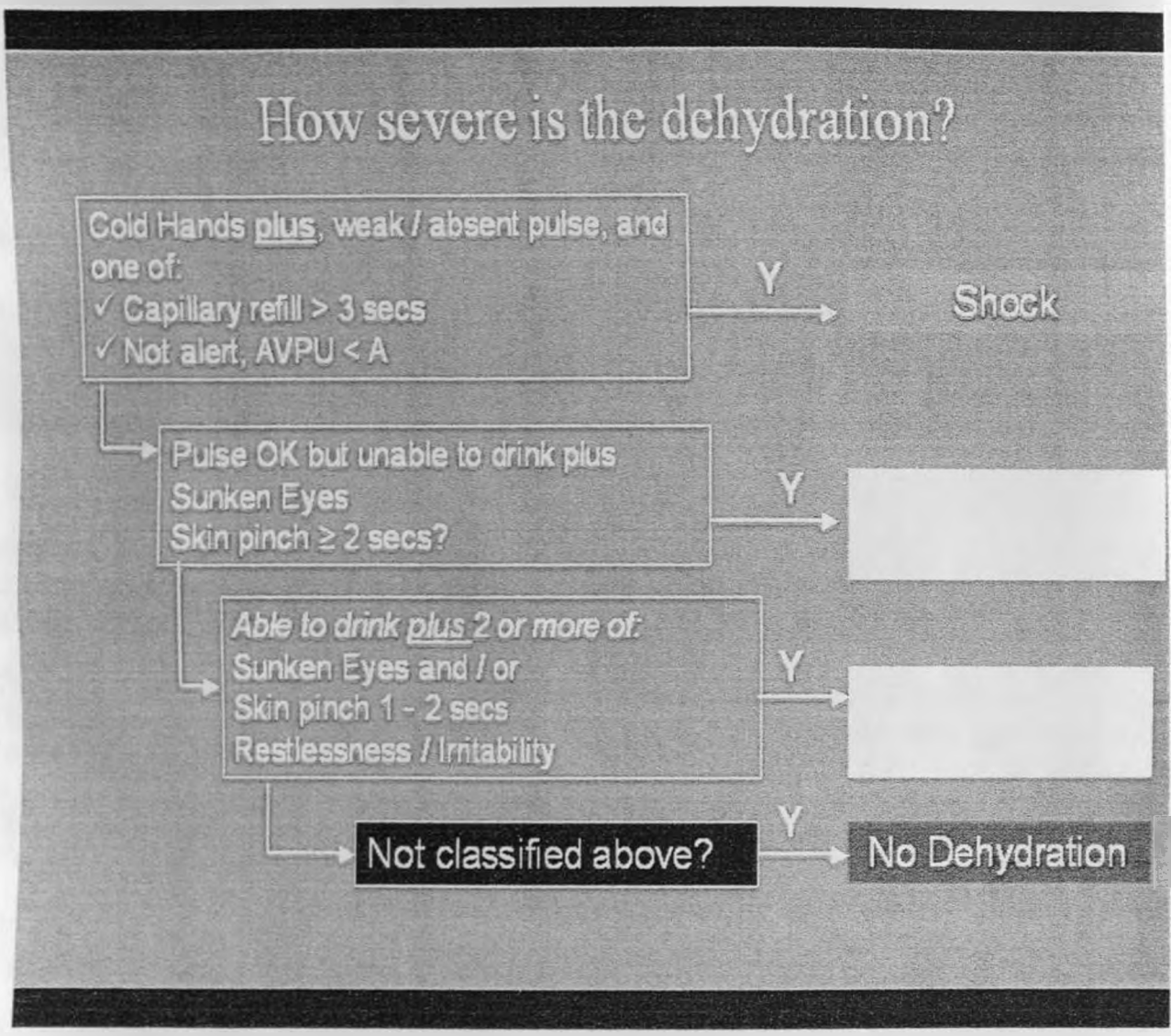
**11. Bacteria cultures results, light microscopy comments**

**12. Stool antigen Elisa for rotavirus** Positive =1 Negative =2 ☐

**Appendix c**


How severe is the dehydration?
Cold Hands plus, weak / absent pulse, and one of:
✓ Capillary refill > 3 secs
✓ Not alert, AVPU < A
Y
Shock
Pulse OK but unable to drink plus
Sunken Eyes
Skin pinch ≥ 2 secs?
Y
Able to drink plus 2 or more of:
Sunken Eyes and / or
Skin pinch 1 - 2 secs
Restlessness / Irritability
Y
Not classified above?
Y
No Dehydration

**Appendix c**


How severe is the dehydration?
Cold Hands plus, weak / absent pulse, and one of:
✓ Capillary refill > 3 secs
✓ Not alert, AVPU < A
Y
Shock
Pulse OK but unable to drink plus
Sunken Eyes
Skin pinch ≥ 2 secs?
Y
Able to drink plus 2 or more of:
Sunken Eyes and / or
Skin pinch 1 - 2 secs
Restlessness / Irritability
Y
Not classified above?
Y
No Dehydration

**KENYATTA NATIONAL HOSPITAL**
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: "MEDSUP", Nairobi.
Email: **KNHplan@Ken.Healthnet.org**

**Ref: KNH-ERC/ 01/ 3513**

**Date: 18th May 2006**

Dr. B.W. Gatinu
Dept. of Paediatric & Child Health
Faculty of Medicine
University of Nairobi

Dear Dr.Gatinu

**RESEARCH PROPOSAL: "PREVALENCE OF GROUP 'A' ROTAVIRUS AND ELECTROLYTES PROFILE IN CHILDREN PRESENTING WITH ACUTE DIARRHOEA AT KENYATTA N.HOSPITAL" (P59/03/2006)**

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** revised version of your above cited research proposal for the period 18th May 2006 – 17th May 2007.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

**PROF A N GUANTAI**
**SECRETARY, KNH-ERC**

c.c. Prof. K.M.Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, Faculty of Medicine, UON
The HOD, Medical Records, KNH
The Chairman, Dept. of Paediatrics & Child Health, UON
Supervisors: Dr. Grace Irimu, Dept. of Paediatrics, UON
Prof. W. Macharia, Dept. of Paediatrics,UON
Dr.J.O. Nyangao, KEMRI