PREVALENCE AND ASSOCIATED FACTORS OF ROTAVIRUS GASTROENTERITIS IN CHILDREN AGED 0-5 YEARS IN MOSHI, 2009

By

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A Dissertation submitted in Partial fulfillment of the requirements for the Degree of Master of Science in Epidemiology and Laboratory Management of Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of dissertation entitled **Prevalence and Associated Factors of Rotavirus Gastroenteritis in Children aged 0-5 years in Moshi, 2009,** in fulfillment of the requirements for the degree of Masters of Science in Epidemiology and Laboratory Management of the Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, Yona Kasebele val, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

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DEDICATION

I dedicate this work to my wife Debora, my daughter Tulibake and son Isaack who gave me courage and support when preparing this work.

ABSTRACT

Prevalence and Associated Factors of Rotavirus Gastroenteritis in Children aged 0-5 years in Moshi, 2009.

Background/Introduction: Diarrhea is a leading killer of children around the world. Responsible for 4 to 6 million deaths per year according to the World Health Organization. The disease is characterised by vomiting, fever and watery diarrhoea, associated with dehydration and sometimes death in children. It is associated with high cases of morbidity and mortality and it is estimated that up to 600,000 deaths in young children occur annually in the less developed countries and approximately 150,000-200,000 deaths occur in Africa alone. Rotavirus is the leading viral agent causing gastroenteritis in children.

Rationale: The study aimed at accurately determining the prevalence and associated factors of group A rotavirus disease in a resource-poor setting necessary to make informed decisions on provision appropriate interventions for prevention and control.

Objectives To determine the prevalence and associated factors of rotavirus gastroenteritis in children aged 0-5 years in Moshi Municipal Health facilities.

Methodology: This was cross- sectional descriptive study conducted in a hospital setting, involving children of five years of age and below with diarrhoea, and whose faecal samples were collected and tested for rotavirus by ELISA test at MUHAS Microbiology laboratory. All patients who met case definition were included in the study. Completed questionnaires were coded by numbers and double entered in a computer using Epi info software version 3.5.1. Chi- squire test was used to explore bivariate associations for categorical variables.

Results Of the 249 children with diarrhoea 31(12.4%) showed stool rotavirus positive results among these, 17 (54.8%) were females and14 (45.2%) were males. Prevalence varied by sex being 5.6% in males and 6.8% among females. The over all mean age was 23.3 months; mean age for females was 23.5 months where as for males was 23.0 months. Rotavirus positive specific age group distribution, 30 (96.8%) children are in the age group 7- 24 months and 1(3.2%) child in the age group 25-60 months.

Conclusion and Recommendations: This study shows insignificant prevalence of rotavirus gastroenteritis that could decrease the importance of vaccination by health education to parents on oral rehydration therapy, general sanitation, improvement of water supplies and proper excreta disposal this could reduce the burden of diarrhea diseases including rotavirus.

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LIST OF ABBREVIATION

AFENET	African Field Epidemiology Network
Ca	Calcium
CDC	Centre for Disease Control and prevention
DMO	District Medical Officer
EIA	Enzyme immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
G	Glycoprotein
IDEA	Trade name for ELISA rotavirus test kits
IgA	Immunoglobulin A
MCH	Maternal and Child Health
MUHAS	Muhimbili University of Health and Allied Sciences
NIMR	National Institute for Medical Research
NSP	Non Structural Proteins
ORT	Oral Rehydration Therapy
PAGE	Polyacrylamide Gel Electrophoresis
RMO	Regional Medical Officer
RT- PCR	Reverse Transcriptase- Polymerase chain Reaction
VP	Viral Protein
WHO	World Health Organization

CHAPTER ONE

1.0. Introduction and Literature review

1.1. General introduction

Diarrhea is a leading killer of children around the world. Responsible for 4 to 6 million deaths per year according to the World Health Organization (1), diarrhea is especially dangerous for infants and young children. Globally, it is estimated that 1.4 billion episodes of diarrhea occur in children less than five years of age annually (1).

There are many different diarrhoeal agents, including bacteria such as *V. cholera*, Salmonella species, Shigella species, E. coli, parasites such as *Girdia lamblia*, Cryptosporidium, and enteric viruses such as rotavirus, adenovirus, calicivirus and astrovirus (2). Determining the causes for diarrhea can be difficult with such a variety of infectious agents, and determination of disease burden for individual diarrhoeal agents is complicated by the lack of access to laboratory tests in many developing countries. Until a few years ago pathogenic organisms could be identified in stool in about 25% of patients with diarrhea disease. Today using new technologies, experienced laboratories can identify pathogens in about 75% of cases seen at a treatment facility (2).

Rotavirus has been established as the most important agents of acute gastroenteritis in infants and young children in many countries (3). The situation in East Africa may be similar to this, a preliminary survey of children under three years of age who received treatment in Mombasa and Nyeri districts in Kenya (1981-1983) revealed that up to 50% of cases were due to rotavirus infection (4). Studies in Tanzania on rotavirus gastroenteritis show a prevalence of 18 to 26% (5).

1.2. Types of diarrhea

According to WHO guidelines Diarrhea is defined as the passage of three or more loose or watery stools in 24 hours period (2). However a variety of terms have been used to describe diarrhea depending for example upon whether the stool is loose, watery bloody

or mucoid. The WHO guidelines stipulates three forms of diarrhea namely, one acute watery diarrhea as the diarrhea that begun acutely and lasted less than or equal to 13 days, two, dysentery; defined as mucoid bloody stool associated with anorexia abdominal cramps and tenesmus, and lastly persistent diarrhea, defined as diarrhea with duration of 14 days or more (2).

1.3. Aetiological agent

Rotavirus is a non-enveloped virus of the family Reoviridae with an icosahedral capsid 70nm across. It derives its name from the wheel like appearance when viewed under an electron microscope (rota is latin for wheel) (6). This is an etiologic agent for rotavirus gastroenteritis.

The viral nucleocapsid is composed of three concentric shells that enclose 11 segments of double-stranded RNA. The genome codes for 6 virus proteins (VP1, 2, 3, 4, 6, and 7) and 6 non-structural proteins (NSP1-6) (7). Once in the small intestine, the virus undergoes a change and becomes infective to the villi. Proteins then mediate the invasion of the host cells and replication of the virus genome (6). The outermost layer contains two structural viral proteins (VP): VP4, the protease-cleaved protein (P protein) and VP7, the glycoprotein (G protein). These two proteins define the serotype of the virus and are considered critical to vaccine development because they are targets for neutralizing antibodies that might be important for protection, there are 15 G serotypes. Rotavirus is a highly variable virus even within the subset of those that are infective to humans (8).

Rotaviruses are usually categorized into seven groups A-G, with subgroups I and II based on the VP6 (9). Within these seven groups, A, B, and C are infective to Humans (6). Group A rotavirus is the most common in children and group C has little association with disease in humans. Group A rotaviruses has several serotypes based on antigenic differences on their viral protein 7 (VP7) (10). Rotavirus is further categorized into G and P serotypes. The G serotype is specified by the glycoprotein VP7 of the outer capsid, which is coded by viral genes 7, 8, and 9 (9). The P serotype is specified by

protein VP4, also on the outer capsid. It is a protease cleaved protein coded by gene 4 of the virus genome (9). The most common G serotypes currently are G1, G2, G3, G4, and G9, with G1 being most prevalent and G9 the fastest emerging worldwide (9) (11) (12) (13). Common P serotypes are P1a, P1b, and P2a (8) (14) (15).

1.4. Epidemiology of rotavirus;

Diarrhea is a leading killer of children around the world. Responsible for 4 to 6 million deaths per year world wide according to the World Health Organization (WHO), diarrhea is especially dangerous for infants and young children under five years of age. Globally, it is estimated that 1.4 billion episodes of diarrhea occur in children less than five years of age annually (13). Rotaviruses are a main cause of gastroenteritis in infants and children of five years of age and below and sometimes adults, causing sporadic seasonal severe enteritis.

People of all ages are susceptible to rotavirus infection, but children of 6 months to 2 years of age, premature infants, and immunocompromised individuals are particularly prone to more severe symptoms (16). The peak incidence occurs between 7 and 15 months of age, with approximately 0.8 episodes per child per year (17). Children become most susceptible after 6 months of age when the protection afforded by maternal antibodies wanes. By 15 months of age many have developed some protection after primary infection (18) almost all children get rotavirus at least once before they are three years old (8). In hospital based surveys rotavirus is responsible for 25%-65% gastroenteritis diarrhoea cases and in community - based surveys rotavirus is detected 5%-40% of cases (19). First rates of rotavirus illness among children in industrialised and less developed countries are similar indicating that clean water supplies and good hygiene have little effect on virus transmission, therefore further improvement in water or hygiene are unlikely to have a substantial impact on disease prevention (20).

The median age of children hospitalised with rotavirus gastroenteritis in Africa is 6 months and 81% are under 1 year of age (20). It is associated with high cases of morbidity and mortality and it is estimated that up to 600,000 deaths in young children

occur annually in the less developed countries and approximately 150,000-200,000 deaths occur in Africa alone (21).

It has been estimated that each year rotavirus infection is responsible for an estimated 111 million episodes of diarrhea requiring only home care, 25 million clinic visits, 2 million hospitalizations, approximately 440,000 deaths in children < 5 years of age and most of it in developing countries, about 85% of fatal cases of rotavirus occur in nations defined as "low-income" by the World Bank (22). In temperate climates, rotavirus gastroenteritis is seasonal with peaks in cooler periods while in the tropics transmission occurs throughout the year (23). There have been several studies done in Tanzania namely Dar es Salaam and Ifakara revealing the prevalence of rotavirus to 7-24 age group of 43 % (24).

1.5. Pathogenesis;

Rotavirus is spread mainly by faecal oral route. In a study of Weiss and Clark (25) they found out that rotavirus is relatively acid labile, but can withstand acidic conditions of the stomach. At room temperature (23 degrees centigrade) the virus is rapidly inactivated after one minute at pH 2, slower at pH 3 (about 10 minutes and at pH 4 inactivation is minimal, the rate of inactivation is higher at normal body temperature.

After ingesting the virus, the rotavirus particles are carried to the small intestine where they infect the mature enterocytes in the mid and upper part of the villi of the small intestine, leading to diarrhea. Rotavirus is thought to invade target cells in two possible ways, by direct entry or fusion with the enterocytes, and through Ca^{2+}-dependent endocytosis (15). Rotavirus infection leads to structural changes in the intestinal epithelium. Within 24 hours of infection the rotavirus selectively infects mature enterocytes on the tips of the villi and shape of the villus epithelium changes from columnar to cuboidal, and the villi become stunted and shortened. Changes are most severe in the upper portions of the small intestine, and there is little or no inflammation of the infected areas. The severity of these changes is correlated with the severity of the

resulting illness (15). Replication of rotavirus particles takes place in cytoplasm of small intestine epithelium.

For the purpose of global rotavirus surveillance, a suspected case of rotavirus is a case of acute diarrhoea in a child <5years of age admitted for treatment. A confirmed case of rotavirus diarrhoea is defined as a suspected case in whose stool the presence of rotavirus is demonstrated by means of an enzyme immunoassay (26).

1.6. Clinical features;

Following an incubation period of 1-3 days, the illness can begin abruptly, and vomiting often precedes 24 hours the onset of other symptoms then followed by severe acute watery diarrhea, irritability and low grade fever (20). Up to one third of patients present with fever. Gastrointestinal symptoms generally resolve in 3-7 days. Because the clinical features of rotavirus gastroenteritis do not differ from those of gastroenteritis caused by other pathogens, confirmation of rotavirus infection by laboratory testing of fecal specimens is necessary for reliable rotavirus prevention and control. Common physical features seen in rotavirus gastroenteritis diarrhea are isotonic dehydration and compensated metabolic acidosis. Rotaviruses are shed in high concentrations in the stools of infected children and are transmitted primarily by the fecal-oral route, both through close person-to-person contact and through fomites (27). People of all ages are susceptible to rotavirus infection, but children 6 months to 2 years of age, premature infants, and the elderly and immunocompromised individuals are particularly prone to more severe symptoms (32).

1.7. Laboratory Diagnosis;

Because it was difficult to identify rotavirus infection by clinical signs alone, therefore there was great demand for rapid reliable laboratory methods to confirm rotavirus diagnosis. Electron microscopy was first used as a laboratory diagnostic test, but it is laborious and costly (18). Various immunoassay techniques have been developed for this purpose. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method for detection of rotaviruses is highly sensitive; however the system needs costly equipments,

reagents and trained manpower and cannot be performed as a routine laboratory diagnostic test for detection of rotaviruses in developing countries. The only diagnostic procedure, the Enzyme Linked Immunosorbent Assay (ELISA) is considered to be highly sensitive tool for screening of rotaviruses from hospital or field samples, because of its ability to detect positive isolates even at low concentrations. It is estimated that ELISA tests require 10^6 rotavirus particles per milliliter, RT-PCR requires 10^4, and Polyacrylamide Gel Electrophoresis (PAGE) requires 10^{11}(28). The most widely available method for the diagnosis is through antigen detection in the stool by an Enzyme Immunoassay (EIA) directed at an antigen common to all group A rotaviruses (29). Latex agglutination tests are also useful rapid screening tests, they are specific but less sensitive, viral culture on tissue can also be done in research settings (29), other methods include nucleic acid hybridization and sequence analysis.

The pitfalls of ELISA test include failure to detect viral antigen in stool samples containing a high titer of the corresponding antibody and false positive results. However, regardless of its limitations, ELISA is still the method of choice in almost all laboratories for the identification of rotaviruses (20). Currently there are no routine laboratory diagnostic tests for rotavirus in Tanzania.

Serologic methods that detect a rise in serum antibodies, primarily enzyme immunoassay for rotavirus serum immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies have been used to confirm recent infections. In vaccine trials the immunogenicity of rotavirus vaccines has been assessed by measuring rotavirus specific- IgA and neutralizing antibodies to vaccine strains (34).

1.8. Control treatment and prevention;

Young children who are infected are the major source of rotavirus infection in the community. They excrete the virus in very high titres up to 10^9-$100x10^9$ viral particles per gram of faeces (30). The burden of diarrhoeal disease can and must be reduced by improving sanitation and educating parents on rehydration therapy to prevent children

deaths from dehydration and developing vaccines to prevent disease. Since improving sanitation is complicated by lack of infrastructure and funding in many countries where diarrhoea is particularly devastating, and is insufficient in combating some pathogens, vaccines offer hope for reducing the toll that diarrhea takes on the world's children.

Intravenous treatments with fluids are an important supportive measure in the treatment of rotavirus diarrhea and are widely used to treat very young children, the severe dehydrated children and the elderly depilated patients. These treatments are largely unavailable to the developing world's 575 million children under age five (31). While the alternative treatment of oral rehydration therapy (ORT) is more available, there are still significant setbacks in distributing ORT or instructions for its production. Due to all of these issues, a vaccine is the much more cost-effective solution (31).

There is no cure or natural immune state to rotavirus, though primary infection by the virus induces production of rotavirus-specific memory B and T cells, these are not normally sufficient to prevent reinfection by the virus. However, they do serve to reduce the severity of secondary infections. It has shown that serum IgA antibody titers correlate with protection against reinfection (14).

Standard sanitary measures that kill most bacteria and parasites are ineffective in controlling rotavirus as demonstrated by the fact that rotavirus incidence is similar in countries with both high and low sanitation standards (32). Improvements in water supplies and excreta disposal may reduce the transmission of enteric bacteria and parasites, but is unlikely to reduce the incidence of rotavirus. Standard methods of sanitation such as antibiotic soaps are not 100% effective in killing the virus, and because low numbers of viruses can cause infection, transmission is common even with good hygiene practices (33). The most effective antiseptics against rotavirus are alcohols, which have been found to reduce the number of viruses on the hands by greater than 99%. However, tap water alone, or tap water with regular soap reduces the titer by only 72-84% (9). Hand washing can reduce the spread of the virus.Vaccines are therefore being developed as a primary public health intervention to reduce the burden

of diarrhoea caused by rotavirus (34). The need to develop a safe and efficacious rotavirus vaccine to reduce disease burden and prevent deaths remains a very high priority for control of rotavirus diarrhoeal diseases. Vaccine for control of rotavirus diarrhea developed are either against a single serotype of animal or human origin or a quadrivalent vaccine comprising of 4 important serotypes (Serotypes 1-4) to reduce severity of diseases (36). Vaccines directed against this virus have shown promising results in recent trials, and are undergoing effectiveness evaluation in sub-Saharan Africa.

In East Africa limited childhood data are available on the incidence and clinical characteristics of severe rotavirus group A rotavirus disease. Advocacy for vaccine intervention and interpretation of effectiveness following implementation will benefit from accurate base-line estimates of the prevalence and severity of rotavirus pediatric admissions in Moshi populations.

1.9. Statement of the problem

Vaccines directed against rotavirus have shown promising results in recent trials, and are undergoing effectiveness evaluation in sub-Saharan Africa. In East Africa limited childhood data are available on the incidence and clinical characteristics of severe group A rotavirus disease. Advocacy for vaccine intervention and interpretation of effectiveness following implementation will benefit from accurate base-line estimates of the prevalence and severity of rotavirus pediatric attended in Moshi populations.

Rotavirus caused gastroenteritis is a major cause of children hospitalization and death, however currently little is known about the prevalence of rotavirus infections among children who were attending health facilities in Moshi (that is, how many children develop severe disease every year) or about the clinical characteristics of the disease in the Hospital. The disease has the prevalence of about 43% in Tanzania according to hospital based studies done in Dar es Salaam and Ifakara. There are multiple association

factors which cause diarrhoea of unknown prevalence which are routinely treated with antibiotics which prolong viral diarrhea.

1.10. Study rationale

The study aimed at accurately determining the prevalence and associated factors of group A rotavirus disease in a resource-poor setting for proper control and prevention. This baseline information may be used to make informed decisions about necessary preventive measures and assess on the need for mass introduction of rotavirus vaccination. This study examined the prevalence and clinical characteristics of rotavirus infections among children of five years of age and below who had diarrhoea and attended treatment at Mawenzi hospital, Majengo and Pasua health facilities. The study also provided an opportunity for a TFELTP resident to learn.

1.11. Study Objectives

1.11.1. Broad objectives

To determine the prevalence and associated factors of rotavirus gastroenteritis in children of five years of age and below in Mawenzi hospital and satellite health centres of Moshi municipality, Kilimanjaro region, Tanzania.

1.1 1.2. Specific objectives

1. To determine the proportion of 5 years of age children and below who had diarrhoea that are caused by Rotavirus infection in Mawenzi Hospital,Pasua and Majengo health facilities
2. To determine the clinical symptoms of children aged 5 years and below with diarrheas caused by rotavirus infection.
3. To describe the social demographic characteristics of children under 5 years of age with rotavirus gastroenteritis diarrhea.

CHAPTER TWO

2.0. Methodology

2.1. Epidemiological Methods

2.1.1. Study site

The study was conducted in Moshi municipal at Mawenzi regional hospital, Pasua and Majengo health centres, in Kilimanjaro region located in the Northern part of Tanzania with the population of approximately 1.4 million inhabitants. The Mawenzi hospital has a bed capacity of 300 beds. The number of children of five years of age and below admitted last year (2008) was 4337 children and out of these 2316 children (53%) were admitted due to diarrhoeal diseases. Common etiologic agents for diarrhoeal diseases included bacteria, parasites and viruses (40). The Mawenzi Hospital catchments population is 1.6 million per year coming from the districts which include Rombo, Hai, Moshi rural, Moshi urban, Mwanga and Same. Data was collected from in patient as well as outpatient children.

Initially data collection was scheduled to be done at Mawenzi Hospital alone, after observing the trend of recruitment it was necessary to add extra data collection sites because of two major reasons, one was that during the study period it was not the peak period for diarrhoeal diseases in the region, and there were few cases. The second reason was administrative, that Mawenzi MCH clinics were decentralised to respective Municipal health centres namely Pasua and Majengo, there fore the number of children attending Mawenzi Hospital clinic decreased compared to the baseline information obtained during research proposal development. In order to attain the estimated sample size within the prescribed time of 2 months and recruit the sample from the original target population in the catchment area, it was necessary to add Majengo and Pasua Health facilities as data collection sites.

2.1.2. Study design

This was a cross- sectional descriptive study.

2.1.3. Study population

Children of five years of age and below with diarrhoea who attended Mawenzi hospital paediatric Clinic, Majengo and Pasua health centre with diarrhoea. The study period was for two months, which was December 2009 and January 2010.

2.1.3.1. Inclusion criteria

A child of five years of age and below who presented with watery diarrhoea.

2.1.3.2. Exclusion criteria

Recurrent readmission due to diarrhea in a specified time.

Children with uncertain age.

2.1.4. Case definition

A suspected case of rotavirus acute diarrhoea was defined as a child of 5 years of age and below who attended for treatment for acute gastroenteritis/diarrhoea at Mawenzi hospital, Pasua and Majengo health centres during December 2009 and January 2010.

A confirmed case of rotavirus diarrhoea was defined as a suspected case in whose stool the presence of rotavirus was detected by means of an ELISA test at MUHAS microbiology laboratory. The IDEA^{TM} rotavirus test kits were used which utilizes a polyclonal antibody to detect specific group A rotavirus proteins.

2.1.5. Sample size

Sample size was calculated using the following formula:

$$n = \frac{z^2 p(100-p)}{\varepsilon^2}$$

$$n = \frac{1.96^2 \times 0.43(1-0.43)}{0.5^2}$$

4 x 2.304x0.2 =

1.7664 =7.0656

0.25

=248

n=Minimum sample size.

p = Proportion/prevalence of the study variable =43% (24).

E = Tolerable error / level of significance = 5%.

z =Standard deviation of the normal distribution = 1.96.

2.1.6. Sampling technique and Data collection

All patients who met the criteria for the case definition were recruited in the study. After informed consent was given, children participating in the study were seen by a physician and underwent a routine medical examination, including assessment of dehydration status, temperature and disease severity. They were screened and recruited at Mawenzi Hospital paediatric clinic, Majengo and Pasua Health centres, after seeing the Clinicians for routine diagnosis, every case subject who qualified the case definition criteria were enrolled and referred to an assistant at the clinic. The assistant was provided with consent form which was read and signed by the mother /guardian. Children whose parents/guardians did not consent and those whose age could not be ascertained were excluded from the study. Three of these children were excluded from the study due to lack of consent or uncertainty of age.

Data was collected from December 2009 to January 2010. Tools for data collection included a structured annexed questionnaire which had the following variables, part one had social demographic information; name, age, sex, identification number, ward, and domicile address. This part was filled by the assistant at the data collecting sites. The questionnaire also asked questions that are important to know the associated factors of diarrhoea.

Mother/guardian was interviewed concerning demographics, medical history and clinical presentation/characteristic by use of a standard structured questionnaire. The assistant then enrolled the subjects in a special log book and assigned a unique identification number which was the only identifier. The research subjects were then provided with a clean dry, wide mouthed plastic container to collect stool samples and submit to a key person in the laboratory for storage at -20 degrees centigrade. After obtaining the required sample size, the specimen were shipped in a cool box by air to Dar es Salaam for analysis at MUHAS microbiology laboratory.

The second part was laboratory processing information which included; date of specimen collection, date of processing, macroscopic appearance, IDEA^{TM} rotavirus group A ELISA results and name of processing technician. This part was filled in the laboratory. The questionnaire was pre-tested before being administered to affirm its completeness to collect data; amendments were made. This provided a feedback of the errors that need rectification by the data collection team. To avoid mistakes and to monitor completeness of questionnaires, data collected was entered into the computer on a daily basis.

2.2. Laboratory Methods

2.2.1. Specimen collection and storage

Fecal specimens were collected in clean, wide-mouthed dry containers, free of calf or bovine serum or detergents. Approximately 0.1 g (0.1 ml) is sufficient to perform the test. For best results samples were collected immediately from patients upon arriving to the health facility.

2.2.2. Specimen Storage

Specimen were secondarily packed in nunc tubes and stored at -20°C awaiting air shipment to MUHAS microbiology laboratory for rotavirus ELISA test. The viral particles are viable for indefinite time at -20°C; the quality of ELISA test is not altered at this temperature. The samples were shipped in a cool box maintaining the low

temperature, freezing and thawing was avoided. It was made sure that the specimens were not treated with solutions containing formaldehyde or its derivatives which compromise test results.

2.2.3. Virus detection

The virus was detected by immunologic specific commercial ELISA test kits at MUHAS microbiology laboratory. There was regular counterchecking during stool samples collection to ensure that proper samples were collected. During specimen processing the following steps were adhered to user manufacturer's instructions, description of macroscopic appearance, and the ELISA test was performed as follows.

2.2.3.1. Detection of Rotavirus in Stool by Enzyme Immunoassay

The IDEA™ rotavirus test kits utilizes a polyclonal antibody to detect specific group A rotavirus proteins especially the internal capsid protein (VP6). This assay is able to detect rotavirus at a low concentration as 7.8x 10 viral particles /ml and shows good correlation, specificity and sensitivity in comparison with electron microscopy (99.5%, 100%, and 99.2%) respectively.

Samples were processed according to manufacturer's instructions. Two positive controls and four negative controls were used for each kit which was used for testing. Briefly 100µl of fecal suspension in a diluent buffer provided was added to each microwell, then 100µl of conjugate was added to each well. After incubation for 1 hour the microwells were automatically washed five times with 500µl of wash buffer. After the five washes the specifically bound conjugate was detected by addition of a chromogenic substrate and incubation was done at room temperature for ten minutes. The absorbance was read at 450 nm using an ELISA reader. Specimen with absorbance value equal or greater than cut off value were considered positive. (Cut off value was calculated on each kit by adding 0.100 absorbance units to the negative control value).

2.3. Data analysis

Completed questionnaires were coded by numbers, data double entry, editing and analysis was done using Epi-info software version 3.5.1. Descriptive analysis was done to document the distribution of study subjects by place, time and person. The risk factors were assessed using logistic regression or bivariate analysis. Odds ratios (OR) was used as the measure of association between associated factors and rotavirus diarrhoea. Corrected Mantel-Haenszel chi square was used to calculate 2 tailed p-values with the level of significance being set at ≤ 0.05.

2.4. Ethical issues

The ethical clearance was obtained from the Muhimbili University of Health and Allied Sciences research ethical committee. All information obtained during the study was kept confidential. An informed written consent was obtained from research subject's parents / guardians' prior enrolment to the study. The samples were de-linked from the study subjects, no names were used in the data collection process only unique identity numbers were used. Permission to collect data was sought from The Regional Medical Officer and in charge of data collection sites.

CHAPTER THREE

3.0. RESULTS

During the study period December 2009 to 2010 February, 249 children with diarrhoea were recruited in the study from three study sites namely Mawenzi hospital, Majengo and Pasua health centres. Three cases were not enrolled in the study due to refusal or age uncertainty. The expected number of children was 386 so the response rate was 64.5%, but the calculated sample size was 248 cases. The study months was not the peak period for diarrhoeal diseases, this could explain the low response rate.

Table 1. Age and sex distribution of the studied population in Moshi municipal, 2009

Age group in months	Male n, (%)	Female n, (%)	Total n, (%)
0 - 6	18 (43.9%)	23 (56.1%)	41 (16.5%)
7- 24	44 (39%)	69 (61%)	113 (45.4%)
25 – 60	41(33.2%)	54 (56.8%)	95 (38.1%)
Total	**103 (41.4%)**	**146 (58.6%)**	**249 (100.0%)**

Age was stratified in three categories according to clinical signs, immunological status and exposure risks.

Table 1 shows age and sex over all distribution of diarrhoea among the studied population, 146 (58.6%) were females and the rest were males 103 (41.4%). The over all mean age was 23.3 months mean age for females was 23.5 months where as for males was 23.0months

Table 2. Socio-demographic characteristics of parents /caretakers of the studied population in Moshi Municipal, 2009 (n=249)

1. Mother/Guardian employment status	Number	Percent
None employed	108	43.4%
Employed	141	56.6%
2. Knowledge on diarrhoeal diseases		
Yes	66	26.5%
No	183	73.5%
3. Mother/caretaker level of education		
No formal education	15	6.0%
Primary education	170	68.3%
Secondary education	62	24.9%
Post secondary education	2	0.8%

All parents/ guardians were females, those with no formal education were 15 (6.0%), with primary education were 170 (68.3%), secondary and post secondary education were 62 (24.9% and 2 (0.8%) respectively (Table2). Regarding knowledge on diarrhoeal diseases; most parents/caretakers of the children with diarrhoea had no knowledge on diarrhoea diseases 183 (73.5%). Among 249 parents/caretakers of the children with diarrhoea 141 (56.6%) were employed where as 108 (43.4%) were not employed (Table2).

Table 3: Sex distribution and risk of rotavirus diarrhoea cases in Moshi municipal, 2009

Sex	Rotavirus Positive	Rotavirus Negative	OR	P-value
Female	17(6.8%)	129(51.8%)	0.84	0.64
Male	14(5.6%)	89(35.7%)		
Total	**31(12.4%)**	**218(87.6%)**		

Of the 249 children with diarrhoea 31 (12.4%) stool positive rotavirus, among these 17 (54.8%) were females and 14 (45.2%) were males. The rest 218 (87.6%) had no rotavirus antigen in their stool specimen. There was no statistical significant difference on rotavirus infection among male and female children thus, sex does not influence infection by rotavirus.

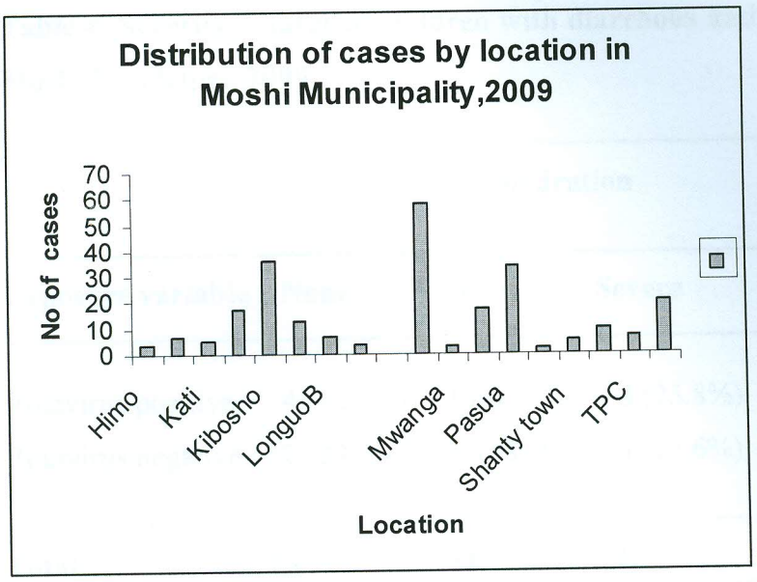

Figure.1. Distribution of diarrhoeal cases by location in Moshi Municipal, 2009.

Among 249 population of children studied 58 (23.3%) were from Majengo location followed by Kibosho 36 (14.5%) and Pasua suburban 34 (13.7) Uru (Figure1)

Table 4. Severity status of children with diarrhoea and rotavirus positive cases in Moshi Municipal, 2009

	Dehydration					
Exposure variable	**None**	**Mild**	**Severe**	**Total**	X2	P-value
Rotavirus positive	4 (12.9%)	19(61.3%)	8 (25.8%)	31	4.42	0.20
Rotavirus negative	52 (20.9%)	132(53.0%)	34 (13.6%)	218		
Total	**56**	**151**	**42**	**249**		

Table 4 shows severity of diarrhoea using hydration status. Out of 249 children included in the study, 52 (20.8%) had severe dehydration, 161 (64.6%) had mild dehydration and 56 (22.5%) had no dehydration. With regard to rotavirus positive diarrhoeal cases 18 (58.0%) had mild dehydration while 9 (29.1) had severe dehydration and 4 (12.9%) had none. Children with rotavirus infections 4 (12.9%) had no dehydration, 19(61.3%) had mild dehydration and 8 (25.8%) had severe dehydration. There was no significant difference on the hydration status between children with or without Rotavirus diarrhea.

Table 5. Knowledge on diarrhoeal diseases in relation to rotavirus enteritis

Knowledge on Diarroeal diseases	**Rotavirus Positive**	**Rotavirus negative**	**P-value**	**OR**	**95% CI**
Yes	6 (19.4%)	59 (27.0%)	0.36	0.65	0.23, 1.77
No	25 (80.6%)	159 (73.0%)			
Total	**31 (100.0%)**	**218 (100%)**			

Table 5 shows parent/ guardian level of health education on diarrhoeal diseases in relation to acquiring rotavirus diarrhoea where 25 (80.6%) parents/guardian had no knowledge compared to 6 (19.4%) who had some knowledge. The prevalence of rotavirus in parents/guardian with knowledge on diarrhoeal diseases was 2.4% and 10.0% in parents/guardian with no diarrhoeal diseases knowledge. There was no association between parent /guardian knowledge on diarrhoeal diseases and acquiring rotavirus infection.

Table 6. Parents/guardian level of education in relation to rotavirus diarrhea in Moshi Municipal, 2009

Education level of parent/caretaker	**Rotavirus positive**	**Rotavirus Negative**	**P- value**	**X2**
No formal education	1 (3.3%)	14 (6.5%)	0.80	0.98
Primary school completed	21 (67.7%)	149 (68.3%)		
Secondary education	9 (29.0%)	53 (24.3%)		
Post secondary education	0 (0.0%)	2 (0.9%)		
Total	**31 (100.0%)**	**218 (100.0%)**		

Table 6 shows Parents/guardians of rotavirus positive children 21 (67.7%) completed primary education, 9 (29.0%) completed secondary education and 1 (3.3%) had no formal education, (table 5). There is no association between parent/guardian education and acquiring rotavirus infection.

Table 7. Parent/guardian employment status in relation to rotavirus positive diarrhoea in Moshi Municipal, 2009

Employment status of parent/guardian	Rotavirus positive	Rotavirus negative	P-value	OR	CI
Employed	18 (58.1%)	124 (56.9%)			
Not employed	13 (41.9%)	94 (43.1%)	0.45	1.05	0.48, 2.24
Total	**31(100.0%)**	**218 (100.0%)**			

Table 7 shows Parent/ guardian employment status in relation to rotavirus positive diarrhoea. Majority of parents / guardians of rotavirus positive children were employed 18 (58.1%) where as 13 (41.9) were unemployed.

Table 8: Relationship between water treatment and rotavirus diarrhea

Water treatment	Rotavirus negative	Rotavirus positive	P- value	Chi square
Boiling	98 (44.9%)	16 (51.6%)		
Chlorination	20 (9.2%)	1 (3.2%)	0.49	1.40
Nothing	100 (45.9%)	14 (45.2%)		
Total	**218 (100.0%)**	**31(100.0%)**		

Table 8: Shows relationship between water treatment and rotavirus positive diarrhea where we find 17 (54.8%) of rotavirus positive children house holds boil drinking water, and 14 (45.2%) do nothing to their drinking water. There was no significant difference in relation to water treatment between general diarrhea and getting Rotavirus diarrhea.

Table 9: Distribution of all diarrhoeal diseases and rotavirus diarrhea by age group in Moshi Municipal, 2009

Age group In months	Rotavirus negative	Rotavirus positive	P-value	Chi-square
0 – 6	3 (1.4%)	1 (3.2%)		
7 – 24	138 (63.3%)	29 (93.6%)	0.001	13.2
25 – 60	77 (35.3%)	1 (3.2%)		
Total	**218 (100.0%)**	**31 (100.0%)**		

Table 9 shows all diarrhoea cases and rotavirus positive specific age group distribution, 30 (96.8%) children are in the age group 7- 24 months and 1 (3.2%) child is in the age group 25-60 months. Children of age group 7 to 24 months are more at risk of Rotavirus diarrhea.

Table 10: Rotavirus positive cases stools macroscopic appearance

Macroscopic findings	Number	Percent
Loose brownish	15	48.4%
Loose yellowish	8	25.8%
Mucoid	2	6.5%
Watery	6	19.4%
Total	**31**	**100**

Stools from children who tested positive for rotavirus had the following macroscopic appearance and frequency, loose brownish 15 (48.4%), loose yellowish 8 (25.8), watery 6 (19.4%) and mucoid 2 (6.5%).

CHAPTER FOUR

4.0. DISCUSSION

This was a descriptive study which investigated rotavirus gastroenteritis prevalence at Moshi municipal Mawenzi hospital and two health centres namely Pasua and Majengo. This study was conducted during a dry season from December 2009 to February 2010.
A total of 249 children with diarrhoea were recruited in the study from three study sites namely Mawenzi hospital, Majengo and Pasua health centres. The expected number of children was 386 so the response rate was 64.5%, however the calculated sample size was 248. The study months was not the peak period for diarrhoeal diseases, this could explain the low response rate. The low response rate could also give wrong impression of the prevalence of rotavirus gastroenteritis and the factors that are associated with it. There is no indication of severity of disease of in the rotavirus positives and negatives and this could be attributed to the children sampled.

In this study, there was no significant difference on rotavirus infection among male and female children the difference was not statistically significant with $p > 0.05$.

The more affected age group with rotavirus diarrhoea was in 7 months to 24 months age group, they constituted 93.6% of all cases. This was in concurrence the study by Sabrina *et al* which was done in Dar es Salaam (24). There was a decline of diarrhoea cases in the age group 25-60 months 98 (39.3%) (Table1). Age stratification was based on clinical signs and symptoms. This age specific pattern reflects the combined declined of rotavirus Anti-VP6 Secretory Immunoglobulin A which contributes to protection via intracellular neutralization but not via immune exclusion and the introduction of foods that may be contaminated by faecal rotavirus particles (24). Most rotavirus gastroenteritis cases occurred among children aged <2 years (93.6%). This is consistent with other recent European surveillance data that found the proportion of rotavirus

gastroenteritis in children presenting with acute gastroenteritis to be 56.7% to 74.2% in children aged 6 to 23 months (1).

. This also shows that health education to parents/guardians on general hygiene, improved sanitation, use of oral hydration therapy and proper excreta disposal can control most diarrhoeal diseases as cited by Umesh *et al.*

The prevalence of rotavirus proven gastroenteritis by laboratory enzyme immunoassay was 12.4% which is within range with the study of Sabrina *et al* who conducted such a study in Dar es Salaam. This prevalence is relatively low compared with findings in Tanzania which reported rotavirus to account for up to 43 % (39). The difference can be explained in part by seasonal variation and by the fact that previous studies used agglutination tests which have been reported to be less specific and may lead to false positives results compared to ELISA. False positive and false negative results may give false high and low prevalence respectively.

Most children who were brought for treatment to the clinics presented with watery diarrhoea, vomiting and some with fever with mild to severe dehydration.

In this study boiling drinking water alone is not preventive to all diarrhoeal diseases because there other contributing factors for diarrhoea such as poor hand hygiene, poor sanitary conditions, and prolonged storage of cooked food at room temperature. Host factors include malnutrition, undercurrent illness and compromised immunity (24) in addition age had been cited as another factor with most rotavirus diarrhoea episodes occurring during the first two years of life especially 2-11 months age group when weaning and starting supplementary feeds which may be contaminated.

Parents/guardians with education above primary education had fewer children with rotavirus diarrhoea than those with primary education and below this shows that good education is preventive of diarrhoeal diseases, still this study also explains that there was no association between parent/guardian knowledge on diarrhoeal diseases and acquiring

rotavirus infection. The prevalence of rotavirus in parents/guardian with knowledge on diarrhoeal diseases was 2.4% compared to 10.0% for parents/guardian who had no diarrhoeal diseases knowledge. This shows that health education to parents/guardians on general hygiene, improved sanitation, use of oral hydration therapy and proper excreta disposal can control most diarrhoeal diseases as cited by Umesh *et al* (37). There was no significant frequency difference of rotavirus diarrhoea among employed and none employed parents/guardians.

In this study dehydration was among the clinical symptoms presented, but there is no statistical difference between dehydration due to rotavirus diarrhoea and other diarrhoea caused by other etiological agents.

The findings of this study demonstrate the amount of illness caused by rotavirus disease. The prevalence of rotavirus disease is similar in children in both developed and developing nations. However, children in developing nations die more frequently, possibly because of several factors, including poor access to health care and poor management hydration therapy and a greater prevalence of malnutrition. An estimated 1,205 children die from rotavirus disease each day, and 82% of these deaths occur in children in the poorest countries (1).

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

Infants and young children under 2 years of age are most vulnerable to rotavirus infection that often results in severe diarrhea and dehydration, causing hospitalization and deaths. This age specific pattern reflects the combined declined of rotavirus Anti-VP6 Secretory Immunoglobulin A which contributes to protection via intracellular neutralization but not via immune exclusion and the introduction of foods that may be contaminated by faecal rotavirus particles (24).

The primary public health intervention of rotavirus diarrhoea 1s hygiene. This study suggests rotavirus vaccination could not be useful to reduce rotavirus gastroenteritis among children in our country, Based on our findings, it is reasonable to expect rotavirus vaccination to have a minor impact in reducing the burden of rotavirus gastroenteritis in the country, and lessening acute gastroenteritis diarrhoea due to rotavirus. The data also suggest the need for a vaccine that can provide protection for at least the first 2 years of life after 6 months if resources are available. The role of rotavirus vaccination in this context is to protect children from rotaviruses, which are the leading cause of severe diarrhea among infants and young children. Safe and effective vaccines are needed, especially in poorer countries where most deaths from the disease occur (41). A 2009 review estimated that vaccination against rotavirus would prevent about 45% of deaths due to rotavirus gastroenteritis, or about 228,000 deaths annually worldwide (42).

Health education to parents on oral rehydration therapy, general sanitation, improvement of water supplies and proper excreta disposal could reduce the burden of diarrhoea diseases. Currently there is no routine laboratory diagnostic test in Tanzania thus all diarrhoeal diseases are treated almost entirely by antibiotics rehydration and other medications some which pose risk to some children in the age group typically affected

by rotavirus. Antibiotics are not indicated for rotavirus gastroenteritis if suspected. Rotavirus routine testing for children is suggested if resources are available.

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