PREVALENCE OF ASTROVIRUSES IN CHILDREN UNDER FIVE YEARS WITH ACUTE DIARRHOEA AT THE KENYATTA NATIONAL HOSPITAL

By

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DECLARATION

This project is my original work and has not been presented to any other university/ institution for a degree award

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Date_____

DECLARATION BY SUPERVISORS:

This thesis has been submitted to the University of Nairobi Institute of Tropical and Infectious Diseases with our approval as University supervisors for Jacklyne Ashubwe-Jalemba.

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DEDICATION:

I give all glory to God without whose help I would not have been able to complete this project.

I dedicate this work to my loving husband Dr. Martin Jalemba Aluvaala who has been a role model for me and provided immense support towards its completion. I also dedicate it to Sean and Jude my sons who acted as a source of motivation. My final dedication goes to my mums Margaret Ashubwe and Rose Aluvaala for your consistent prayers and encouragement.

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Contents

ABSTRACT

Background: Diarrhoea is a common childhood malady in developing countries but a majority of cases remain aetiologically undetermined. As such, the epidemiology of childhood diarrhoeal illness is not fully understood. This creates gaps in the attempts to institute prevention and control interventions.

Objective: To determine the prevalence of astroviruses among children below five years with acute diarrhoea.

Methodology: This study was nested within a cross-sectional study titled "Viral etiology of gastrointestinal infections in children in Nairobi". The study population comprised of children who were below five years of age who presented to the Kenyatta National hospital paediatric outpatient clinic. The sample size was 107 participants derived from the primary study's sample population; these participants were selected through random sampling. Stool samples were collected from these participants and molecular methods were used to determine the prevalence of astroviruses. Additionally, a structured case report form was used to extract the data from the records of the primary study. The data obtained was then analysed using SPSS software.

Results: The overall prevalence of astrovirus in children below five years with diarrhoea was found to be 3.7%. The highest prevalence was recorded in the age group between seven and twenty four months, among those who resided in urban areas and among those who consumed tap water. However, in spite of this observed trend no statistically significant association was found between astrovirus prevalence with age, sex, area of residence or water source in this study.

Conclusion: The prevalence of astrovirus in this age group in this study was found to be lower than what was found by a previous study carried in Kenya, but was comparable with other studies in Africa such as Tunisia and Madagascar.

CHAPTER ONE:

Introduction:

Diarrhoea remains a worldwide problem to date afflicting both adults and children. The causes of diarrhoea are variable and include viruses, bacteria, parasites and toxins. Its greatest impact is on children below five years of age. It is one of the leading causes of under-five child mortality. It accounts for 25% - 30% of under-five child deaths globally. By age five almost every child has suffered from several bouts of acute diarrhoea. Annual incidence of diarrhoea is 2.5 – 3.9 episodes per child [(1)]. Diarrhoea is a very important factor in childhood morbidity and mortality in the developing world; it is ranked second to respiratory diseases globally[(2)]. Its impact is greatest in developing countries where it accounts for approximately 1.2 million deaths annually[(3)]. In Kenya, the 2008/2009 Kenya Demographic and Health Survey (KDHS) ranked diarrhoea amongst the three primary causes of mortality in children below five years of age. Seventeen percent of the children in the survey had diarrhoea in the preceding two weeks.

Sub- Saharan Africa, South East Asia and parts of South America are the regions greatly burdened with cases acute diarrhoea in children below five years. These areas are afflicted with high rates of poverty, malnutrition, poor sanitation and inadequate supplies of safe water. Such conditions provide an environment conducive for pathogens causing diarrhoea to flourish. Diarrhoea illness and malnutrition work in synergy to worsen the children's health with increases nutrient losses and poor intake as a result of the illness. This increases the severity of illness contributing to high morbidity and mortality. It also compounds the poverty situation through lost working hours while caring for the sick children and diversion of the household resources towards healthcare costs. As such a vicious cycle is established.

In these regions access to appropriate healthcare is limited meaning there is a delay in accessing the life-saving treatment. Most of these children die from severe dehydration and

its accompanying complications. Improved sanitation, adequate clean water supplies, food safety practices and access to appropriate primary health care in industrialized regions have served to reduce the number of cases of diarrhoea.

Most health facilities in developing regions have inadequately equipped laboratory facilities. This specialised equipment and appropriately trained staff are primarily limited to research laboratories that are situated in the major cities and towns in the countries. As such, the pathogens responsible for the cases of diarrhoea go largely unidentified. This failure to determine the aetiological agents causing diarrhoea results in inadequate understanding of epidemiology of diarrhoea illness in Kenyan children and hampers the development and implementation of prevention and control interventions that are tailored to our local unique needs. Information on the bacteria, viruses and parasites causing diarrhoea in our setting would enable policy makers determine the usefulness of interventions developed in the western countries locally, for example vaccines. It would also serve as grounds to justify subsidization of the costs of the vaccines and inclusion in local vaccination programs. Surveillance is also very important; it facilitates determination of the circulating pathogens causing diarrhoea. This knowledge is important in the management and containment of epidemics of gastroenteritis.

Few studies have been carried out in Kenya and other regional countries in Africa to identify causes of acute diarrhoea; however their scope has been narrow with most focussing on rotavirus and bacteria. These studies reported that only a fraction of the cases of diarrhoea were as a result of the pathogens studied. This leaves an extensive untapped frontier in the determination of aetiology of diarrhoea locally and regionally.

This study is based in an urban setting which has many informal settlements where there is poverty, poor sanitation, inadequate supplies of safe water and limited access to primary

health care. Diarrhoeal illness is endemic in these environments; surveillance carried out here would increase our understanding of diarrhoea pathogens circulating in community.

CHAPTER TWO:

LITERATURE REVIEW:

Aetiology of diarrhoea:

Acute diarrhoea is defined as three or more episodes of loose stools in twenty-four hours for a period not lasting more than fourteen days. The magnitude of the problem associated with the illness varies with regions. In the industrialised countries cases of diarrhoea are lower than those in sub Saharan Africa and the Asian subcontinent. The predominant aetiological agents responsible for diarrhoeal illness also vary with geographical regions and age.

A study done in Tanzania demonstrated that viruses are of equal importance to bacteria in causation of diarrhoea in children below the age of five years[(4)]. There are many virus families capable of causing disease in humans. The four main families are Reoviridae (rotavirus), Caliciviridae (Norovirus, Sapovirus), Astroviridae (genus Mamastrovirus: astrovirus) and Adenoviridae (Adenovirus). Other viruses include coronaviruses, toroviruses, picobirnaviruses and cytomegaloviruses[(1,5)]. Viral diarrhoea is usually profuse watery with no erythrocytes or leucocytes. Its incubation period is usually short, two to three days from time of exposure and lasts less than two weeks. Viral shedding continues after resolution of the clinical illness for varying duration depending on the virus and the host's immune status.

Rotavirus is the only virus that has been extensively studied globally as a cause of acute diarrhoeal illness in children. As a result of the wealth of knowledge and understanding about this virus and its epidemiology measures to mitigate its effects have been developed including development of a vaccine that has been rolled out in many countries around the world. This goes to show that equal attention to other viral diarrhoea pathogens could result in enhanced interventions to reduce the resultant morbidity and mortality.

Astrovirus biology:

Astrovirus is an un-enveloped virus whose genome comprises positive sense single stranded RNA 6.8 – 7.3 kb in length. It is divided into three overlapping open reading frames (ORFs), ORF1a which encodes for a serine protease, ORF1b which encodes for the RNA dependent RNA polymerase (RdRp), and ORF2 which encodes for the capsid precursor protein. ORF1a and ORF1b are relatively conserved regions located at the 5' end whereas variations occur in the nucleotide sequence of ORF2 located at the 3' end; analysis of this region forms the basis of sero-typing[6]. Eight serotypes have so far been identified and named HAstV1 to HAstV8. This virus affects a diverse range of animals and birds, humans included.

Epidemiology of astrovirus:

The virus has a worldwide distribution causing diarrhoea in both adults and children. It causes both endemic diarrhoea and outbreaks of gastroenteritis depending on the clinical setting. It accounts for 4 – 10% of endemic diarrhoea in children in whom it causes a mild illness that is detected mostly in community based studies[7,8]. Outbreaks occur in paediatric wards and child care settings; astrovirus is an important agent of nosocomial gastroenteritis outbreaks accounting for 5 – 10%[9–11]. In immune-competent children it causes a mild illness that lasts two to three days. Symptoms comprise primarily diarrhoea with or without vomiting, abdominal pain or fever. Viral shedding persists for up to two weeks after resolution of clinical illness. A more severe disease occurs in immunocompromised patients such as those who are infected with HIV and the elderly living in homes for the aged. In these patients viral shedding in stool persists for as long as three months[9,11]. Treatment of the illness is supportive with fluid therapy and electrolyte supplementation in severe cases. Majority of cases resolve spontaneously prior to presentation to hospital.

Virus transmission:

The virus is transmitted via fecal – oral route either directly or contamination of food and water. The virus has been detected in water sources for human consumption and sewage samples in South Africa[7,11]. In France it was detected in 17% of oyster samples and 50% of mussel samples collected from an area impacted by human sewage. Shellfish contamination has been associated with outbreaks in Europe[11]. Virus transmission occurs throughout the year but has a peak in the cold months in temperate climate regions. Immunity post infection is only partial as a mild illness resulted in adult volunteers who were experimentally infected with the virus[12]. A vaccine is yet to be developed; so the current prevention measures involve removal of a common source of infection such as an infected food handler or a contaminated water source, and interruption of person – person spread through hand hygiene. Control of nosocomial outbreaks relies on isolation of cases and hand hygiene.

Virus distribution:

Studies conducted in different parts of the world have detected astrovirus in 1.4% - 17% of cases[10]. The prevalence varies with geographical region, age limits of the study population and sensitivity of the methods used to detect the virus. In Kenya, a study was carried out to determine the prevalence of astrovirus in children below ten years with acute diarrhoea between February 1999 and September 2005. They found it to be 6.3%. They further determined the prevalence in the sub-group of children aged below five years and determined it to be 5.3%. In this study, a comparison was done between the prevalence of astrovirus in children residing in an urban area versus those residing in a rural area and found a higher prevalence among the urban dwellers. The method used for detecting astrovirus in this Kenyan study was enzyme immunoassay[7].

The virus distribution from studies carried out in other regions is illustrated in the table below[8,10,13]:

COUNTRY (Year of study)	PREVALENCE (%)
TUNISIA (2009)	3.6
MADAGASCAR (2008)	2.1
INDIA (2006)	5.8
AUSTRALIA (2000)	3.7
ARGENTINA (2004)	3.7
CHINA (2007)	9.8
KENYA (2007)	6.3

Table 1: Astrovirus prevalence in different regions

The findings shown in the above table illustrate the varying prevalence of astrovirus with geographical location.

There is also a variation in age distribution of the virus depending on clinical setting. A hospital based study in France found a higher prevalence in seven months' old infants (median 7 – 7.5months), whereas a community based study found astrovirus to be commoner in children older than 3 years[(11)].

A study in Thailand found astrovirus to be present in a population of asymptomatic children albeit at a lower prevalence: 8.6% in children with acute diarrhoea and 2% in children without diarrhoea[(12)]. This is important because these asymptomatic children may serve as a reservoir for epidemics of gastroenteritis in their communities.

Serology:

The astrovirus serotypes are not uniformly distributed around the world. Most studies in which serotyping was carried out found HAstV 1 to be the predominant strain(9,11). However, other strains such HAstV8 are being detected with increasing frequency as in a

study carried out in Madagascar[9,13]. Co-circulation of several serotypes occurs in communities as demonstrated in a study that found different prevalence rates of several serotypes in Mexico[9]:

SEROTYPE	PREVALENCE (%)
HAstV1	10
HAstV2	42
HAstV4	23
HAstV3	13

Table 2: Distribution of astrovirus serotypes in Mexico

This concurrent circulation of several serotypes in a community may lead to recombination events. Recombinant pathogenic strains have been in reported in the US, Mexico and Kenya[6,11]. Novel strains labelled MLB1 and VA_2 were recently identified in a cohort study of children with diarrhoea residing in Egypt[14].

Increasing public health concern about viruses causing sporadic diarrhoea and gastroenteritis outbreaks together with the emergence of new strains has created need for improved surveillance and application of sensitive molecular assays to increase awareness of these enteric pathogens. This study will use a molecular assay to determine the prevalence of astrovirus as opposed to the earlier prevalence study that utilised enzyme immunoassay (EIA)[7].

Problem statement:

Acute diarrhoeal illness is one of the major causes of morbidity and mortality in children aged below five years, yet interventions to mitigate its effects are inadequate. This is due to paucity of information on the nature of the pathogens responsible and the epidemiology. Research to determine the aetiology of diarrhoeal illness in this population will play a vital role in the development of tools to reduce the impact of this problem amongst children below five years of age. It will result in a broadened understanding of these pathogens and how to control them.

Justification:

Despite the improvement in sanitation in industrialised countries and the roll out of rotavirus vaccine globally, cases of acute diarrhoea still present to hospitals for treatment. A review of published studies carried out in Africa on rotavirus reveal a median prevalence of 24%. This leaves a diagnostic gap in the remaining cases of acute non-bacterial diarrhoea. There is a need to elucidate the other causes of acute diarrhoea as such knowledge will have a positive impact on the development and implementation of interventions to mitigate the resultant morbidity and mortality. Maintaining vigilance through continued surveillance is also necessary because changes that occur in the circulating viruses.

Astrovirus has been found in studies carried out in some African countries to play a significant role in the causation of acute diarrhoea in both adults and children. This study sets out to determine its role in acute diarrhoea in young Kenyan children.

Research question:

Is the prevalence of astroviruses among children less than five years old in Kenya higher or lower than in other countries in the region? Is there an age and sex association with the incidence of infection?

Broad objective:

The aim of this study was to determine the prevalence of astrovirus among children less than five years of age who present with acute diarrhoea at Kenyatta National hospital paediatric outpatient clinic.

Specific objectives:

1. To determine the prevalence of astrovirus in children aged less than five years
2. To determine other factors associated with astrovirus infection status among children below five years of age

CHAPTER THREE

METHODOLOGY:

Study site:

The study was carried out at the Kenyatta National hospital where the participants were recruited from the paediatric outpatient clinic. Kenyatta National hospital is a referral hospital and as such receives large numbers of patients from different parts of the country. It also serves as a primary health care facility for a significant proportion of the population in Nairobi in the middle and lower socio-economic classes.

Study period:

Sample collection was carried out for a period of thirteen weeks from the 1/11/2010 to 30/1/2011.

Study population:

Children included in the study were those who had not yet celebrated their fifth birthday attending the paediatric outpatient clinic at Kenyatta National Hospital with acute diarrhoea. These children live in varying environmental conditions. Some come from slums which are informal settlements that are not linked to the main city's water and sewerage systems. As such they have poor sanitation and inadequate supplies of clean water. Others come from urban areas which are more organised settlements but are still prone to overcrowding and shared amenities such as water taps and toilets. Overcrowding in these settlements puts a strain on the water and sewerage infrastructure increasing the risk for cross contamination. Some of the participants also hailed from rural areas which lie outside of the geographical boundaries of the city of Nairobi.

Inclusion criteria:

- Age less than five years
- Signs and symptoms of diarrhoea with or without vomiting for a period of less than fourteen days
- Children whose parents or guidance willingly consented

Exclusion criteria:

- Children with bloody diarrhea
- Children above 5 years of age.
- Children whose parents declined or refused to consent

Ethical considerations:

This was a nested study and permission to use the primary data was obtained from the Principal Investigator of the primary study. The study was carried out under the ethical approval of the primary study that was obtained from the KNH-UON ethics review committee reference number P348/10/2010. In the primary study informed consent was obtained from the participants' parents/ guardian after an explanation of the purpose of the study. In the process of extracting secondary data all efforts were made to ensure confidentiality of the study participants. No identifier information was included in the data collected. The primary data was kept secure to ensure no unauthorised access to it.

Sample size calculation:

$$N = \frac{Z_{\alpha/2}^2 \cdot \pi(1-\pi)}{(\text{precision})^2} = \frac{1.96^2 \times 0.053 \times 0.947}{0.05^2} = 77$$ rounded off to 100, where

$Z_{\alpha/2} = 1.96$ = Z-score for 95% confidence interval

π = estimated prevalence from a local study(7).

Sample collection:

In the primary study, stools samples were collected from each child in a sealable plastic container and stored in a cool box. They were then transported to the laboratory where the stool was aliquoted into three separate tubes and frozen at - 80°C in freezers.

Laboratory methods:

One tube was used for RT-PCR on site at the University of Nairobi to detect astroviruses. Assays to determine other causes of acute diarrhoea were not carried out. Specimens were run in batches with appropriate negative and positive control specimens and appropriate quality assurance procedures.

Data collection:

The data for this study was collected as part of a larger study to determine viral aetiology of gastroenteritis in children in Nairobi. A structured case report form was used to extract demographic information on the child and details of the illness. The results from the tests were recorded on this form. An example is included in the appendix.

The sample size for the primary study was 150; of these, samples from 118 were assayed for astrovirus. In this sample sub-population, eleven participants with incomplete data were excluded leaving 107 participants.

Variables that were measured include age, sex, residence, water source and presence or absence of infection with astrovirus.

Data analysis:

Data collected comprised both categorical and quantitative data. The collected data was then entered into MS Access data base and cleaned for errors and inconsistent answers. All the data analyses were done using Statistical Package for Social Sciences (SPSS). Depending on the type of variable appropriate statistics were presented. Specifically, continuous data with a

near normal distribution was summarized and presented as mean and standard deviation, and data with skewed distribution expressed as median and inter-quartile range. Categorical variables and data with skewed distribution were analyzed by chi-squared and Mann-Whitney test respectively. For all the analyses the 5% level of significance with P values of less than 0.05 were considered to be significant.

CHAPTER FOUR:

RESULTS:

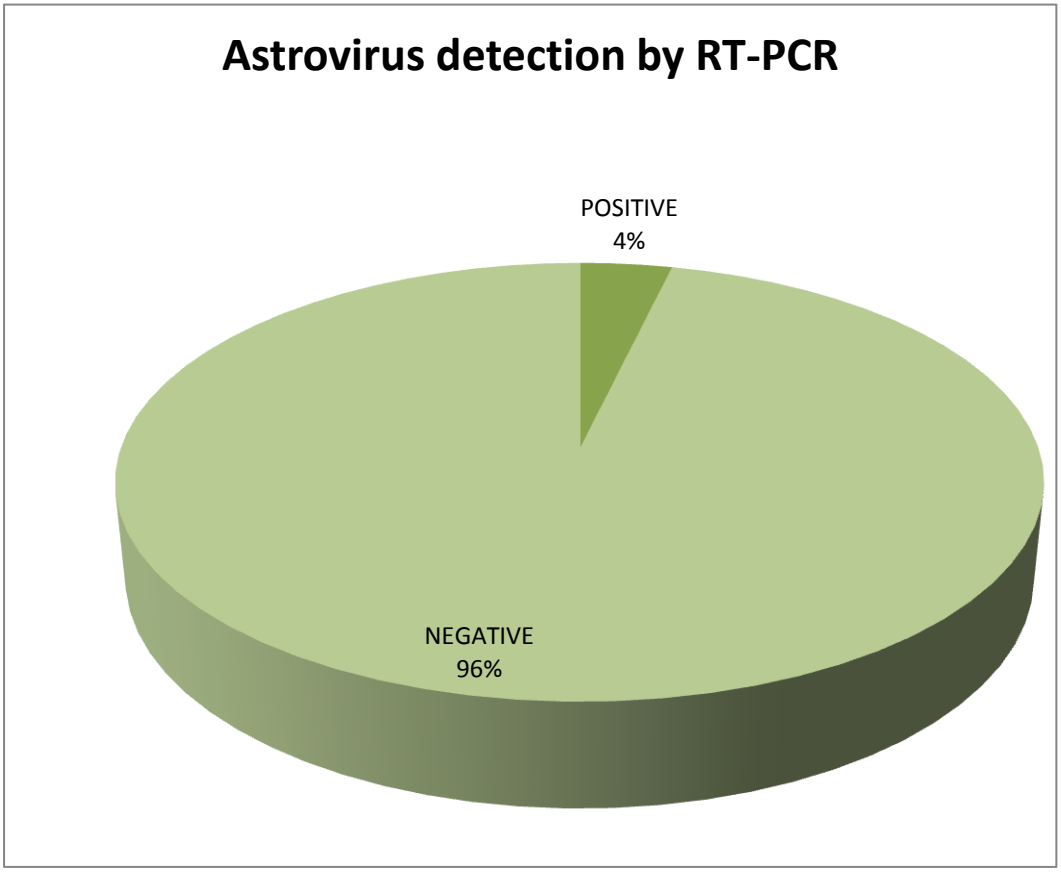
This study aimed at determining the prevalence of astrovirus infection in children below five years of age and establishes any factors that may be associated with astrovirus infection status. Data from 107 participants was analyzed. The age group between seven and twenty four months had the largest number of participants (64.5%), whereas those in the age group 0 – 6 months comprised 26.2% and those 25 – 59 months comprised 9.3% of the study population. The mean age of the study population was twelve months with a standard deviation of $\pm$ 8.7 months. There was little gender disparity within the study population (males: 48.6%, females: 51.4%). Majority of the participants were drawn from an urban setting (79.8%), while the rest came from rural and slum areas.

VARIABLE		**NUMBER (n)**	**PERCENTAGE (%)**
AGE GROUP	0- 6	28	26.2
(months)	7- 24	69	64.5
	25-59	10	9.3
SEX	Male	52	48.6
	Female	55	51.4
RESIDENCE	Urban	83	79.8
	Rural	10	9.6
	Slum	11	10.6
WATER SOURCE	Tap	85	74.5
	Vendor	15	13.16
	Well	14	12.28

Table 3: Description of study population

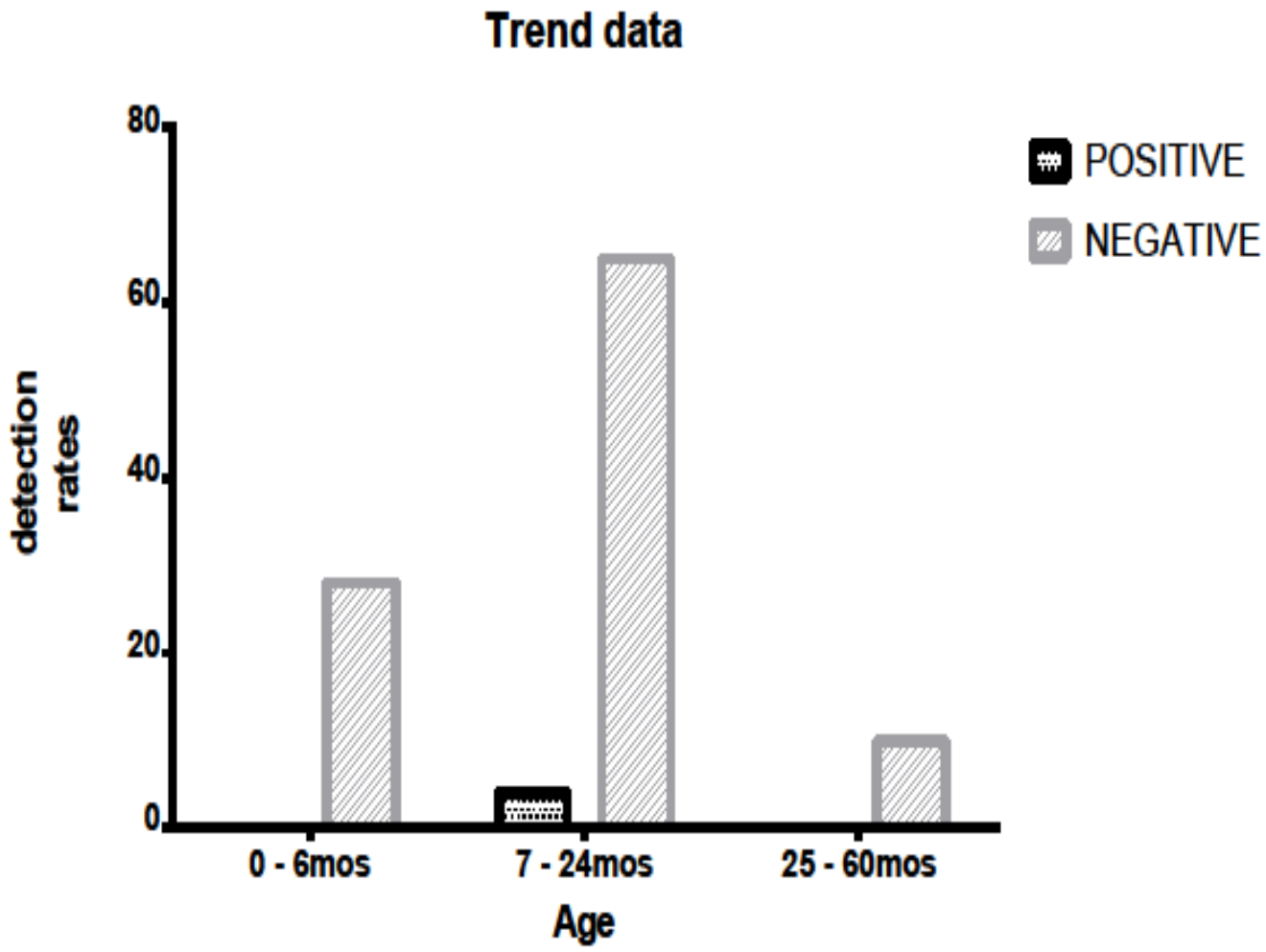
Astrovirus was detected in four out of the 107 cases assayed. This worked out to a prevalence of 3.7%.

Figure 1: Astrovirus prevalence in the study population



All four cases were detected in the 7 – 24 months age group comprising 5.8% of the cases of diarrhoea in this cluster. No cases of astrovirus were detected in the other two age groups.

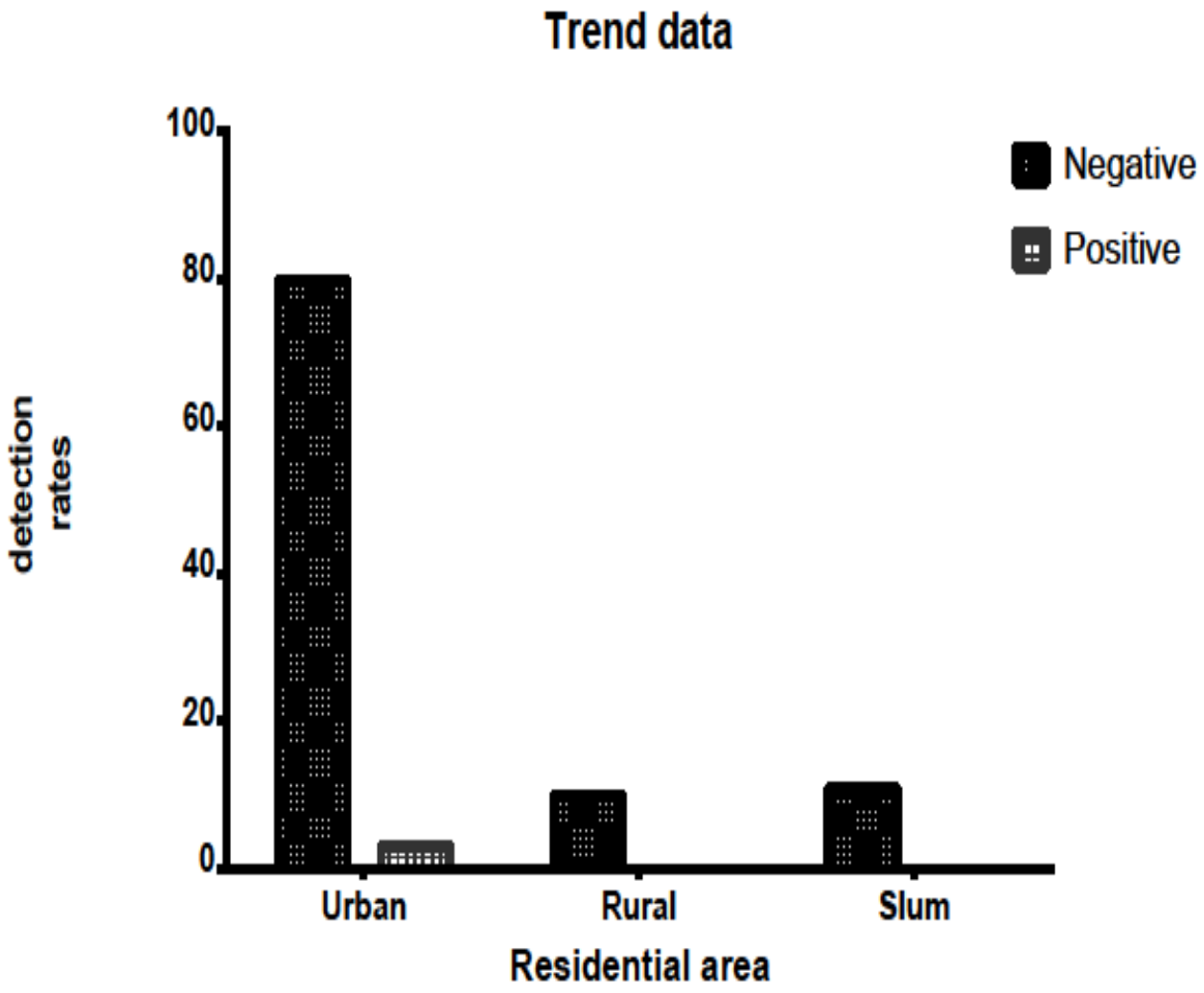
Figure 2: Detection of astrovirus by age in months



Three of the cases positive for astrovirus were from female participants who represented 5.5% of all cases of diarrhoea among this group; there was only 1 positive case in the male participants (2%). This difference was found not to be statistically significant (P-value = 0.336).

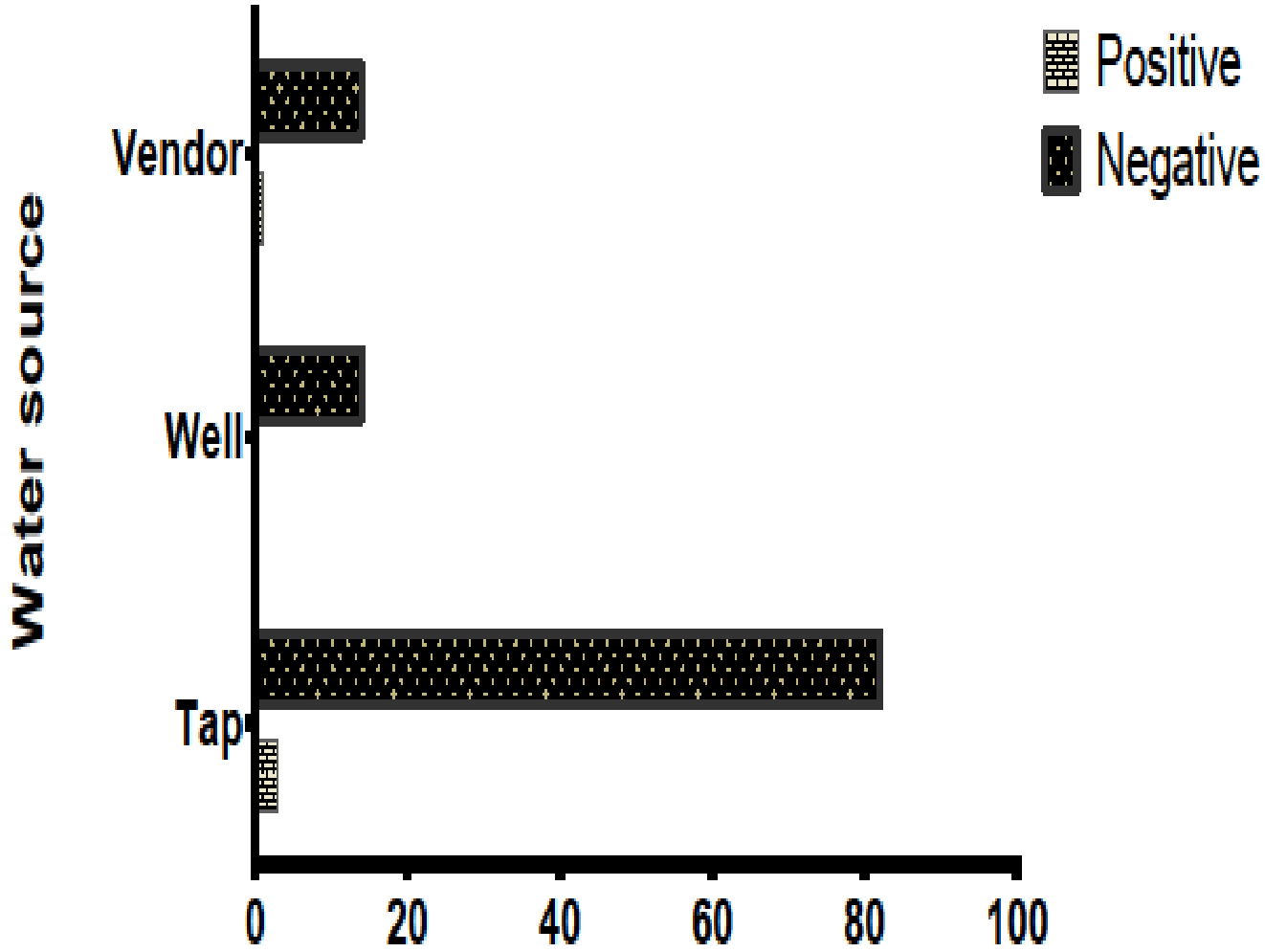
All the positive cases hailed from the urban area and comprised 3.6% of all the cases of diarrhoea from this setting. No astrovirus was detected from the participants residing in the slums or the rural areas. This finding was not of statistical significance however (p-value = 0.677).

Figure 3: Astrovirus prevalence by residence



A higher astrovirus detection rate was found among those who used tap water (75%) as compared to those who bought water from a vendor (25%) (P value = 0.622). No astrovirus was detected in the group that drew water from a well.

Figure 4: Comparison of astrovirus detection by water source



CHAPTER FIVE:

DISCUSSION:

The goal of this study was to determine the prevalence of astrovirus in children below five years with acute diarrhoea and elucidate any association between infection and other factors. Astrovirus infection is acquired via the faecal-oral route; as such this study was investigating the role environmental factors such as water source play in acquisition of infection.

Few studies have been carried out in Kenya prior to this one to determine prevalence of astrovirus as a pathogen causing acute diarrhoeal illness. In this study the prevalence of astrovirus was 3.7%. This finding was however lower than that found in an earlier study carried out in Kenya which found a prevalence rate of 6.3% in children below ten years of age. The prevalence in a sub-group of children aged below five years in this study was 5.3% [(7)]. This may attributable to the period when the study was carried out (1999 – 2005) after which some improvement in sanitation and hygiene may have resulted in a decrease in occurrence of infection. This association is yet to be ascertained. Another possible reason for the variation in detection rates between the two studies is the technique used for detection of astrovirus. The previous study used enzyme immunoassay (Dako IDEIA^{TM} astrovirus kit, DakoCytomation UK), which has a high sensitivity but lower specificity. This results in more false positives. In this study molecular techniques were used for astrovirus detection; this method as has high sensitivity and specificity which reduces the margin of error.

The study prevalence however compares with those from other studies from different parts of the world where it ranges between 1.4% and 17%. In Tunisia it was 3.6%, Madagascar 2.1%, Argentina 3.7%, Australia 3% and India 5.8% [(10)].

The low rate of detection in hospital outpatient departments may be due to the mild nature of illness that results from infection with this virus; most cases resolve spontaneously without need for medical intervention. Carrying out a community based survey may give a more accurate picture of the contribution of astrovirus causation of acute diarrhoea in children.

This study also determined the prevalence to be higher in the age group comprising children aged 7 – 24 months (5.8%). The prevalence by age was higher among the female participants (5.5%) as compared with the male contemporaries (2%). A higher prevalence was measured in the participants who hailed from the urban areas (3.6%) and among those who consumed tap water (75%).

Role of factors in astrovirus infection status:

A higher prevalence was found in the group aged 7 – 24 months, 5.8%, while the virus was not detected in the other age groups (p value >0.05). This was also the age group that had the highest number of cases of diarrhoea in the study group (64.5%). A possible reason for this difference is that in the group below 6months the children have passive immunity acquired from their mothers, whereas the children older than 24 months acquired immunity through prior exposure to the virus. The finding was similar to a hospital based study carried out in France which found a higher prevalence in the median age of 7 months [(11)]. This was a variation from a community based study in the same country which found a higher prevalence in children aged 3 years.

Females were more prone to infection with astrovirus (5.5%) as compared to their male counterparts in whom the virus was detected in 2% (p value >0.05). This differs from a previous study carried out in Brazil which found no difference in infection rates between the two genders [(15)].

Astrovirus infection was found predominantly amongst the urban dwellers (3.6%) as opposed to those who hailed from the rural areas and the slums, among whom the virus was not detected (p value >0.05). The participants from the urban area comprised the larger proportion of the study population (79.8%) which may have influenced the higher detection rates of atsrovirus in this sub-group. This finding is similar to that in the Kenyan study where the prevalence of astrovirus in the children from the urban set up was 5.8% whereas that in the children from the rural setup was 3.7% [(7)].

The participants who consumed tap water were more likely to be infected with astrovirus (75%) than those who bought water from a vendor (25%),or those who drew water from a well (p > 0.05). The study participants are drawn from the lower middle and low socio-economic areas; as such they reside in areas prone to overcrowding and communal sharing of amenities such as water taps and toilets. This creates an environment for transmission of pathogens through the fecal – oral route.

Conclusion:

These findings confirm that astrovirus plays a role in the causation of acute diarrhoeal illness in children albeit to a smaller extent than viruses like rotavirus and norovirus. This highlights the need to carry out investigations to profile the causes of acute diarrhoea in children.

Despite the numerical differences found in the occurrence of astrovirus infection with age, sex, water source and residence these were found not to be of statistical significance. This indicates that none of these factors influenced the likelihood of infection with astrovirus in this population.

Limitations:

- Due to limited resources typing of the strains detected was not carried out.
- The study was based at one site and in a hospital; this may have played a role in low prevalence detected and as such may not be an accurate representation of the true prevalence of astrovirus in this population of children
- The use of secondary data limited the factors that could be measured for an association with astrovirus infection status.

Recommendations:

This study determined that astrovirus plays a role in causation of acute diarrhoea but also reveals an existing knowledge gap in the aetiology of acute diarrhoea in children aged below five years. It is therefore a recommendation that a survey should be carried in the community on a larger scale to identify more diarrhoea causing viruses in circulation. This should be coupled with typing of the strains in the different virus families. There should also be continued surveillance of these viruses to detect new emerging strains in the population.

The information would be important for the implementation of new and more effective interventions to mitigate the impact of diarrhoeal illness on the vulnerable populations.

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APPENDIX I:

CASE REPORT FORM

STUDY NO.: _____

1. AGE GROUP:
    a. 0 – 6 MONTHS _____
    b. 7 – 24 MONTHS _____
    c. 25 – 59 MONTHS _____
2. SEX:
    a. MALE _____
    b. FEMALE _____
3. RESIDENCE:
    a. URBAN: _____
    b. SLUM: _____
    c. RURAL: _____
4. ASTROVIRUS STATUS:
    a. POSITIVE: _____
    b. NEGATIVE: _____