PREVALENCE OF ROTAVIRUSES AND ADENOVIRUSES ASSOCIATED WITH DIARRHOEA AND NUTRITIONAL STATUS OF CHILDREN AGED 0-5 YEARS OLD IN KATSINA STATE, NIGERIA

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AUGUST, 2015

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AUGUST, 2015

Declaration

I hereby declare that the work in this thesis entitled **"PREVALENCE OF ROTAVIRUSES AND ADENOVIRUSES ASSOCIATED WITH DIARRHOEA AND NUTRITIONAL STATUS OF CHILDREN AGED 0-5 YEARS OLD IN KATSINA STATE, NIGERIA"** was carried out by me in the Department of Microbiology, under the supervision of Dr. M. Aminu Mukhtar and Prof. S.E Yakubu. The information derived from the literature has been duly acknowledged and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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Gambo, Mukhtar Lawal.	Signature	Date

Certification

This thesis entitled **"PREVALENCE OF ROTAVIRUSES AND ADENOVIRUSES ASSOCIATED WITH DIARRHOEA AND NUTRITIONAL STATUS OF CHILDREN AGED 0-5 YEARS OLD IN KATSINA STATE, NIGERIA"** by GAMBO, MUKHTAR LAWAL meets the regulations governing the award of Master of Science in Microbiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This thesis is dedicated to my dear parents, Mal. Gambo Lawal and Haj. Binta A. Aliyu (Mrs).

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Abstract

Malnutrition and diarrhoeal morbidity continues to be a public health problem of considerable magnitude in most developing countries; therefore, a study on the prevalence of some diarrhoea associated enteric viruses and nutritional status of children aged 0-5 years old was undertaken in Katsina State, Northwestern Nigeria where there is little or no information. A total of 400 (322 diarrhoiec and 78 non-diarrhoiec) stool specimens were collected from children attending six hospitals located across the three senatorial zones of the state from June 2013 to April 2014. Their socio-demographic information, anthropometric measurements and clinical presentations were also noted with the aid of questionnaire. Viral antigens were detected by enzyme linked immunosorbent assay (ELISA) and all the ELISA positive samples were examined by negative staining electron microscopy. Rotavirus was detected in 5.3% of the diarrhoiec and none in the non-diarrhoiec specimens while adenovirus was detected in 12.4% of the diarrhoiec and 5.1% of the non-diarrhoiec specimens. Generally, children < 2 years old were more vulnerable to rotavirus and adenovirus infection. There was significant association ($p<0.05$) between dehydration and rotavirus and adenovirus infections. There was a significant association between source of drinking water ($p<0.05$) and rotavirus infection. There was however, no significant association between sex of child, socio-economic status, mother's level of education, toilet type, and previous history of diarrhoea and rotavirus and adenovirus infection. Rotavirus and Adenovirus particles were observed via Negative Staining Electron Microscopy. Weight-for- Age Z-Scores (WAZ), Height-for-Age Z-Scores (HAZ) and Weight-for-Height Z-scores (WHZ) were used to estimate the children nutritional status. The Z-scores were determined using the WHO anthropometric Software version 3.0. Overall prevalence of underweight, stunting and wasting among the study population was 59.0, 47.0 and 46.3% respectively. Prevalence of underweight, stunting and wasting among the diarrhoiec children was 63.3, 53.0 and 46.8% respectively. Underweight was the most prevalent nutritional problem identified. Generally, children < 2 years old were more undernourished and prevalence of undernutrition was higher in girls than in boys. The present study indicates that children < 2 years old were more vulnerable to rotavirus and adenovirus infections and were more undernourished. There is further need for a national survey to be undertaken so as to ascertain the magnitude of diarrhoea and malnutrition among this group. This will help in establishing effective strategies for the prevention, control and management of diarrhoea and malnutrition caused by rotavirus and adenovirus respectively.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Study Background

Acute infective gastroenteritis is a major global health problem which manifests as three or more watery or loose bowel evacuations in a 24 h period that may last several days, with fever and vomiting. Children under 5 years of age are particularly susceptible, and global estimates indicated a mean of between 3.5 and 7 episodes of severe diarrhoea during the first 2 years of life, and the greatest burden is in the developing countries because of poor sanitation, lack of safe drinking water, and bad sanitary habits (WHO, 2009).

Diarrhoeal disease is a leading cause of child mortality and morbidity in the world, and mostly results from contaminated food and water sources. Worldwide, 780 million individuals lack access to improved drinking-water and 2.5 billion lack improved sanitation. Diarrhoea due to infection is widespread throughout developing countries. In developing countries, children under three years old experience on average three episodes of diarrhoea every year. Each episode deprives the child of the nutrition necessary for growth. As a result, diarrhoea is a major cause of malnutrition, and malnourished children are more likely to fall ill from diarrhoea (WHO, 2009).

Diarrhoea is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene (WHO, 2009). Among these causative agents of diarrhoea, viral diarrhoea is associated with severe disease and hospitalization and is one of the major causes of mortality in children in sub-Saharan Africa (Estes, 2001).

Rotavirus has long been acknowledged to be a major etiological agent of gastroenteritis and responsible for a large proportion of morbidity and mortality associated with diarrhoeal illnesses (Parashar *et al.,* 2003). Recent estimates generated by Parashar and colleagues attribute 527 000 deaths in children less than five years of age to rotavirus annually, with 145 000 occurring in sub-Saharan Africa. This is likely to be under-estimation because only hospital-based studies have been considered. In Nigeria, a high incidence of childhood diarrhoea is estimated to account for over 160 000 of all deaths in children less than 5 years of age annually and of this number approximately 20% are associated with rotavirus infection (Parashar *et al.,* 2003).

Adenoviruses belonging to the Mastadenovirus genus in the family Adenoviridae cause a variety of diseases and are prevalent throughout the world. Adenoviruses, particularly enteric adenoviruses (EAds) type 40 (Ad40) and type 41(Ad41), can cause acute and severe diarrhoea in young children worldwide (Seranti *et al.,* 2004).

Nutrition plays a major role in maintaining health and malnutrition appears to generate vulnerability to a wide variety of diseases and general ill-health (Garba and Mbofung, 2010). The potential impact of diarrhoea on nutritional status through the negative impacts of stool losses, vomiting, anorexia, withholding of food, and the catabolic effect of infection are well accepted and the synergistic interactions of diarrhoea and malnutrition are well recognized (Gracey 2013). On the other hand, growth assessment is the single measurement that best describes the health and nutritional status of children; because disturbances in health and nutrition, regardless of their etiology, invariably affect child growth. Anthropometry has become a practical tool for evaluating the nutritional status of populations, particularly of children in developing countries (Hakeem *et al.,* 2004) and nutritional status is the best indicator of the global well-being of children (Onis *et al.,* 2000).

1.2 Statement of Problem

Diarrhoeal diseases are a major source of morbidity and mortality among young children in developing countries. They have assumed a very special significance in the developing world, where they are responsible for 15-30% of deaths in children less than 5 years of age (Kosek *et al.*, 2003). Diarrhoea in association with malnutrition constitutes major cause of morbidity and mortality and the second leading cause of death in children. Rotaviruses remain the major cause of morbidity and mortality in developing countries and are associated with approximately 400,000-600,000 deaths annually in infants and young children under 5 years of age (Miller and McCann, 2000; WHO, 2005a).

In sub-Saharan Africa, recent estimations show that rotavirus infection is responsible for the death of approximately 110,000-150,000 children under 5 years old annually (Molbak *et al*., 2001; Jain *et al*., 2001; Glass *et al*., 2005). Rotavirus infection is associated with 33,000 deaths in children less than 5 years of age annually in Nigeria (Parashar *et al*., 2003). Nigeria was recently ranked third among the 10 countries with the greatest number of rotavirus disease-associated deaths per year in the same age group (Glass *et al*., 2005).

In Nigeria, few available studies conducted in the Southern and North central regions have associated adenoviruses with 3.8%and 6.7% of pediatric diarrhoea. In Northwestern Nigeria, there is little or no information on the epidemiology of EAds which some studies have implicated as the second most important viral agent associated with gastroenteritis in children (Nimzing *et al*., 2000).

One of the major global health problem faced by the developing countries, today is malnutrition (UNICEF 2004, WHO 2004). Of course, Nigeria too, is not an exception to this problem of malnutrition (Abidoye *et al., al* 2001; Udunayo *et al.*, 2006). Children who die from diarrhoea often suffer from underlying malnutrition, which makes them more vulnerable to diarrhoea. Each diarrhoeal episode, in turn, makes their malnutrition even worse. Diarrhoea is a leading cause of malnutrition in children under five years of age (WHO, 2013).

Worldwide, about a half of mortalities in children are directly or indirectly attributable to malnutrition (Sufyan *et al.,* 2012). While nearly 12 million children under five die each year in developing countries mainly from preventable causes, the death of over 6 million (55%), are either directly or in directly attributable to malnutrition; mainly under nutrition (UNICEF 2012).

In Nigeria, malnutrition has been reported to be associated with increased morbidity and mortality, such that 30-40% of deaths in the preschool age group are associated with malnutrition. The major causes of childhood mortality in Nigeria include malaria (30%), vaccine preventable diseases (22%), diarrhoea 19%), acute respiratory tract infections (16%), etc., with malnutrition underlying about 60% of these childhood deaths (FMOH 2006).

1.3 Justification

At present, the widespread occurrence of infectious diarrhoea has become one of the major public health problems worldwide (WH0, 2009). Therefore, a rapid response, which includes identification of the pathogens and prevention of the spread of these pathogens in the community, is crucial for the control of disease outbreak and case investigations (Yuanhai *et al.*, 2008).

The number of diarrhoeal episodes in the first two years of life has been shown not only to affect growth but also fitness, cognitive function, and school performance (Torres *et al.,* 2000). These suggest that diarrhoea is of great public significance and therefore increased attention should be given to its study in children aged less than 5 years.

Child malnutrition is a major contributor to the burden of disease in developing countries. For this reason, a better understanding of the relationship between diarrhoea and malnutrition will help in the development of effective prevention programmes.

Understanding the nutritional status of children has far-reaching implications for promoting the health of future generations.

1.4 Aim

The aim of the study is to determine the prevalence of rotaviruses and adenoviruses associated with diarrhoea and the nutritional status of children aged 0-5 years in Katsina State, Nigeria.

1.5 Specific objectives

The specific objectives of this study are to:

(1) Determine the prevalence of rotaviruses and adenoviruses in children with diarrhoea in the study area.

(2) Examine the ELISA positive samples for rotavirus and adenovirus particles using negative staining electron microscopy.

(3) Determine some of the risk factors associated with diarrhoea in the study area.

(4) Determine the nutritional status of children in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diarrhoea

Diarrhoea is generally defined as the occurrence of three or more loose liquid or watery stools or at least one bloody loose stool in a 24-h period (Gondwe *et al.,* 2005). There are three major forms of diarrhoea namely: acute watery diarrhoea, acute bloody diarrhoea and persistent diarrhoea (Guarrant *et al.,* 2001). Acute watery diarrhoea is diarrhoea with a high volume of watery stool occurring over a period of less than 14 days. It usually results in severe dehydration if intervention measures are not sought. Acute bloody diarrhoea is diarrhoea manifested by loose or watery stools with the shedding of red blood cells. Persistent diarrhoea is usually associated with loose or watery stools with or without visible blood occurring over a period of 14 days.

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, "pasty" stools by breastfed babies. Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene (WHO, 2009). Diarrhoea can last several days, and can leave the body without the water and salts that are necessary for survival. The most severe threat posed by diarrhoea is dehydration. During a diarrhoeal episode, water and electrolytes (sodium, chloride, potassium and bicarbonate) are lost through liquid stools, vomit, sweat, urine and breathingng.

Diarrhoea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual. Though most episodes of childhood diarrhoea are mild, acute cases can lead to significant fluid loss and dehydration, which may result in death or other severe consequences if fluids are not replaced at the first sign of diarrhoea.

Most people who die from diarrhoea actually die from severe dehydration and fluid loss (WHO, 2009). The loss of fluids through diarrhoea can cause severe dehydration which is one cause of death in diarrhoea patients. Along with water, patients also lose large amounts of important salts, electrolytes, and other nutrients (Alam and Ashraf, 2003). The major determinants of diarrhoeal deaths are dehydration and malnutrition (Gondwe *et al.*, 2005).

2.1.1 Global Burden of Diarrhoea Illnesses

Diarrhoea remains the second leading cause of death among children under five globally. Nearly one in five child deaths – about 1.5 million each year – is due to diarrhoea. It kills more young children than AIDS, malaria and measles combined (WHO, 2004).

Each year, an estimated 2.5 billion cases of diarrhoea occur among children under five years of age, and estimates suggest that overall incidence has remained relatively stable over the past two decades. More than half of these cases are in Africa and South Asia where bouts of diarrhoea are more likely to result in death or other severe outcomes. The incidence of diarrhoeal diseases varies greatly with the seasons and a child's age. The youngest children are most vulnerable: Incidence is highest in the first two years of life and declines as a child grows older (Black *et al*., 2003).

Diarrhoeal illnesses are constantly ranked as one of the top six causes of all deaths, one of the top three causes of death from an infectious disease, and one of the top two causes of death each year in the under –five in developing countries (Walker *et al.*, 2005).

Mortality from diarrhoea has declined over the past two decades from an estimated 5 million deaths among children under five to 1.5 million deaths in 2004, which parallel downward trends in overall under-five mortality during this period.

Despite these declines, diarrhoea remains the second most common cause of death among children under five globally, following closely behind pneumonia, the leading killer of young children. Together, pneumonia and diarrhoea account for an estimated 40 per cent of all child deaths around the world each year (UNICEF/WHO, 2009).

Diarrhoeal diseases have assumed a very special significance in the developing world, where they are responsible for 15-30% of deaths in children less than 5 years of age (Kosek *et al.,* 2003). The impact of diarrhoea illnesses is more severely felt in sub-Saharan Africa where of the 25 million children born each year, 4.3 million are expected to die by the age of 5 years and about 20% of these deaths will be from diarrhoea (Cunliffe *et al.*, 1998). As a sub-Saharan African country, Nigeria not surprising has an appalling under-five mortality rate. The UNICEF ranked Nigeria in 2000 as 17^{th} by the under-five mortality rate with diarrhoea accounting for 20-26% all mortality (Black *et al.*, 2003; Parashar *et al.*, 2003; UNICEF, 2005).

2.1.2 Causes of Diarrhoea

Diarrhoea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria, viruses and protozoa. However, just a handful of organisms are responsible for most acute cases of childhood diarrhoea. Among these causative agents of diarrhoea, viral diarrhoea is associated with severe disease and hospitalization and is one of the major causes of mortality in children in sub-Saharan Africa. The major viral aetiological agents of gastroenteritis in humans are: rotaviruses (*Reoviridae*), astroviruses (*Astroviridae*), adenoviruses (*Adenoviridae*) and caliciviruses (*Caliciviridae*) (Estes, 2001). Rotavirus is the leading cause of acute diarrhoea, and is responsible for about 40 per cent of all hospital admissions due to diarrhoea among children under five worldwide.

The major bacterial pathogens include *Escherichia coli*, *Shigella*, *Campylobacter* and *Salmonella*, along with *Vibrio cholerae* during epidemics. Among these bacterial pathogens of diarrhoeal diseases, the most commonly implicated in the endemic form of childhood diarrhoea on a global scale are strains of *Escherichia coli* (*E. coli*). At least six groups of diarrhoeagenic strains of *E. coli* are described to cause diarrhoea. These are enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enterotoxigenic *E. coli* (ETEC) (WGO, 2008). Among these strains of *E. coli*, EPEC is an important cause of infantile diarrhoea in developing countries (Robins-Browne *et al*., 2004). In Nigeria, EHEC and EAEC have been shown to be significantly associated with diarrhoea (Iruka *et al*., 2003).

The important protozoan species implicated in diarrhoea illnesses are; *Entamoeba histolytica, Giardia lamblia and Cryptosporidium parvum.* Among these parasitic causes of diarrhoea, *Cryptosporidium parvum* is increasingly recognised as the major pathogen responsible for parasitic diarrhoea in both developed and developing areas in immunocompetent as well as immunocompromised individuals (WGO, 2008).

2.1.3 Management and Control of Diarrhoea

The latest recommendations for treating childhood diarrhoea in the developing world are set out in a UNICEF and WHO joint statement issued in 2004. These interventions are proven, affordable and relatively straightforward to implement. Since the 1970s, oral rehydration therapy has been the cornerstone of treatment programmes to prevent life-threatening dehydration associated with diarrhoea. It is estimated that in the 1990s, more than 1 million deaths related to diarrhoea may have been prevented each year, largely attributable to the promotion and use of these therapies.

However, treatment recommendations have changed over time to reflect a better understanding of what works to reduce child deaths from diarrhoea as well as new insights into treatment feasibility.

The revised recommendations formulated by UNICEF and WHO in collaboration with the United States Agency for International Development (USAID) and experts worldwide, emphasize family and community understanding of preventing, recognizing and appropriately managing diarrhoea. When they become routine practice, caretakers will act quickly at the first sign of diarrhoea, rather than waiting before treating the child (UNICEF/ WHO, 2004).

Mothers and other caregivers should improve hygiene through hand washing with soap, increase use of improved sources of drinking water and sanitation facilities, prevent dehydration through the early administration of increased amounts of appropriate fluids available in the home, and ORS solution, if on hand, provide children with 20 mg per day of zinc supplementation for 10-14 days as part of the diarrhoea treatment, promote exclusive breastfeeding and increase breastfeeding and all feeding during and after the episode of acute diarrhoea, recognize the signs of dehydration and take the child to a health care provider for ORS or intravenous electrolyte solution, as well as familiarize themselves with other symptoms requiring medical treatment (e.g. bloody diarrhoea) (UNICEF/ WHO, 2004).

2.2 Viral Agents of Gastroenteritis

The major viral aetiological agents of gastroenteritis in humans are rotaviruses (Reoviridae), adenoviruses (Adenoviridae), astroviruses (Astroviridae), and caliciviruses (Caliciviridae) (Estes, 2001).

2.2.1 Rotavirus

2.2.1.1 Morphology/Structure

The term rotavirus is derived from the Latin word "rota," meaning wheel. Rotaviruses are non-enveloped viruses with icosahedral symmetry. Their electron microscopic appearance shows a 60-80nm wheel with radiating spokes (Baron, 1996). The intact virus is made up of 3-capsid structures: an inner core, an immediate capsid and an outer capsid with short radiating spikes (Figure 2.1).

The viral genome is structured in 11 segments of double-stranded RNA. Each segment encodes structural and non-structural viral proteins (Morris *et al.*, 2001). Major structural proteins are the outer structural proteins VP7 and VP4 which have antigenic properties. VP4 is the viral hemagglutinin and forms spikes from the surface. The middle capsid is made up of VP6 which forms more than 50% of the virion. The inner structural protein core is composed of VP1, VP2 and VP3 proteins. The VP3 and VP6 proteins are necessary for the RNA transcription and for a correct viral structure ((Morris *et al*., 2001). In addition to the 6 structural proteins, there were identified 5 nonstructural proteins (NSP 1-5).

2.2.1.2 Classification and Antigenic Types

Rotavirus is a genus in the family Reoviridae. Rotaviruses have three important antigenic specificities: group, subgroup, and serotype, which are determined by the proteins of the viral capsid (Coluchi *et al.*, 2002). There are seven different groups (A to G) based on the antigenicity (each group shares common antigens) and the electrophoretic mobility of their RNA segments (Figure 2.2). Groups D, E and F have not been found in humans.

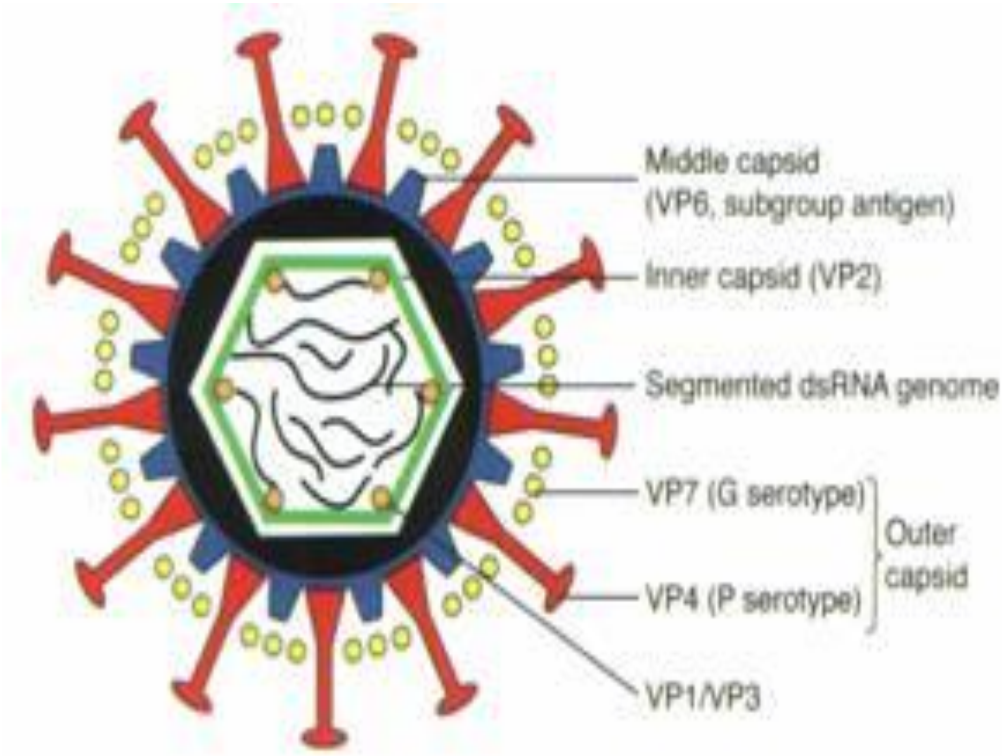

Figure 2.1: Schematic representation of the rotavirus virion (Cunliff *et al.*, 2002).

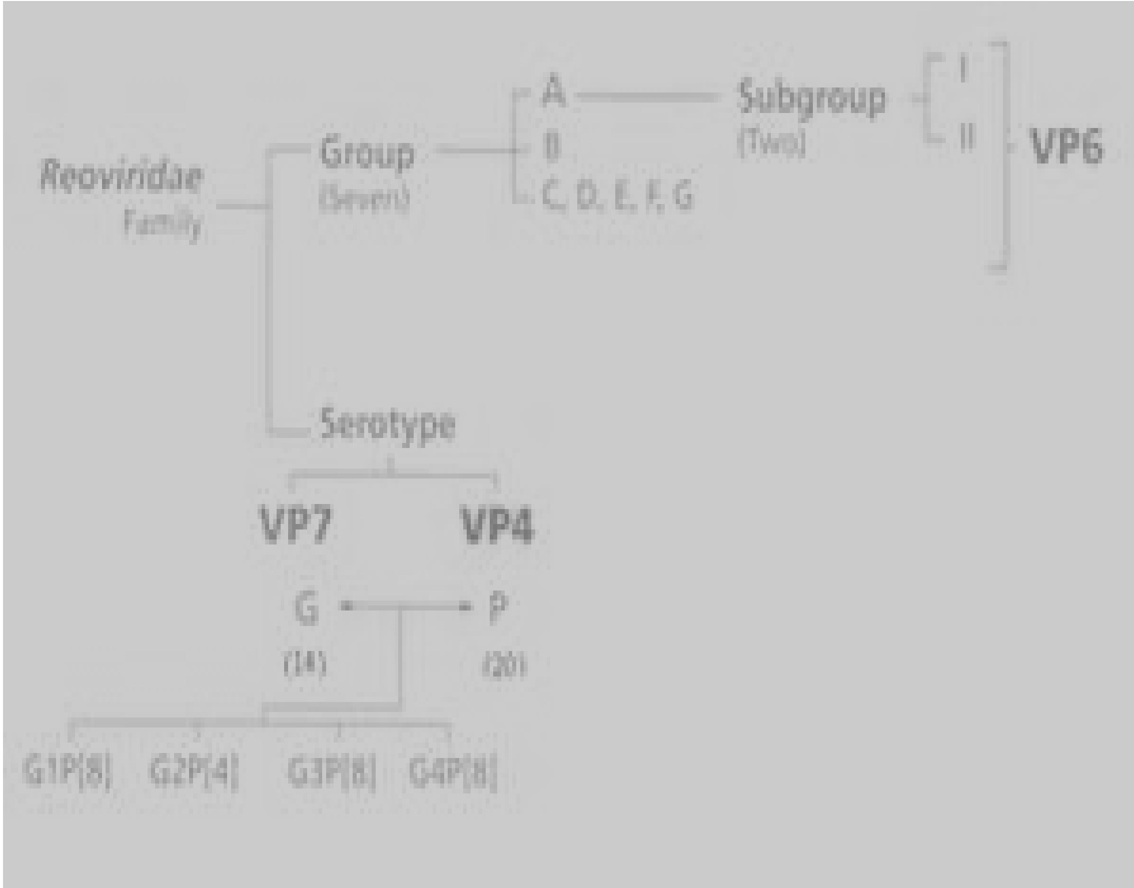

Figure 2.2: Classification of rotaviruses, modification (Coluchi *et al.*, 2002).

Within the A serotype, depending on the proteins of the outer capsid, there were described P serotypes (depending on the VP4 protein) and G serotypes (depending on the VP7 protein). So far, there were identified 20 P serotypes and 14 G serotypes which, for man, are pathogens: P 4, P 6, P 8, P 9 and G 1- G 4. Both proteins, VP4 and VP7, stimulate the synthesis of neutralizing antibodies and can be involved in protective immunity; they also represent important targets for the vaccine production (Coluchi *et al.*, 2002). A large variety of rotaviruses can result from different combinations of G and P serotypes which infect people. Only 4 strains are more spread: G 1 P[8], G 2 P[4], G 3 P[8], G 4 P[8]. Most of the illnesses are attributed to the G 1 P[8] type (Gentech *et al.*, 1996). Important studies have shown that these 4 strains account for more than 88% of all rotavirus infections (Santos and Hoshino, 2005).

2.2.1.3 Transmission and Pathogenesis

The rotaviruses are highly contagious and the main transmission mode is the fecal-oral route (Parashar *et al.,* 1998). Since the virus is stable in the environment, the transmission can occur through person-to-person spread, ingestion of contaminated water or food, through contact with contaminated surfaces, such as toys or food preparation counters (Dennehey, 2000).

From clinical studies, the incubation period of rotavirus diarrhoeal illness was estimated to be less than 48 hours. Large numbers of virus particles are shed in the stool following multiplication in epithelial cells of the small intestine. Shedding may persist for 10 days or more after the illness, but peak shedding appears to occur within 8 days of illness. Affected host cells are mature enterocytes lining the middle and upper end of the intestinal villi. In laboratory animals, hepatocytes are also infected. The infectious particle is thought to be an "intermediate sub-viral particle" (ISVP).

The viral attachment protein is probably exposed after protease digestion in the GI tract removes some or all of the outer capsid protein (VP4). Rotaviruses replicate in the host cell cytoplasm. Virions enter the host cell by endocytosis and viral mRNA is transcribed using the viral RNA polymerase that is already present in the virion to form structural protein units of the capsid. The mRNA segments are assembled into the immature capsid and then replicated to form the double stranded RNA genome. Large amounts of viral particles are shed in diarrhoeal stools (Baron, 1996).

2.2.1.4 Clinical Manifestations of Rotavirus Diarrhoea

The incubation period for rotavirus diarrhoea is short, usually less than 48 hours. The clinical manifestations of infection vary and depend on whether it is the first infection or reinfection. Rotaviruses induce a clinical illness characterized by vomiting, diarrhoea, abdominal discomfort, fever, and dehydration (or a combination of some of these symptoms) that occurs primarily in infants and young children and may lead to hospitalization for rehydration therapy. Fever and vomiting frequently precede the onset of diarrhoea. Infection may be asymptomatic, may cause self-limited watery diarrhoea, or may result in severe dehydrating diarrhoea with fever and vomiting. Up to one-third of infected children may have a temperature greater than 102°F (39°C).The gastrointestinal symptoms generally resolve in 3-7 days. The duration of hospitalization ranges from 2 to 14 days with a mean of 4 days. The highest attack rate is usually among infants and young children 6 to 24 months old and the next highest in infants less than 6 months old.

It has been reported that rotavirus diarrhoea is frequently associated with dehydration (Pennap and Umoh, (2010) and Parashar *et al*., (2006), vomiting and fever (Aminu *et al.*, 2008). Rotavirus disease is more severe than diarrhoea caused by other enteric pathogens (Albano *et al*., 2007), with symptoms including an average of six stools per day (Castello *et* *al.*, 2006), severe dehydration which is 14 times more frequent in children with rotavirus diarrhoea than among those with the disease from other causes (Kapikian and Channock, 1996) and vomiting and fever (Nguyen *et al.*, 2007; Aminu *et al.*, 2008; Ahmed *et al.*, 2009). Vomiting followed by fever appears to be more common with rotavirus diarrhoea. Rotavirus excretion was highest when all three symptoms (diarrhoea, fever and vomiting) occurred in the same child (7.5%) and lower when 2 symptoms occurred together (diarrhoea and vomiting) with 3.8%, diarrhoea and fever with 1.3% and lowest when diarrhoea occurred alone with 1.3% (Junaid *et al.*, 2011).

Pennap and Umoh (2010) in a study in Zaria reported the most common clinical features as dehydration (1.9%), vomiting and fever (41.3%), vomiting (34.6%), fever (19.2%), respiratory symptom (1.9%), others (2.9%). Watery stool is typical of rotavirus diarrhoea. Several studies have reported highest occurrence of watery stool among rotavirus infected children. Pennap and Umoh, (2010) reported that; of the 104 rotavirus positive diarrheic children, the most frequent type of stools observed were watery stool (65.4%) and watery and mucoid stool (18.3%). Aminu (2006) and Junaid *et al*, (2011) have also reported the occurrence of different stool types with watery stool being the most frequent.

2.2.1.5 Epidemiology

Rotavirus is highly transmissible and virtually all children will have experienced at least one rotavirus infection by the age of 5 years (White *et al.*, 2008). Re-infection is also common (Fischer *et al.*, 2002), although previous infections reduce the risk of severe disease (White *et al.*, 2008). Adults are also known to experience rotavirus diarrhoea (Paul *et al.*, 2008).

Rotavirus infection is the single most important cause of infectious, severe, dehydrating diarrhoea and death globally in children aged 5 years and below (Ahmed *et al.*, 2009; Mast *et al*., 2009; Dhama *et al*., 2009), and continues to have a great impact on childhood morbidity and mortality (Dennehy, 2008).

Each year rotavirus infection leads to about 600000 deaths globally (Parashar *et al*., 2006; Mast *et al*., 2009) with more than 85% of these deaths occurring in Africa and Asia (CDC, 2008). In 2004, six countries, including Nigeria, accounted for more than half of all rotavirus diarrhoea deaths in children under 5 years of age (Ahmed *et al*., 2009).

In sub-Saharan Africa, recent estimations show that rotavirus infection is responsible for the death of approximately 110,000-150,000 children under 5 years old annually (Molbak *et al*., 2001; Jain *et al*., 2001; Glass *et al*., 2005). Rotavirus infection is associated with 33,000 deaths in children less than 5 years of age annually in Nigeria (Parashar *et al*., 2003). Nigeria was recently ranked third among the 10 countries with the greatest number of rotavirus disease-associated deaths per year in the same age group (Glass *et al*., 2005).
Rotavirus has long been acknowledged to be a major etiological agent of gastroenteritis and responsible for a large proportion of morbidity and mortality associated with diarrhoeal illnesses (Parashar *et al.*, 2003). Studies published between 1986 and 1999 indicated that rotavirus cause approximately 22% (range 17%-28%) of childhood diarrhoea hospitalizations. From 2000 to 2004, this proportion increased to 39% (range 29%-45%). Application of this proportion to the recent World Health Organization (WHO) estimates of diarrhoea-related childhood deaths gave an estimated 611,000 (range 454,000-705,000) rotavirus-related deaths (Parashar *et al*., 2006) indicating increase in the incidence of rotaviruses worldwide. In epidemiological studies conducted in developing countries, rotavirus accounted for a median of 8% of all diarrhoeal episodes, 28% of outpatients or clinic visits for diarrhoea, and 34% of hospitalizations of young children for diarrhoea (WHO, 2005a).

There are quite a number of studies regarding the epidemiology of rotavirus in West Africa (Asmah *et al*., 2001, Akran *et al*., 2002). Some hospital-based studies (Audu *et al*., 2002; and Pennap *et al*., 2002) have shown rotaviruses to be associated with 3.6-24.2% of childhood diarrhoea in Nigeria. Prevalence of 9.0% in Northwestern Nigeria, 11.0% in Jos, 11.9% in Maiduguri, 16.3% in Zaria, 13.0% in Ibadan and 13.8% in Jos, (Aminu *et al*., 2008; Nimzing *et al.*, 2000; Adah *et al.*, 2001; Pennap and Umoh 2010; Ojeh *et al*., 1995; Junaid *et al.*, 2011) have been reported.

Rotaviruses are ubiquitous and 95% of children worldwide are infected by three to five years of age (Parashar *et al*., 1998). Because natural infection confers some immunity to disease and protection increases with each subsequent exposure, the highest rates of rotavirus disease occur early in life, between three months and two years of age (WHO, 2005). A number of studies in Nigeria (Anochie *et al*., 2013; Junaid *et al*., 2011;Aminu *et al*., 2008; Audu *et al*., 2002) and most parts of the world (Basu *et al*., 2003; Kargar *et al*., 2012; Almusawi *et al.*, 2013; Magzoub *et al.*, 2013) have reported higher infection rate in children less than two years of age. Furthermore, some studies have found the peak incidence at 6-12 and 9-14 months. Anochie *et al* (2013) in a study in Lagos have reported the highest prevalence of rotavirus infection in the age-group 13-24 months. In Jos, Nigeria, 90.9% of children with rotavirus gastroenteritis were under 2 years of age with a peak of infection in children 7-12 months old (Junaid *et al*., 2011). In South Western Iran, a peak was found in 7-12 months old with 37.2% prevalence (Kajbaf *et al*., 2013). However, in Sudan, the peak incidence of rotavirus disease occurs in 3-12 months old (Magzoub *et al*., 2013) while François *et al.*, (2009) on their study on incidence and clinical presentation of infantile rotavirus diarrhoea in Sierra Leone found the peak incidence of rotavirus infection in children 3-9 months old.

2.2.1.6 Diagnosis

The clinical features and stool characteristics of rotavirus diarrhoea are nonspecific, and similar illness may be caused by other pathogens. As a result, confirmation of a diarrhoeal illness as rotavirus requires laboratory testing. Because the clinical manifestations of rotavirus gastroenteritis are not distinct enough to permit a specific diagnosis, specimens must be examined in the laboratory. Therefore, it requires the detection of virus or viral antigen and/or demonstration of a serologic response (Estes, 2001). The most common specimen used in diagnosis is the stool, although the virus has also been found in sera, cerebrospinal fluid and throat swabs (Ushijima *et al.*, 1994). Numerous methods to detect rotavirus in stool and rectal swab specimens have been described. Since the early 1980s, by means of rapid diagnosis techniques such as ELISA or EIA, the viral antigen (capsid protein) has been detected in stool specimens. The most widely available method for confirmation of rotavirus infection is detection of rotavirus antigen in stool by enzyme immunoassay (EIA). Several commercial test kits are available that detect an antigen common to human rotaviruses. These kits are simple to use, inexpensive, and very sensitive. Other techniques (such as electron microscopy, reverse transcription polymerase chain reaction, nucleic acid hybridization, sequence analysis, and culture) are used primarily in research settings. Rotavirus antigen has also been identified in the serum of patients 3–7 days after disease onset, but at present, routine diagnostic testing is based primarily on testing of faecal specimens (Jawetz *et al*., 2007).

2.2.1.7 Treatment, Prevention and Control

The primary aim of treatment of rotavirus gastroenteritis is the replacement by the intravenous or oral route of fluids and electrolytes lost by vomiting or diarrhoea. Oral rehydration is more readily available and has gained widespread use worldwide as a life-saving treatment. In patients with severe dehydration and shock, intravenous rehydration is indicated for efficient replacement of fluid loss. The oral rehydration therapy is recommended for the mild and medium forms of the disease; in severe forms of dehydration, parenteral and then oral rehydration is recommended. The rehydration therapy treats the disease symptoms and not the cause and does not reduce the spread of the virus to other individuals (Subbotina *et al*., 2003).

As with all viral infections, antibiotics are not indicated, and at present there are no efficient antiviral agents against rotaviruses. Passive immunity from the parentally transferred maternal antibodies and breast-feeding plays an important role in the protection against the occurrence of the rotavirus disease in young infants (Singer *et al*., 2010).

Rotavirus vaccine protects against the moderate and severe forms of disease, it also prevent deaths, hospitalization, and reduce morbidity and the associated socio-economic costs. At present, there are two types of vaccine with an increased efficiency and demonstrated safety. RotaTeq (Merck&Co) is a recombined, pentavalent, human-bovine vaccine, with a live virus and an oral administration. It contains 5 human–bovine recombined strains. Each strain contains gens of the bovine (WC_3 strain) and human virus which encodes either the VP4 (P_1A) protein, or the VP7 (G1, G2, G3 and G4) protein. Both surface proteins (VP4 and VP7) stimulate the production of specific neutralizing antibodies. Rota Teq is administered in 3 oral doses in the first 6 months of life, at a time interval of at least 4 weeks between the doses; the first dose is administered in the 6 and 12 weeks of life interval (Singer *et al.*, 2010).

Rotarix (GlaxoSmithKline) is a vaccine with a human monovalent, live-attenuated virus. It was approved in more than 63 countries and will be available in many others. It was developed by using cloning and passaging in cell cultures from a precursor- attenuated strain 89-12, which was initially obtained from a child infected with rotavirus in Cincinnati.

This strain 89-12 has the G_1P_1A serotype (genotype P[8]) (Berstein *et al.*, 1999). Although Rotarix contains only 1 $G_1P[8]$ type, it shares protecting epitopes, including those involved in neutralizing the virus, with numerous other types of human rotavirus belonging to other G types. This is due, first of all, to the presence of 11 human genes and the common epitopes of the VP4 protein which was identified as the neutralizing immune-dominant protein which occurs after the Rotarix inoculation (Ward, 2003). 2 oral doses are administered, in the first 6 months of life. The first dose can be administered from as early as 6 weeks of age, while the second after a minimum interval of 4 weeks. The vaccination must be completed by the age of 24 weeks (Singer *et al.*, 2010).

2.2.2 Adenovirus

Adenoviruses belonging to the Mastadenovirus genus in the family Adenoviridae cause a variety of diseases and are prevalent throughout the world. Common clinical manifestations resulting from adenovirus infection include pneumonia, cystitis, conjunctivitis, diarrhoea, hepatitis, myocarditis, intussusceptions and encephalitis (Baum *et al.*, 2000; Seranti *et al.*, 2004).

Adenoviruses are increasingly recognized as agents of life-threatening infection in immunocompromised patients, particularly in human immunodeficiency virus positive individuals and allogeneic bone marrow transplant recipients often with very high mortality (Slatter *et al.*, 2005). To date, 51 human adenovirus serotypes that are divided into six species (A to F) based on their ability to agglutinate various types of erythrocytes are distinguished. Although, adenoviruses are more often associated with respiratory infections, some serotypes, denominated enteric adenoviruses (EAds) are related to diarrhoeal disease. Adenoviruses, particularly enteric adenoviruses (EAds) type 40 (Ad40) and type 41(Ad41), can cause acute and severe diarrhoea in young children worldwide

(Seranti *et al.,* 2004). More so, Ad40 and Ad41 primarily affect the gut, contributing to 5%-20% of hospitalizations.

2.2.2.1 Structure of Adenovirus particle

Adenoviruses are 70–90 nm in diameter and display icosahedral symmetry, with capsids composed of 252 capsomeres. There is no envelope. Adenoviruses contain 13% DNA and 80% protein. The particle has an estimated molecular weight of 150–180 x 10^6. Adenoviruses are unique among icosahedral viruses in that they have a structure called a "fiber" projecting from each of the 12 vertices, or penton bases (Figure 2.3). The rest of the capsid is composed of 240 hexon capsomeres.

The hexons, pentons, and fibers constitute the major adenovirus antigens important in viral classification and disease diagnosis (Jawetz *et al.*, 2007).

2.2.2.2 Epidemiology of Adenoviruses in Diarrheoa Illnesses

Adenoviruses are often recovered from the stools of young children and are considered to be the second most important viral agent associated with infantile gastroenteritis (Grimwood *et al*., 1995; Audu *et al*., 2002; Basu *et al*., 2003; Oh *et al*., 2003). Further studies indicate that adenoviruses are associated with 4-15% of all hospitalized cases of viral gastroenteritis (Grimwood *et al.,* 1995). Adenoviruses have been associated with 3.1% to 22.2% of cases of paediatric gastroenteritis in studies from Australia, Europe, and North America (Brown, 1990; Grimwood *et al*., 1995; Hársi *et al*., 1995). 14% was reported in Bangladesh (Kim *et al*., 1990), 13% in Mexico (Maldonado *et al.,* 1998), 16.2% in Khartoum (Elhag *et al.*, 2013). In Africa and other parts of the world, lower prevalence of 3.8% in Jos, Nigeria (Nimzing *et al.,* 2000), 7.8% in Gaborone (Basu *et al.*, 2003), 5% in Northern France (Tran *et al.*, 2010), and 3.7% in Najra region, Saudi Arabia (AlAyed *et al.*, 2013) have been reported.

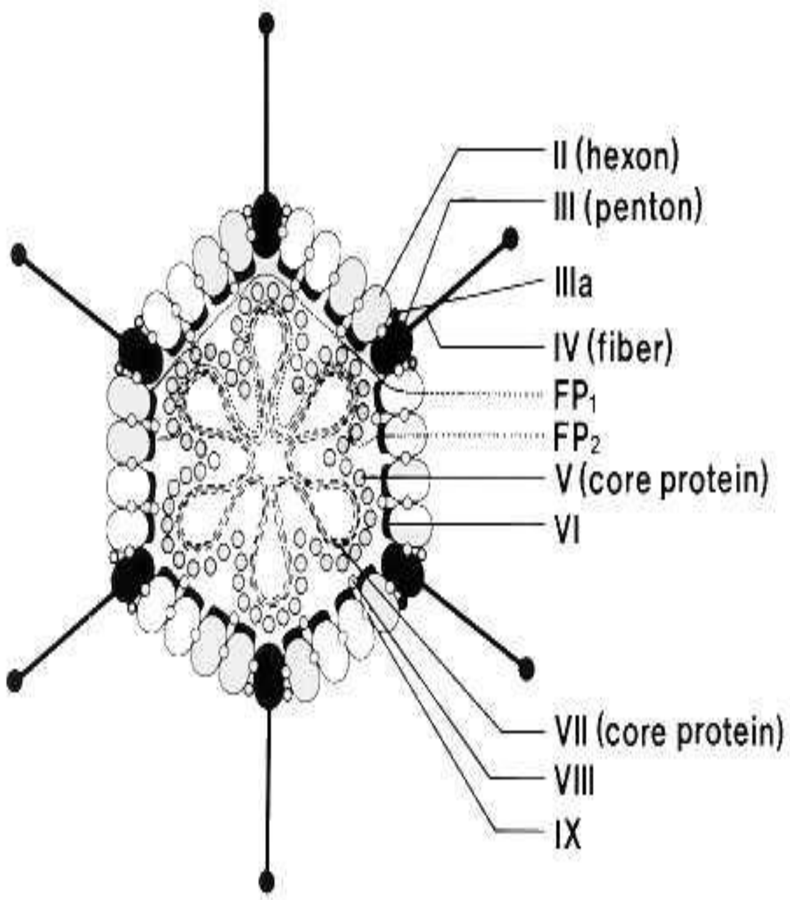

Figure 2.3: Structural model of the adenovirus virion (Brown *et al*., 1975).

In Nigeria, few available studies conducted in the Southern and North central regions have associated adenoviruses with 3.8% (Nimzimg *et al.*, 2000) and 6.7% (Audu *et al.*, 2002) of pediatric diarrhoea. In Northwestern Nigeria, however, a higher prevalence of 22.3% in Nigeria has been reported (Aminu *et al.*, 2007). Some studies have implicated enteric adenoviruses as the second most important viral agent associated with gastroenteritis in children (Nimzing *et al.*, 2000).

2.2.2.3 Transmission and Clinical Features of Adenovirus Diarrhoea

Adenovirus transmission in diarrhoeal illnesses is believed to be by faecal-oral route. Adenoviruses have an incubation period of 8-10 days, followed by viral shedding for an approximate period of 7-14 days (Narayan and Albrecht, 2011).

The main symptoms are diarrhoea and vomiting, however, fever is also seen in 40-90% of the cases. Diarrhoea resulting from enteric adenoviruses is longer in duration than that caused by rotaviruses, usually lasting 7-8 days (Grimwood *et al.*, 1995). This is the leading reason why patients seek medical attention for this condition.

2.3 DIARRHOEA AND MALNUTRITION

One of the major global health problem faced by the developing countries, today is malnutrition (UNICEF 2004, WHO 2004). Of course, Nigeria too, is not an exception to this problem of malnutrition (Abidoye *et al., al* 2001; Udunayo *et al.*, 2006). Children who die from diarrhoea often suffer from underlying malnutrition, which makes them more vulnerable to diarrhoea. Each diarrhoeal episode, in turn, makes their malnutrition even worse. Diarrhoea is a leading cause of malnutrition in children under 5 years of age (WHO, 2009).

In developing countries, children under three years old experience on average three episodes of diarrhoea every year. Each episode deprives the child of the nutrition necessary for growth. As a result, diarrhoea is a major cause of malnutrition, and malnourished children are more likely to fall ill from diarrhoea. The potential impact of diarrhoea on nutritional status through the negative impacts of stool losses, vomiting, anorexia, withholding of food, and the catabolic effect of infection are well accepted and the synergistic interactions of diarrhoea and malnutrition are well recognized (Gracey, 2013). A study from Colombia estimates that the average two to eight diarrhoeal episodes per year experienced by children in developing countries during the first 3 years of life, causes a total negative effect on body height of between 2.5 and 10 cm at 3 years of age (Lutter *et. al*, 1989). The influence of malnutrition on diarrhoeal episodes seems to be variable. A community based, prospective study of more than 700 children in a rural, lowland area of Bangladesh showed a diarrhoeal incidence of 4.6 episodes per year. About three-quarters of the children were below 2 standard deviations ("Z" scores) underweight and height for age and about one third were below - 2"Z" scores for weight-for height. About 20% of the children were anergic, and these children had a 50% increased incidence of diarrhoea (Baqui *et. al*, 1993).

Malnutrition has been noted as important in increasing the severity of the clinical manifestation of human rotavirus infection. It has been observed that repeated diarrhoea enhances malnutrition by damaging intestinal mucosa thus making absorption difficult over an extended period (Mata *et al*., 1982). Generally this viral infection shows a high morbidity but relatively low mortality rates. This fact can be attributed to the development in the 1940s of replacement for body fluids and electrolytes usually lost in course of the illness. This improvement in therapy showed a marked decrease in childhood diarrhoea mortality rates (Kapikian and Chanock, 1996). However, Dodet *et al*. (1997) concluded that the greater severity of infection in developing countries is associated with concomitant malnutrition, malabsorption, zinc and vitamin A deficiencies and the synergistic action of mixed infection with other pathogens.

Persistent diarrhoea is part of a vicious cycle between nutrition, poverty, poor hygiene, environmental contamination, inappropriate feeding practices and early weaning. The association between the immune system and the gut is important for the development of malnutrition (Ochoa *et al.,* 2004) and when parents refrain from taking their children, with diarrhoea to a health facility to be treated, the risk for the development of malnutrition increases (Abate *et al*., 2001). Persistent diarrhoea also affects growth and intellectual function (Ochoa *et al.,* 2004).

The relationship between diarrhoea and malnutrition is bidirectional: diarrhoea leads to malnutrition while malnutrition aggravates the course of diarrhoea. On the one hand, severe and prolonged episodes of diarrhoea cause malnutrition in individual patients; on the other hand, malnourished children are more likely to develop complications with diarrhoea. Many studies, addressing the relationship between diarrhoea and malnutrition, have demonstrated a detrimental effect of diarrhoea on nutritional status. The contribution of diarrhoea to growth failure in children in developing countries was estimated to be as high as 25–30%. The same effect is, however, not seen in children in developed countries. This lack of effect may be due to the lower burden of disease in developed countries, the better nutrition of children in these countries, and bias in measuring episodes of morbidity (Nel, 2010).

2.3.1 Pathophysiology

Many factors contribute to the detrimental effect of diarrhoea on nutritional status. Reduced intake (due to anorexia, vomiting, and with-holding of feeds), decreased absorption of nutritients, maldigestion, increased nutrient losses, and the effects of the inflammatory response are some of the factors involved (Nel, 2010). Childhood infections are frequently associated with reduced dietary intake. This effect is most prominent in children with diarrhoea. Nutrient intake may decline by 30% or more during the first days of acute diarrhoea (Taiwo *et al.,* 2012). Overall nutrient absorption is also reduced by about 30% during acute diarrhoea, the impairment being greater for fats and proteins than for carbohydrates. Greater impairment can occur in undernourished children with persistent diarrhoea, reflecting more extensive damage to the gut mucosa. Acute diarrhoea causes increased losses of fluid and electrolytes. These are accompanied by varying degrees of nutrient maldigestion, malabsorption and losses. Children with high stool losses have significant malabsorption of protein, carbohydrates, and fat. Additionally, enteric protein losses are often increased. Studies of children with acute and persistent diarrhoea in developing countries have increased our knowledge of these effects (Taiwo *et al.,* 2012).

2.4 Anthropometry

Anthropometry has become a practical tool for evaluating the nutritional status of populations, particularly of children in developing countries (Hakeem *et., al* 2004) and nutritional status is the best indicator of the global well-being of children (Onis *et., al* 2000). Anthropometry is the single most universally applicable, inexpensive and non-invasive method available to assess the size, proportion and composition of human body (WHO, 1995). According to WHO (2005b); the ultimate intention of nutritional assessment is to improve human health.

Nutritional status can be measured using anthropometric measurements even in less advanced cases of malnutrition. The benefit of these measurements is that they are less invasive and costly than biochemical evaluation (Zere and McIntyre, 2003). The nutritional status of children under five years is one of the best predictors of child survival (WHO, 2005b).

The choice of which anthropometric measurements to use depends on their simplicity, accuracy and sensitivity. The availability of measuring instruments and the existence of reference standards for comparison are also important. International or universal standards, such as the NCHS and newer WHO standards for children under five years can be used because of the following: most children have the potential to grow the same regardless of ethnic background; the relationship of weight and height stays relatively constant in healthy children and the reference standards are not an ideal or target but just used for comparison (Torun and Chew, 1994; Torun, 2006). Both the NCHS and WHO standards use SD from the median and the results are referred to as Z-scores. A child who has Z-scores within -+1SD is within the normal range. Children with the lower portion of these ranges are classified as “moderately malnourished”. Children who are more than 3SD below the normal have severe malnutrition. The accepted anthropometric cut-off for the diagnosis of undernutrition is –2SD (Z-score) and indicates an increased risk of morbidity and mortality (WHO, 2006).

The new WHO reference standards can be used globally and came into effect in April 2006. Six countries growth standards were used to develop these standards, whereas the NCHS standards were only based on the standards of one country. The main idea of the new WHO standards is to see how children should be growing for the best health outcome, rather than just showing how the average child is growing. The new standards also take into consideration the use of length and height and body mass index (BMI), which was never used in the NCHS standards. The growth charts therefore include length or height for age, weight for age and weight for length or height. The growth charts are also available for boys and girls, infants to one year and children to five years and the BMI of infants to five years of age. The standards used were for healthy, breastfeeding children and their growth patterns. The new WHO standards also look at the milestones that children should reach at specific ages, whereas milestones were not part of the NCHS standards (WHO, 2006).

The three combinations of anthropometric measurements that are usually used to categorize malnutrition are: low weight-for-age, an indicator of underweight; low height-for- age, an indicator of stunting; and low weight-for-height, an indicator of wasting.

2.4.1 Underweight (low weight-for-age)

Low weight-for-age index identifies the condition of being underweight, for a specific age. The advantage of this index is that it reflects both past (chronic) and/or present (acute) undernutrition. Undernourished children are more likely to suffer from diarrhoea and its consequences, which, in turn, increase their chances of worsening nutritional status. Today, 129 million children under the age of five in the developing world are underweight for their age. More than 4 out of 5 children who are underweight for their age live in Africa or South Asia. Globally, more than one third of deaths among children under five are attributable to undernutrition (WHO, 2004). Together, Africa and South Asia account for more than 80 per cent of total underweight children (25 and 57%, respectively) (UNICEF/WHO, 2012).

2.4.2 Stunting (low height-for- age)

Stunting is an indication of the height of the child compared to the height of a normal child of the same age (Golden, 2000).Stunting is a greater problem than underweight and wasting usually in infants and children younger than five years (UNICEF, 2009). Stunting is the first clinical sign of malnutrition and affects about 195 million children younger than 5 years in the developing world, where stunting affects about one in three children in Africa (UNICEF, 2009).

A “stunted” child is small for his or her appropriate height for age. A height-for-age smaller than 85% of the median (50%) represents an SD score of minus (-) 3SD and is classified as severe stunting (Williams, 2005). The worldwide variation of the prevalence of low height for-age is considerable, ranging from 5-65% among the less developed countries (de Onis *et al.*, 1993). Globally, an estimated 165 million children under-five years of age, or 26%, were stunted (i.e height-for-age below –2 SD). More than 90% of the world’s stunted children live in Africa and Asia (United Nations Children’s Fund, 2009). About 40 per cent of children under five years of age are stunted in Africa, and nearly half in South Asia.

There exist variations in the patterns of childhood stunting within Nigeria. Victor *et al.*, (2013) used data from birth histories including the 2008 Nigerian Demographic and Health Survey to estimate childhood stunting in the 36 states of Nigeria including the Federal Capital Territory. They reported that childhood stunting in Nigeria on the average was as high as 39% and ranged from 11.5% in Anambra state to as high as 60% in Kebbi State. A prevalence of 41% among children under five has also been reported in Nigeria (UNICEF, 2009).

2.4.3 Wasting (low-weight-for height)

Wasting is indicated as a low weight for height, occurring at any age and is used as an indicator for identifying severe acute malnutrition (UNICEF, 2009). Wasting is the weight of the sick child compared to that of a normal child of the same height (Duccan and Golden, 2000). Typically, the prevalence of low weight-for-height shows a peak in the second year of life. Of the children younger than five years old in the developing countries, 13% are wasted and 5% are severely wasted (about 26 million). Africa and Asia are the two countries with high rates of wasting and exceed 15%. Out of 134 countries, 32 of these countries have wasting prevalence of 10% or more. And ten countries are contributing to about 60% of all wasted children (UNICEF, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Katsina State (Figure 3.1). Katsina State, located at the extreme northern margin of Nigeria, covers a total area of about 23,938sqkm (3,370sq) with a total population of 5,801,584 people, going by 2006 census (FGN 2007). It lies between latitudes 11°08'N and 13°22'N and longitudes 6°52'E and 9°20'E. The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. Katsina State has predominantly Hausa-Fulani indigenes. About 75% of the people are farmers and others are traders and livestock owners. There are also some other Nigerian settlers from Southern Nigeria especially the Yorubas and Ibos in the state.

The state has varying climate with cool dry season (harmattan) from October to February, hot dry season from March to May and a warm wet season from June to September. Water supply in Katsina State is sourced through damming of rivers and digging of wells and boreholes. The state has thirty four (34) local governments. The local governments are divided into three (3) senatorial zones according to their geographical locations, Namely; Funtua zone (South), Katsina zone (Central), and Daura zone (North) (Dauda *et al.*, 2011).

3.2 Study Population

The study population consisted of children between 0-5 years of age with or without diarrhoea. Those without diarrhoea served as control. Two hospitals were selected from each of the three senatorial districts of the state.

The hospitals selected for the studies were; General Hospital Funtua (Katsina South), Children's and Maternity Hospital Malumfashi (Katsina South),

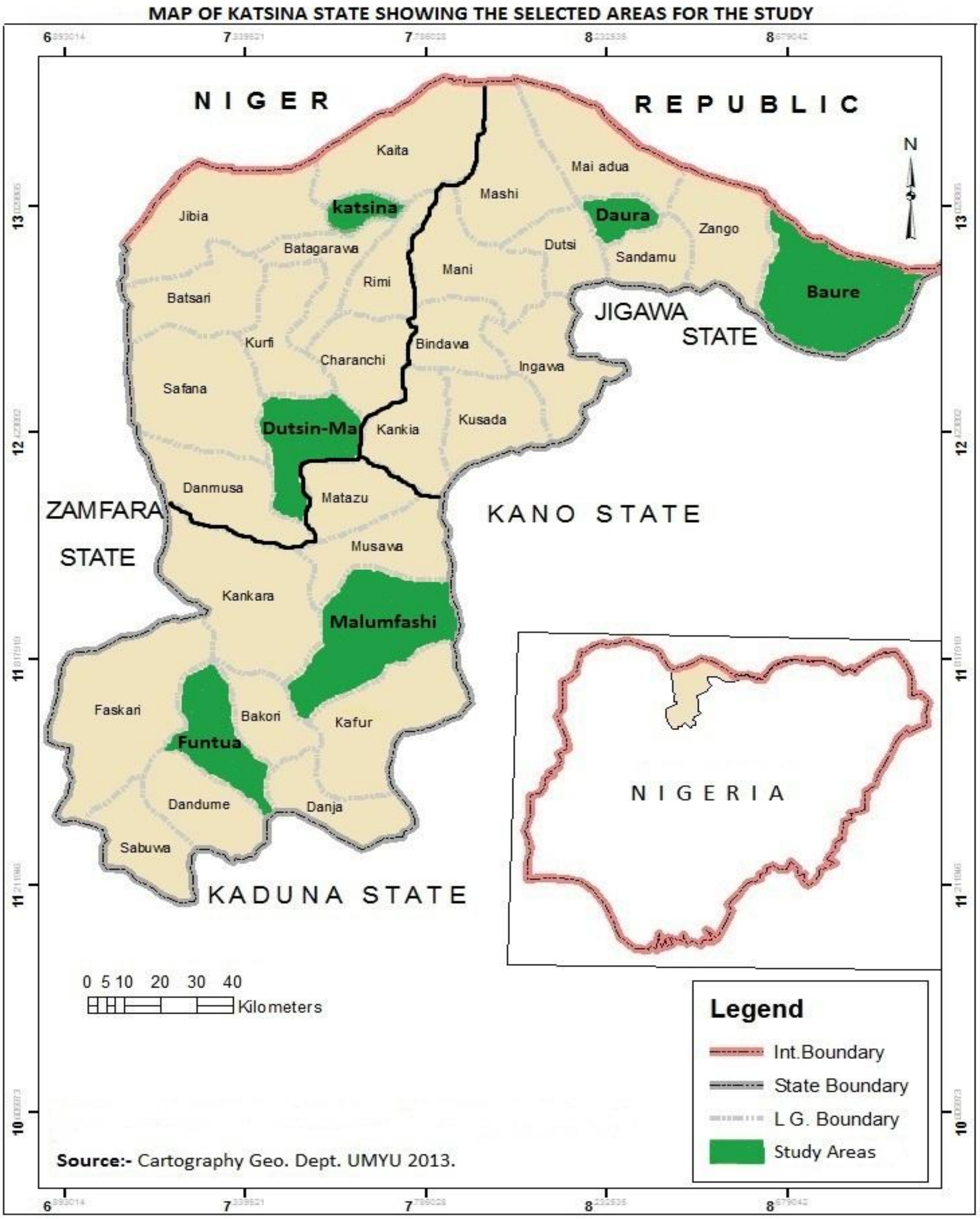

Figure 3.1: Map of Katsina State showing selected areas for the study

General Hospital Dutsinma (Katsina Central), Turai Yardua Children's and Maternity Hospital Katsina (Katsina Central), General Hospital Daura (Katsina North) and General Hospital Baure (Katsina North).

3.3 Inclusion Criteria

The inclusion criteria entailed recruiting any child between the ages of 0-5 years of both sexes who presented or was admitted for diarrhoea illness in the selected hospitals.

3.4 Exclusion Criteria

The exclusion criteria include children above 5 years of both sexes.

A diarrhoea case in the study was defined as a child passing loose, watery or a bloody loose stool three or more times in a 24-h period as reported by the parents. The control was any child who presented for an illness other than diarrhoea and with no history of it on the day of, or in three weeks prior to sampling.

The socio-economic status of a child's parent was determined using education and occupation as indices. A child with high socio-economic background was any child whose parent had tertiary education and is a civil servant while those with parents with primary/secondary education and are not civil servants were said to belong to a low socio-economic background.

3.5 Ethical Approval

Ethical approval (Appendix 5) was obtained from the ethical committee of Katsina State Ministry of Health.

3.6 Sample Size

The sample size for the study was determined using the formula of Sarmukaddam and Gerald (2006) at 95% confidence level and a reported prevalence of 18% of Rotavirus infection in children with diarrhoea in four states in Northwestern Nigeria (Aminu *et al.*,2008).

$$N = \frac{Z^2pq}{d^2}$$

Where, N= Sample size

Z= Statistics for a level of 95% confidence interval= 1.96

P= prevalence rate of rotavirus infection from previous studies = 18%

d = level of significance (allowable error) = 5% or 0.05

q= 1-p

$$\text{thus, } N = \frac{(1.96^2) \times 0.18 \times (1-0.18)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.18 \times 0.82}{0.0025}$$

$$= 226.8$$

The calculated sample size was 226.8; hence a total of 400 samples were collected from the children with and without diarrhoea and used for the study.

3.7 Collection and Transportation of Specimens

Between June 2013 and April, 2014, three hundred and twenty two (322) faecal specimens were collected from children between the ages of 0-5 years that were presented or admitted at clinics or hospitals for acute diarrhoeal illness, and seventy eight (78) from non diarrhoea patients as controls. The specimens were collected in a clean, labeled screw capped containers and transported immediately in ice-cooler boxes to Postgraduate Microbiology Laboratory Ahmadu Bello University, Zaria and were stored at -20°C. Prior to specimen collection, informed consent (Appendix 4) was obtained from the parent/guardian of each child. Basic demographic data, history of illness and clinical information concerning the children were obtained using a designed questionnaire (Appendix 1).

3.8 Analysis of Samples

3.8.1 Viral Antigen Detection using ELISA

All the 400 feacal specimens collected were analysed for the presence of rotavirus and adenovirus antigens using commercially available enzyme immunoassay (Diagnostic Automation/Cortez Diagnostics Inc, USA) ELISA kits. The assay was done according to the manufacturer's instruction in order to determine enteric virus positive specimens.

3.8.1.1 Principle of ELISA Procedure

During the first incubation, viral antigens presents in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-viral antibody that ''sandwiches'' the antigen. The third incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue colour in the presence enzyme complex and peroxide. The stop solution ends the reaction and turns the blue colour to yellow.

3.8.1.2 ELISA Test Procedure

A 1:5 dilution of each solid specimen was prepared by adding 1 gram (approximately the size of a pea) to 4 ml of diluted wash buffer, for liquid stools, lower dilution of 1:2 was prepared. The suspension was mixed thoroughly and heavy particles were allowed to settle for about 20 minutes. Ninety six wells microtitre ELISA plate and other reagents included in the kits were brought to room temperature. A micropipette was used to dispense one hundred (100μl) of the negative and positive control into well 1 and 2 respectively. One hundred (100μl) of the stool supernatant was added to all the appropriate test wells and incubated at room temperature for 30 minutes.

The wells were then washed vigorously with distilled water and excess wash buffer was removed after the last washing by slapping the plate on a clean absorbent tissue. Two (2) drops of reagent 1 (blue solution) was added to each well and incubated at room temperature for 5 minutes. The wells were washed as described above and 2 drops of reagent 2 (red solution) was added to each well, incubated for 5 minutes and then washed. Two (2) drops of chromogen were then added to each well and incubated for 5 minutes. Finally, two (2) drops of stop solution was added to each well and was mixed gently by tapping the side of the plate with index finger.

Results were read visually and with ELISA plate reader. Any sample well that was obviously more yellow than the negative control was interpreted as positive. Any sample well that was colourless or not obviously more yellow than the negative control well was interpreted as negative. Any sample well with absorbance reading of 0.15 and above was regarded as positive while sample wells with absorbance reading of less than 0.15 was regarded as negative.

3.8.2 Negative Staining Electron Microscopy

Negative staining electron microscopy (EM) was performed on the ELISA positive specimens to further confirm the presence of the viral particles. This was carried out at Biological Science Department of Umaru Musa Yar`dua University, Katsina and Department of Chemical Engineering, Ahmadu Bello University, Zaria.

0.1 g of sodium phosphotungstate (PTA) powder was added to 10 ml of distilled water. The pH of the PTA solution was checked with pH meter and was adjusted to pH of 7.2-7.4 using 0.1 N NaOH. Application of the stain to the sample was done based the sequential two droplet method where a drop of the sample suspension was placed on the sample stub and a drop of the PTA stain was then applied. The sample stub was allowed to air dried at room temperature for at least 15 minutes (Hayat and Miller, 1990). The stubs were examined in a Phenom^{TM} scanning electron microscope.

3.9 Anthropometric Measurements

Nutritional status was assessed by anthropometric indices of Height-for Age (HAZ), Weight-for-Age (WAZ), and Weight-for-Height (WHZ). Heights and weights were measured according to standard anthropometric methods (WHO, 2006). Height was measured to the nearest 0.1 centimeters (cm) using a mounted measuring board, similarly the weight was measured to the nearest 0.1 kilogramme (kg) with participants lightly dressed using a portable digital scale (Appendix 2).

In order to reduce intra-individual error, all measurements were done twice and the mean value was used for the analysis.

The measurements obtained were entered into the WHO anthropometric software version 3.0 were Z-scores were calculated (Appendix 2). The cut-off point of -2 Z-score was use to classify the nutritional status of the children as recommended by the World Health Organization reference growth standard (2006) into underweight (WAZ), wasting (WHZ), Stunting (HAZ) which signifies acute malnutrition, acute severe malnutrition and chronic malnutrition respectively.

3.10 Data Analysis and Presentation

Data obtained from questionnaires and the results obtained from laboratory analysis were reduced to percentages by descriptive statistics. SPSS version 20.0 and Graph pad statistical software were used to analyse the data. Chi-square and odds ratio were used to determine association between variables and t-test was used to test significant difference.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of Rotavirus and Adenovirus in the Study Area

Of the 400 stool samples collected, 5.3% (17/322) were positive for rotavirus and no viral antigen was detected in the non-diarrhoeic samples. Rotavirus was significantly associated with diarrhoea in this study (χ^2= 4.3008, df= 1, p= 0.038095). Adenovirus was detected in 12.4% (40/322) of the children with diarrhoea and in 5.1% (4/78) of the non-diarrhoeic children. There was no statistically significance difference of adenovirus infection between the diarrhoeic and non-diarrhoeic children (χ^2= 3.4124, df= 1, p= 0.064707), however, those with diarrhoea were about three times more likely to be infected with adenovirus than those without diarrhoea (OR = 2.6241, 95% CI= 0.9098-7.5685) (Table 4.1).

Analysis of the result by sex among the children showed that rotavirus shedding occurred more frequently in males with a prevalence of 5.5% (10/182) than in females (5.0%: 7/140). However, the differences observed in the prevalence according to sex was not significant (χ^2= 0.005, df= 1, p= 0.944). Analysis by sex for adenovirus showed that viral shedding occurred more in females with a prevalence of 13.2% (24/140) than in males with 11.0% (20/182) prevalence (Table 4.2). However, the differences observed in the prevalence by sex was not statistically significant (χ^2= 3.1942, df= 1, p= 0.073901).

Analysis by age-group showed that rotavirus was most prevalent among children aged 7-12 months (7.7%: 5/65) while adenovirus infection occurred with the highest incidence in children in the age group 0-6 months old (20.0%: 5/25). There was no significant association (p>0.05) between rotavirus and adenovirus infection and age-group. However, generally, children under the age of 2 years were most affected by rotavirus and adenovirus diarrhoea (Table 4.3).

Table 4.1: Prevalence of Rotavirus and Adenovirus Infection in Diarrhoeic and Non-diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

	Rotavirus		**Adenovirus**		
Population	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Total**
Diarrhoeic	17 (5.3)	305 (94.7)	40 (12.4)	282 (80.5)	322
Non-Diarrhoeic	0 (0.0)	78 (100)	4 (5.1)	74 (19.5)	78
Total	**17 (5.3)**	**383 (95.7)**	**44 (11.0)**	**356 (89)**	**400**

RVχ2 = 4.3008, df= 1, p= 0.03; AdVχ2= 3.4124, df= 1, p= 0.06; (OR = 2.6241, 95% CI= 0.9098-7.5685)

Key: RV =Rotavirus; AdV =Adenovirus

Table 4.2: Distribution of Rotavirus and Adenovirus Infection in Relation to Sex in Diarrhoiec Children 0-5 Years Old in Katsina State, Nigeria

	Rotavirus		Adenovirus		
Sex	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Total**
Male	10 (5.5)	172 (94.5)	20 (11.0)	162 (89.0)	182
Female	7 (5.0)	133(95.0)	24 (13.2)	116 (63.7)	140
Total	**17 (5.3)**	**305 (94.7)**	**44 (12.4)**	**278 (87.6)**	**322**

RVχ2= 0.005, df= 1, p= 0.944; AdVχ2= 3.1942, df= 1, p= 0.073901

Key: RV =Rotavirus; AdV =Adenovirus

Table 4.3: Age Distribution of Rotavirus and Adenovirus Infection Among the Under 5 years Children with Diarrhoea in Katsina State, Nigeria

	Rotavirus		**Adenovirus**		
Age	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Total**
0-6	0 (0.0)	25	5 (20.0)	20	25
7-12	5 (7.7)	60	8 (12.3)	57	65
13-18	1(3.0)	32	6 (18.1)	27	33
19-24	8(7.0)	107	11 (9.6)	104	115
25-36	2(4.0)	48	8 (16.0)	42	50
37-60	1 (3.0)	33	2 (5.9)	32	34
Total	**17 (5.3)**	**305**	**40 (12.4)**	**282**	**322**

RV$\chi 2$= 3.666, df= 5, p= 0.598; AdV$\chi 2$= 5.114 df= 5, p= 0.402

Key: RV =Rotavirus; AdV =Adenovirus

Analysis of rotavirus infection by hospital showed the highest prevalence (10.9%: 5/46) in General Hospital Dutsin-ma, followed by General Hospital Funtua (10.7%: 6/56) prevalence. No viral antigen was detected in General Hospital Baure and Maternal and Children Hospital Malumfashi respectively. Conversely, analysis of adenovirus infection by hospital (Figure 4.1) showed the highest prevalence in Maternal and Children Hospital Malumfashi (21.5%: 14/65), followed by General hospital Baure (17.8%: 8/45) (Appendix 6).

Distribution of rotavirus infection by Senatorial District (Figure 4.2) showed the highest prevalence (8.3%: 9/109) in Katsina Central while the least was found in Katsina North with a prevalence of 2.2% (2/92). However, there was no significance difference in the prevalence of rotavirus infection by Senatorial Zone (χ^2= 3.542, d.f= 2, p= 0.170163). Analysis of adenovirus infection by Senatorial District (Figure 4.2) showed the highest prevalence in Katsina South (15.7%: 19/121) while the least was found in Katsina Central with prevalence of 9.2% (10/109). However, the difference observed in the prevalence rates was not significant (χ^2= 0.0049, df= 1, P= 0.94395) (Appendix 7).

Analysis of stool specimen in rotavirus infected children showed watery stool in 70.6% (12/17), stool with mucus in 23.5% (4/17), stool with mucus and blood in 5.9% (1/17). Analysis of stool specimen in adenovirus infected children showed watery stool in 54.5% (24/44), stool with mucus in 22.7% (10/44), stool with mucus and blood in 13.6% (6/44) and normal stool 9.1% (4/44) (Figure 4.3).

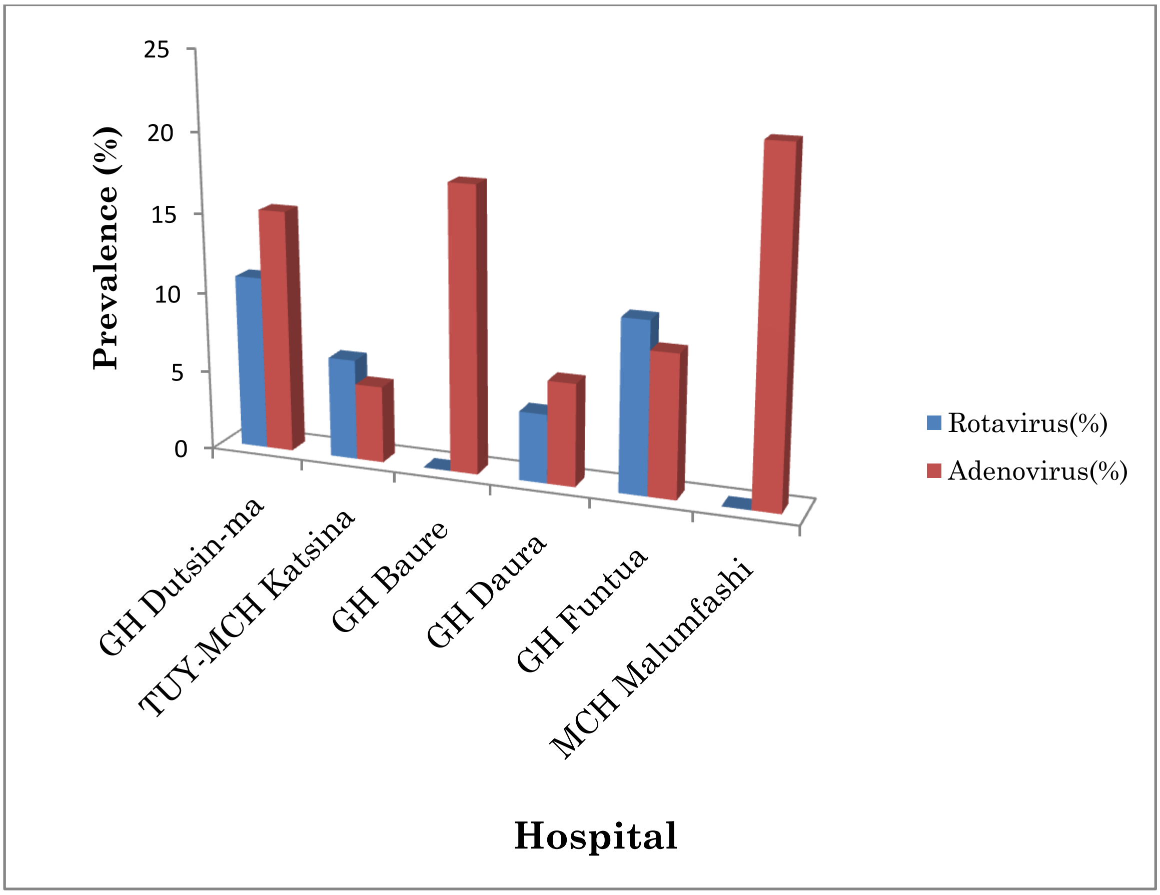

Figure 4.1: Distribution of Rotavirus and Adenovirus Infection by Hospital in Children 0-5 Years Old in Katsina State, Nigeria

Key:
GH= General Hospital
TUY-MCH= Turai Umaru Yardua- Maternal and Children Hospital
MCH= Maternal and Children Hospital

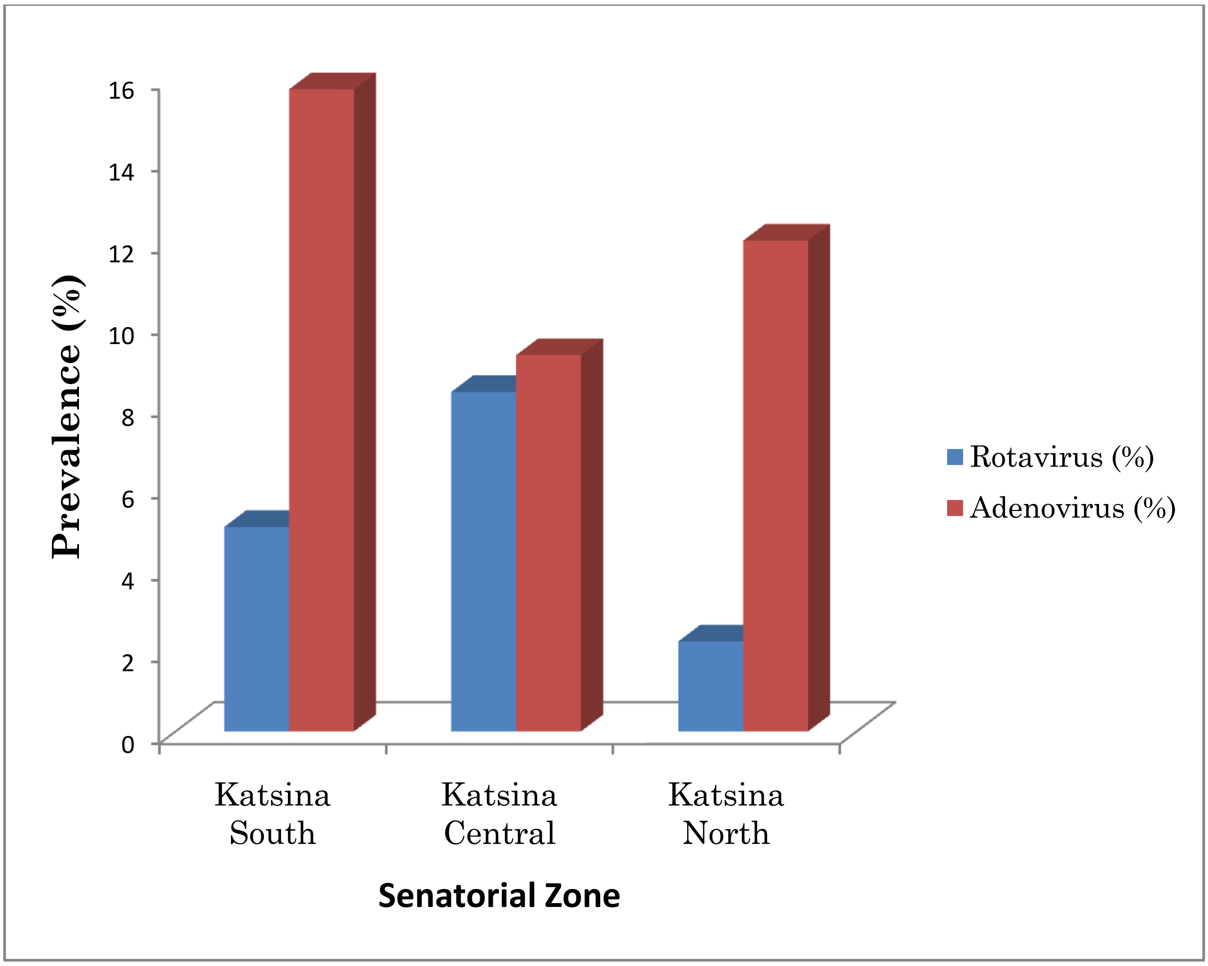

Figure 4.2: Distribution of Rotavirus and Adenovirus Infection by Senatorial Zone in Diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

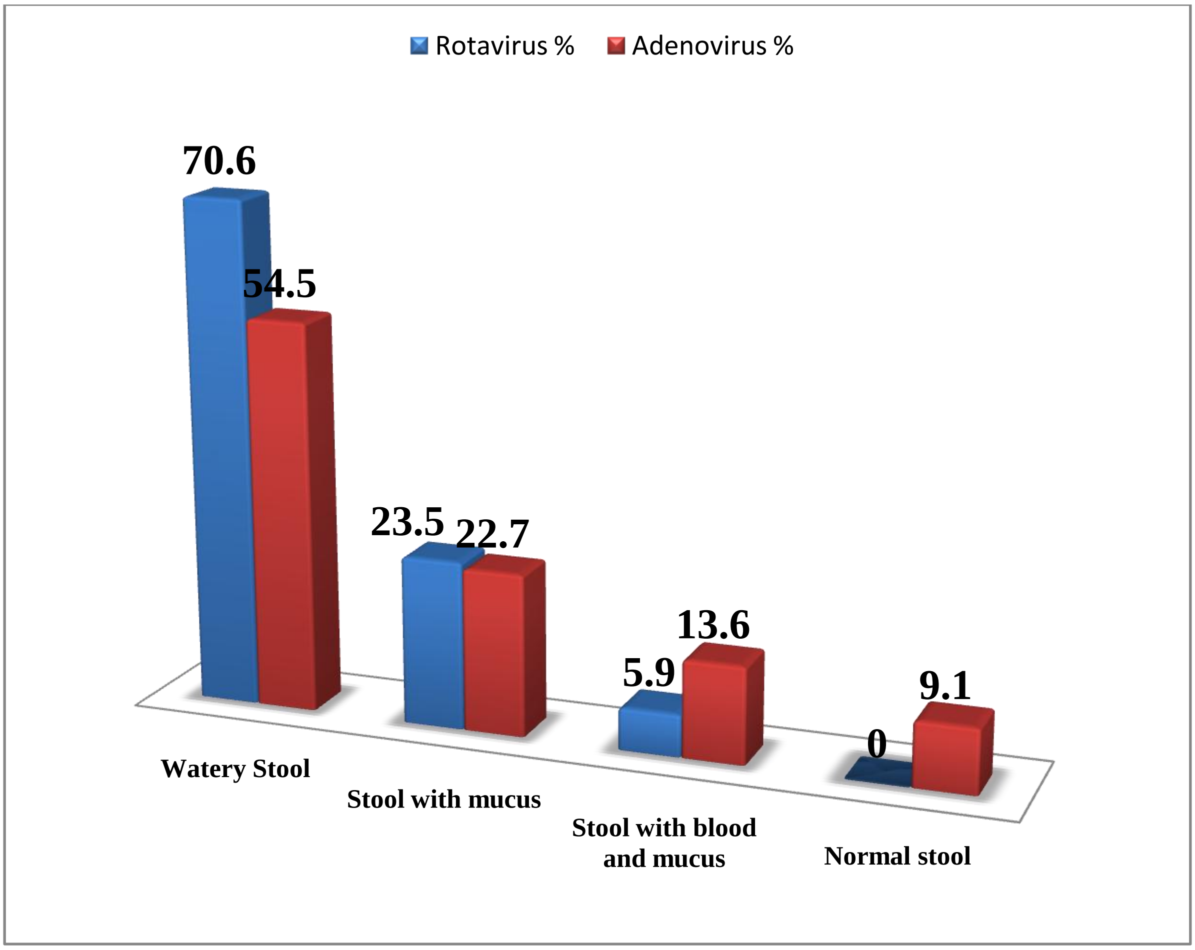

Figure 4.3: Stool type in Rotavirus and Adenovirus Infected Children 0-5 Years old in Katsina State, Nigeria

The frequency of stool passed per day varied among the children, about 52.9% (9/17) of the children infected with rotavirus passed stool 4-6 times a day, 35.3% (6/17) passed stool 1-3 times a day and 11.8% (2/17) 7-10 times a day respectively. Of the 44 adenovirus positive diarrhoeic children, 60% (24/40) passed stool 4-6 times a day, 32.5% (13/40) passed stool 1-3 times day and 7.5% (3/40) passed stool 7-10 times a day (Figure 4.4).

The major clinical features among the rotavirus positive children included a combination of diarrhoea, fever and vomiting (35.2%: 6/17), diarrhoea and fever (29.4%: 5/17), dairrhoea and vomiting (23.5%: 4/17) and diarrhoea only (11.8%: 2/17). In adenovirus infected children, a combination of diarrhoea, fever and vomiting occurred in 52.5% (21/40) of the children, diarrhoea and fever in 17.5% (7/40), diarrhoea and vomiting in 20.0% (8/40) and diarrhoea only in 10.0% (4/40) (Table 4.4).

Among the diarrhoeic children positive for rotavirus, the degree of dehydration among the children was found to be mild, moderate, severe and absent in 13.5% (5/37), 2.8% (4/145), 8.3% (7/84) and 1.8% (1/56) of cases respectively. In this study, rotavirus infection was significantly associated with dehydration (χ^2= 9.792, df= 3, p= 0.02). Among the diarrhoeic children positive for adenovirus, the degree of dehydration among the children was found to be mild, moderate, severe and absent in 27% (10/57), 9.7 % (14/145), 15.5% (13/84) and 5.4% (3/56) of children respectively. In this study, adenovirus infection was found to be significantly associated with dehydration (χ^2= 11.564, df= 1, p= 0.009) (Table 4.5).

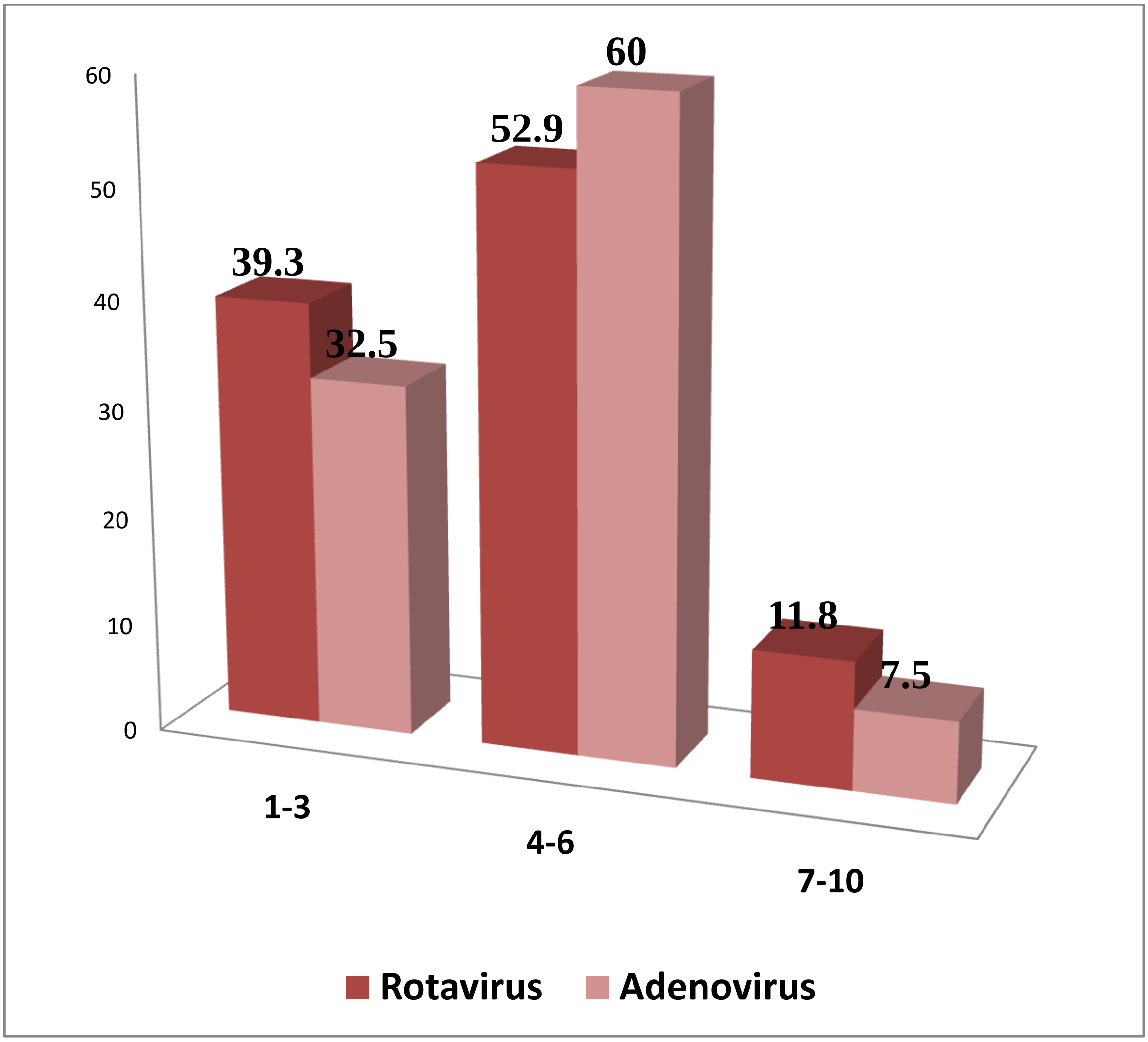

Figure 4.4: Frequency of Stool Passed in 24 hour period (per day) Among the Rotavirus and Adenovirus Infected Children 0-5 Years Old in Katsina State, Nigeria.

Table 4.4: Clinical features among the Rotavirus and Adenovirus Infected children 0-5 Years Old in Katsina State, Nigeria

Clinical feature	Rotavirus N (%)	Adenovirus N (%)
Diarrhoea, fever and vomiting	7 (41.2)	21 (52.5)
Diarrhoea and fever	4 (23.5)	7 (17.5)
Diarrhoea and vomiting	4 (23.5)	8 (20.0)
Diarrhoea only	2 (11.8)	4 (10.0)

Key; N= Number of children positive

Table 4.5: Degree of Dehydration in Relation to Rotavirus and Adenovirus infection in Diarrhoeic Children 0-5 years old in Katsina State, Nigeria

	Rotavirus		**Adenovirus**		
Degree-of Dehydration	**Positive (%)**	**Negative**	**Positive (%)**	**Negative**	**Total**
Mild	5 (13.5)	32	10 (27.0)	27	37
Moderate	4 (2.8)	141	14 (9.7)	131	145
Severe	7 (8.3)	77	13 (15.5)	71	84
None	1 (1.8)	55	3 (5.4)	53	56
Total	**17 (5.3)**	**305**	**40 (12.4)**	**282**	**322**

$^{RV}\chi^2$= 9.792, df= 3, p= 0.02; $^{AdV}\chi^2$= 11.564, df= 1, p= 0.009
Key: RV= Rotavirus; AdV= Adenovirus

In this study, there was no statistically significant relationship between socio-economic background and rotavirus infection (χ^2= 0.0684, df= 1, p= 0.79371). However, the highest prevalence rate (5.5% (12/218) of rotavirus infection predominated among children from parents whose socio-economic status is low. Similarly, there was no statistically significant relationship between adenovirus infection in children and socio-economic background (χ^2= 0.177 df= 1, p= 0.674). However, the result showed that most of the adenovirus positive children were from parents whose socio-economic status was low with 12.8% (28/218) prevalence (Table 4.6).

The level of education of a child's parent had no relationship with rotavirus infection in this study (Table 4.6). Even though, there was no significant relationship observed between mother's level of education and viral infection (χ^2= 7.082, df= 3, p= 0.069331), there was differences in the prevalence rate. The highest rate of rotavirus infection was 9.5% (2/21) among children from mothers with tertiary education, followed by children from illiterate mothers with 5.5% (9/165) prevalence. The highest rate of adenovirus infection was 14.3% (11/77) among children from mothers with primary education. Similarly, there was no significant relationship observed between mother's level of education and adenovirus infection (χ^2= 1.166, df= 3, p= 0.761).

Rotavirus was found to be significantly associated with source of drinking water in this study, (χ^2= 5.6347, df= 4, p= 0.03). The main source of drinking water in most of the rotavirus infected children was public well with 8.7% (9/104) prevalence. Adenovirus infection was not associated with source of drinking water in this study, (χ^2= 4.6819, df= 4, p= 0195). However, the main source of drinking water in most of the children was river with 14.2% (4/28) prevalence (Table 4.8). Most of the rotavirus and adenovirus infected children used open field as their toilet type. However, there was no association between toilet type and viral infection in this study (Table 4.6).

Table 4.6: Relationship between Some Possible Risks Factors Studied and Rotavirus and Adenovirus Infection in Children 0-5 Years old in Katsina State, Nigeria

Factor	Total	Rotavirus Positive N (%)	Rotavirus Negative	Adenovirus Positive N (%)	Adenovirus Negative	P-value
Socio-economic status						
High	104	5 (4.8)	99	12 (11.5)	92	0.74^{RV}
Low	218	12 (5.5)	206	28 (12.8)	190	0.67^{AdV}
Mother`s level of education						
None	165	9 (5.5)	153	21 (12.0)	144	
Primary	77	4 (5.2)	73	11 (14.3)	60	0.06^{RV}
Secondary	59	3 (5.1)	57	7 (13.0)	52	0.74^{AdV}
Tertiary	21	1 (4.8)	19	1 (4.8)	20	
Source of drinking water						
Public well	104	9 (8.7)	95	13 (12.5)	91	
Bore-hole	38	3 (7.9)	35	3 (7.9)	35	
Pipe-borne	61	2 (3.3)	51	8 (13.1)	53	0.03^{RV*}
Private well	81	1 (1.2)	80	11 (13.4)	70	0.195^{AdV}
River/stream	28	2 (7.1)	26	4 (14.2)	24	
Others	10	0 (0.0)	10	1 (10.0)	9	
Type of toilet						
Pit-latrine	223	9 (4.0)	214	29 (13.0)	194	
Water-closet	62	5 (8.0)	57	6 (9.70	56	0.32^{RV}
Open-field	37	3 (8.1)	34	9 (24.3)	28	0.12^{AdV}
Previous history of diarrhoea						
Yes	205	10 (4.9)	195	18 (18.8)	187	0.66^{RV}
No	117	7 (6.0)	110	26 (22.2)	91	0.32^{AdV}

^{RV}= Rotavirus; ^{AdV}= Adenovirus, *= Significant
Key: RV= Rotavirus; AdV= Adenovirus, N= Number of children positive

Analysis of the previous history of diaarhoea and rotavirus infection showed no significant relationship (χ^2= 0.1818, df= 1, p= 0.669808). However, rotavirus was most prevalent (6.0%) 7/117 among children with no history of diarrhoea. Similarly, adenovirus infection predominated among children with no history of diarrhoea with 22.2% prevalence. Also, there was no association between previous history of diarrhoea and adenovirus infection (χ^2= 0.1818, df= 1, p= 0.32) (Table 4.6).

4.2 Electron Microscopy

Rotavirus particles were observed in only two of the seventeen ELISA positive samples examined (Figure 4.5 and 4.6) while adenovirus was detected in only one of the forty specimens examined by negative staining electron microscopy (Figure 4.7).

4.3 Co-Infection of Rotavirus with Adenovirus

Rotavirus and adenovirus co-infection was observed in 0.6% (2/322) of the diarrhoiec children (Figure 4.8). Analysis by sex and age showed that co-infection was found in one male and one female with 24 and 36 months old respectively. All the co-infected children were found in the diarrhoeic population. Analysis of their stool samples showed that they both had watery stool and both passed watery stool atleast 1-3 times in 24 hour period. The male patient had both fever, vomiting with severe dehydration while the female patient had vomiting, no fever and mild dehydration. Socio-economic and clinical characteristics of the co-infected children is shown on Table 4.7.

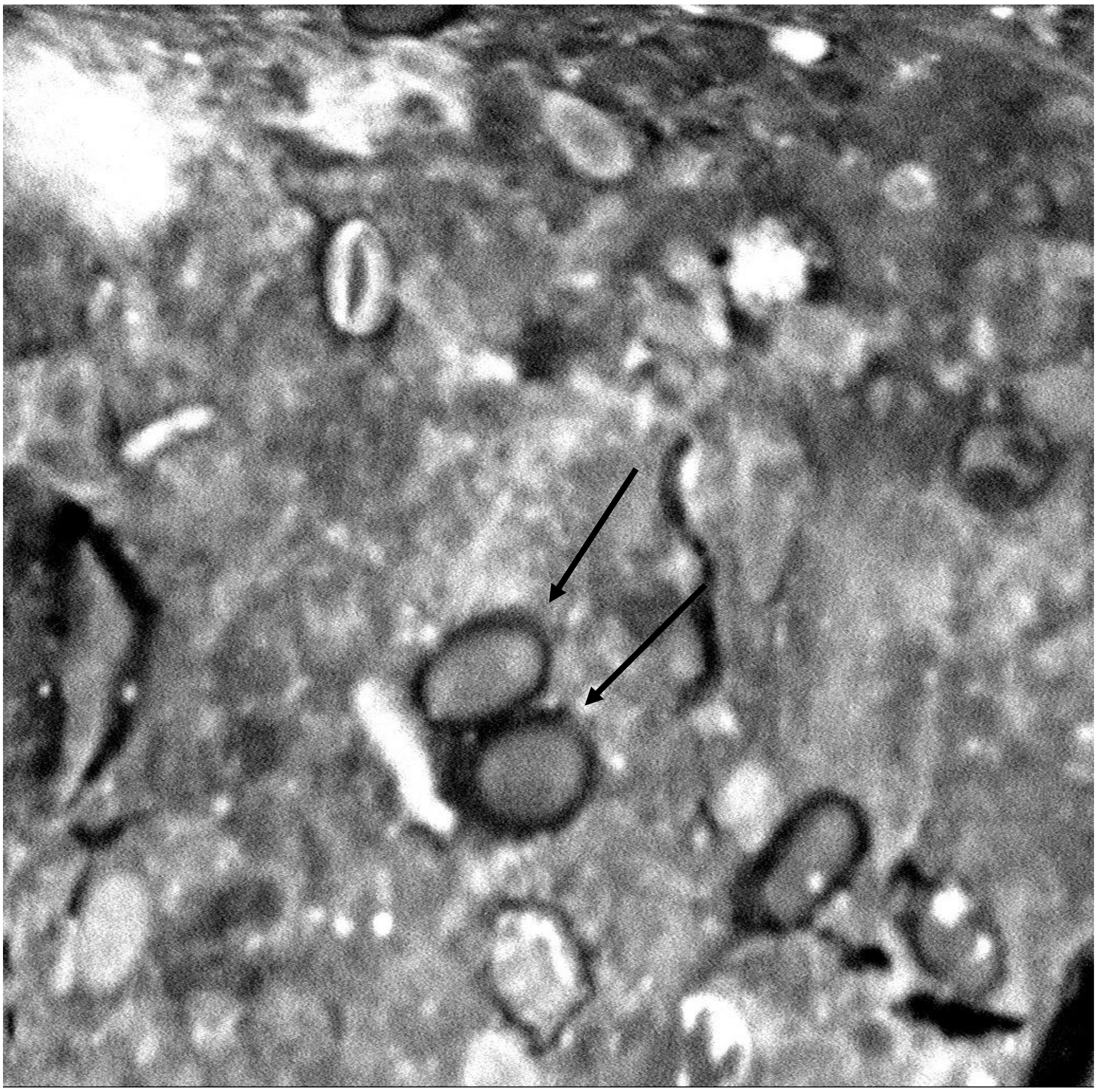

Figure 4.5: Electron Micrograph of Rotavirus particles observed in stool specimen of diarrhoiec child in Katsina State.

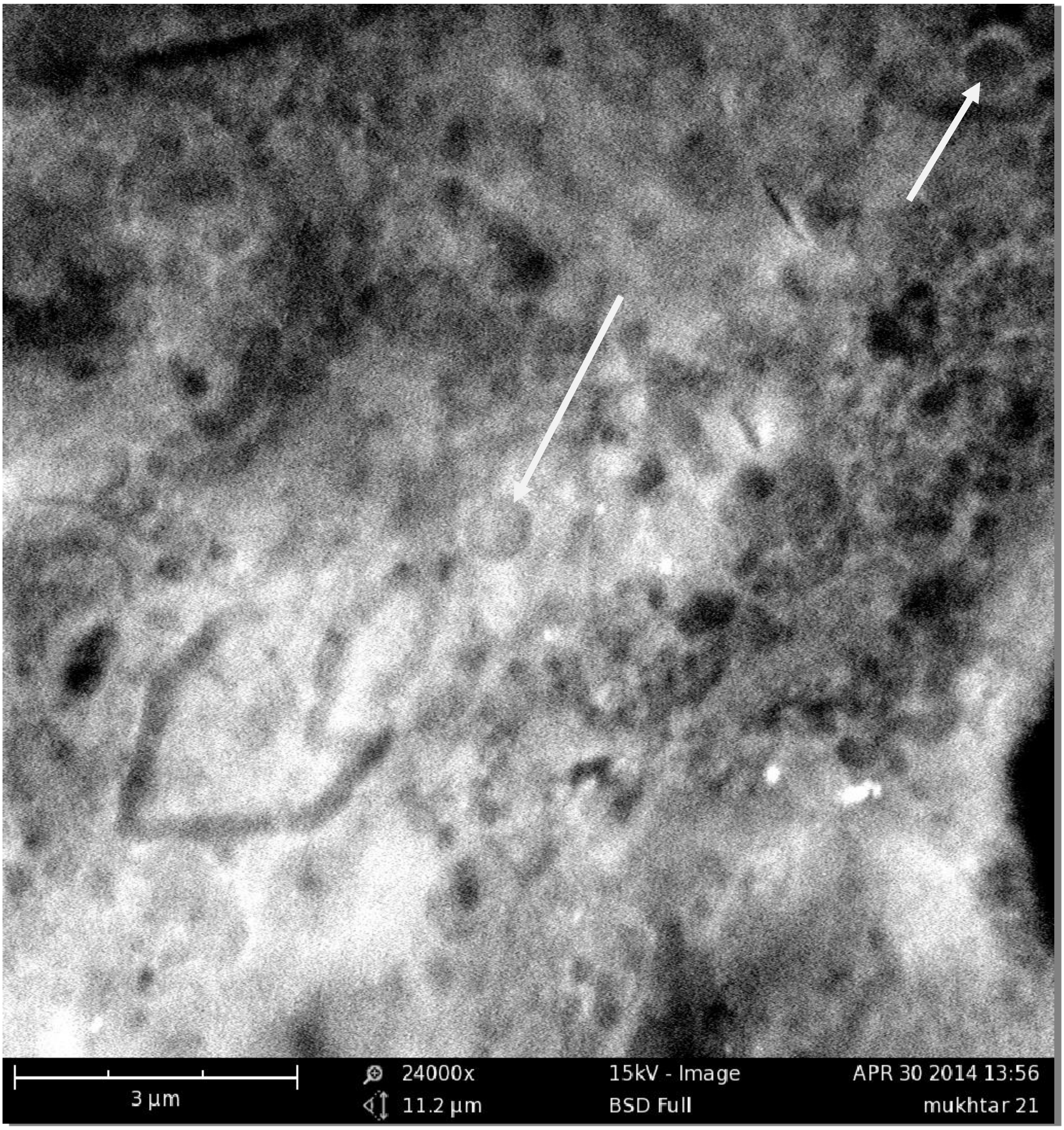

Figure 4.6: Electron Micrograph of Rotavirus particles observed in stool specimen of diarrhoiec child in Katsina State.

Figure 4.7: Electron Micrograph of Adenovirus particles observed in stool specimen of diarrhoiec child in Katsina State.

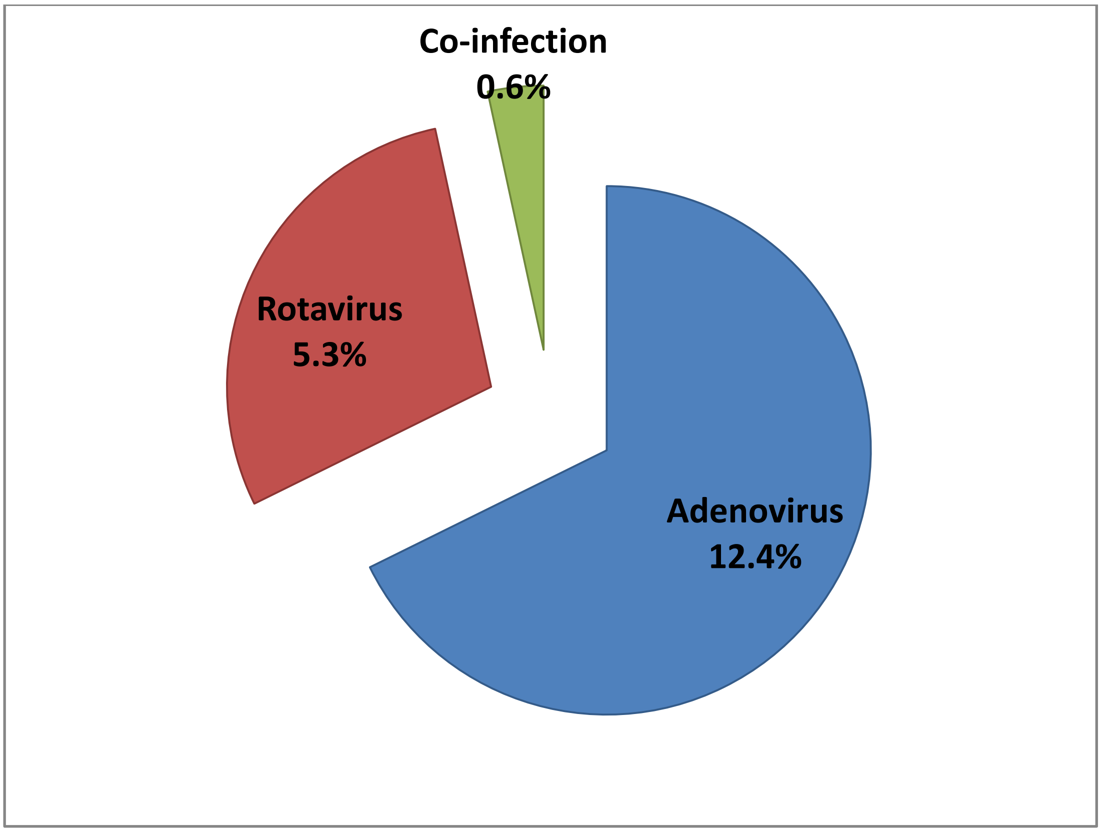

Figure 4.8: Co-infection of Rotavirus with Adenovirus in Diarrhoiec Children 0-5 Years Old in Katsina State, Nigeria

Table 4.7: Clinical and Socio-demographic Characteristics of the Co-infected Children 0-5 years old in Katsina State, Nigeria

	Rotavirus and Adenovirus Co-infection	
Factor	**Male**	**Female**
Age (Months)	24	36
Stool type	Watery	Watery
Vomiting	Yes	No
Fever	Yes	Yes
Dehydration	Severe	Mild
Frequency of stool per day	1-3	1-3
Mother`s level of education	None	None
Socio-economic status	Low	Low
Source of drinking water	Public well	Public well
Type of toilet	Pit-latrine	Pit-latrine
Previous history of diarrhoea	None	Yes

4.4 Nutritional Status

4.4.1 Anthropometric Characteristics

The study included 322 diarrhoeic and 78 non-diarrhoeic children aged 0-5 years. The mean height and weight values of the diarrhoeic children were 77.5 cm and 8.4 kg respectively while that of the non-diarrhoeic children were 78.6 cm and 9.01 kg respectively (Table 4.8).

There was no significant difference in the mean height and weight values (p= 0.4394 t= 0.774, df= 398), (p= 0.07, t= 1.815, df= 398) of the diarrhoeic and non-diarrhoiec children. The non-diarrhoeic children had significantly higher mean WAZ, HAZ and WHZ values (p= 0.003, t= 3.6697, df= 398; p= 0.0281, t=2.2046, df=398; p= 0.0052, t= 2.8120, df= 398) compared to the diarrhoeic children (Table 4.9).

4.4.2 Prevalence of Undernutrition among the Study Population

Comparison of the Z-score values of the children (Appendix 2) with the New 2006 WHO reference growth standards showed that the prevalence of underweight, stunting and wasting among the study population was 59.0, 47.0 and 46.3% respectively (Figure 4.9). Plot of graph of the Z-scores of the children in relation to WHO standard curves shows deviation (skewness) from normal distribution (Appendix 3).

4.4.3 Prevalence of Undernutrition among Diarrhoeic and Non-diarrhoeic children

Comparison of the Z-score values of the children with the New 2006 WHO reference growth standards showed that 63.3% (60.2% for boys and 67.2% for girls) of the diarrhoeic children were underweight while prevalence of underweight in non-diarrhoeic children was 48.7% (51.1% for boys and 45.5% for girls) (Table 4.10). There was significant difference (χ^2=4.235, df= 1, p= 0.0396) between the prevalence of underweight among the diarrhoeic

Table 4.9: Mean Anthropometric Characteristics of the Diarrhoeic and Non-diarrhoeic Children 0-5 Years in Katsina State, Nigeria

Anthropometric Indices	Diarrhoeic Mean Value (SD)	Non-diarrhoeic Mean Value (SD)
Height (cm)	77.5 (11.09)	78.6 (11.95)
Weight (kg)	8.35 (2.9)	9.01 (2.84)
WAZ	-2.77 (1.82)*	-1.98 (1.11)
HAZ	-2.07 (1.91)*	-1.56 (1.47)
WHZ	-2.33 (2.34)*	-1.54 (1.67)

KEY:
WAZ= Weight for Age Z-score
HAZ= Height for Age Z-score
WHZ= Weight for Height Z-score
***Significant (P<0.05) by t-test comparison**
SD= Standard Deviation

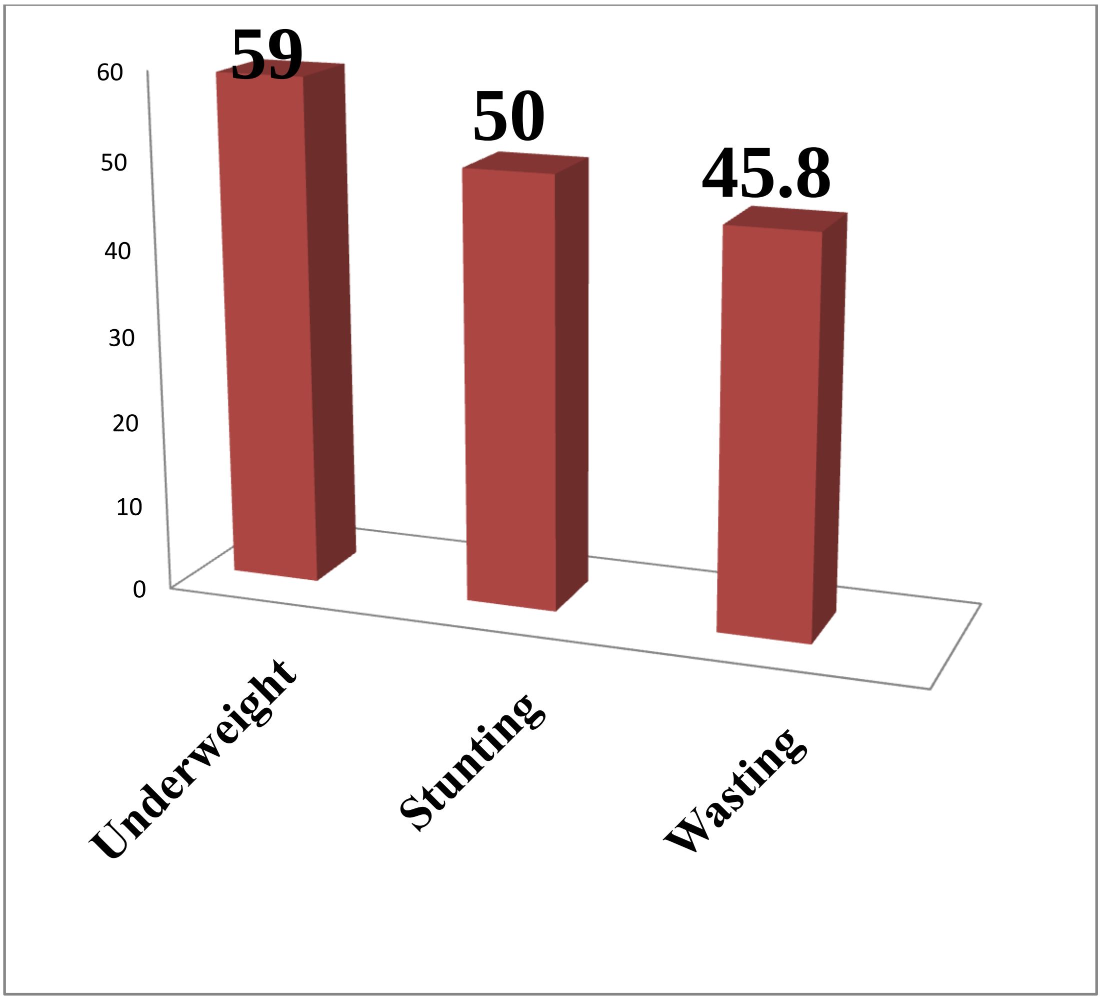

Figure 4.9: Prevalence of Undernutrition Among the Children 0-5 Years Old in Katsina State, Nigeria

Table 4.10: Prevalence of Underweight among the Diarrhoeic and Non-diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

	Low-Weight for Age (Underweight)	
	Diarrhoiec	**Non-diarrhoeic**
Variable	**N (%)**	**N (%)**
Gender		
Male	106 (60.2)	23 (51.1)
Female	92 (67.2)	15 (45.5)
Age-group		
0-6	16 (80.0)	3 (50.0)
7-12	35 (59.3)	11 (91.7)
13-18	25 (53.2)	4 (33.3)
19-24	83 (72.2)	14 (46.7)
25-36	26 (63.4)	4 (40.0)
37-60	13 (41.9)	2 (25.0)
Total	**198 (63.3)***	**38 (48.7)***

(χ^2=4.235, df= 1, p= 0.0396)

KEY: N= Number of children

***= Significant**

and non-diarrhoiec children. Analysis of underweight by age among the diarrhoeic children showed that the proportion of underweight was highest (80%) in the age-group 0-6 month.

Underweight levels were lowest (41.9%) in the age-group 37-60 months. Among the non-diarrhoeic children, underweight was highest (91.7%) in the age-group 7-12 and lowest (25%) in the age-group 37-60 months.

There was no significant difference (χ^2=1.019, df= 1, p= 0.3127) between the prevalence of stunting among the diarrhoeic and non-diarrhoiec children. However, the level of stunting was higher among diarrhoiec children as compared to the non-diarrhoeic children. Prevalence of stunting among the diarrhoiec children was 53.0% (54.2% for boys and 51.4% for girls) while it was 44.9% (53.3% for boys and 33.3% for girls) among the non-diarrhoeic children. Stunting levels was highest (61.4%) in children 19-24 months old among the diarrhoiec and (83.3%) in 7-12 months old among the non-diarrhoeic children (Table 4.11).

There was no significant difference (χ^2=0.4802, df= 1, p= 0.5228) between the prevalence of wasting among the diarrhoeic and non-diarrhoiec children. However, the level of wasting was higher among diarrhoiec children as compared to the non-diarrhoeic children. Wasting was prevalent in 46.8% (42.3% for boys and 52.5% for girls) of the diarrhoeic children and 41.0% (45.5% for boys and 54.5% for girls) of non-diarrhoeic children. Analysis by age showed that wasting was highest (60.0%) among children 19-24 months old with diarrhoea and 72.7% in 7-12 months old among the non-diarrhoeic children (Table 4.12).

Table 4.11: Prevalence of Stunting among the Diarrhoeic and Non-diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

	Low-Height for Age (Stunting)	
	Diarrhoiec	**Non-diarrhoeic**
Variable	**N (%)**	**N (%)**
Gender		
Male	95 (54.2)	24 (53.3)
Female	70 (51.4)	11 (33.3)
Age-group		
0-6	12 (60.0)	3 (50.0)
7-12	25 (41.9)	10 (83.3)
13-18	23 (48.9)	3 (25.0)
19-24	70 (61.4)	10 (33.3)
25-36	23 (55.0)	5 (50.0)
37-60	13 (43.8)	4 (50.0)
Total	**165 (53.0)****	**35 (44.9)****

(χ^2=1.019, df= 1, p= 0.3127)

KEY: N= Number of children

**** = Not significant**

Table 4.12: Prevalence of Wasting among the Diarrhoeic and Non-diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

	Low-Weight for Height (Wasting)	
	Diarrhoiec	**Non-diarrhoeic**
Variable	**N (%)**	**N (%)**
Gender		
Male	74 (42.3)	19 (42.2)
Female	71 (52.5)	13 (39.4)
Age-group		
0-6	9 (44.4)	3 (50.0)
7-12	25 (42.0)	8 (66.7)
13-18	25 (53.2)	5 (41.0)
19-24	52 (60.0)	14 (46.7)
25-36	19 (45.2)	2 (20.0)
37-60	9 (30.0)	0 (0.0)
Total	**145 (46.8)****	**32 (41.0)****

(χ^2=0.4802, df= 1, p= 0.5228)

KEY: N= Number of children

**** = Not significant**

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of Rotavirus and Adenovirus

In this study, rotavirus infection occurred in 5.3% of children 0-5 years old that presented with diarrhoea and did not occur in the control group. This implies that rotavirus is significantly associated with diarrhoea. Rotavirus has been shown to be the most important cause of viral diarrhoea in children less than five years of age in Nigeria (Pennap *et al.*, 2010; Aminu *et al.*, 2008; Kuta *et al.*, 2014), Ghana (Armar *et al.*, 2003) and Cameroon (Esona *et al.*, 2003).

The prevalence of rotavirus infection in this study is comparable to the report of Kuta *et al*, (2014) where a prevalence of 4.5% was reported in their study in three North Central States and Federal Capital Territory Abuja, Nigeria. However, the result is lower than previous reported studies in other parts of the country such as 13.0% in Ibadan, 11.0% in Jos, 11.9% in Maiduguri, 18.0% in Northwestern Nigeria, 16.3% in Zaria and 13.8% in Jos, (Ojeh *et al*., 1995; Nimzing *et al.*, 2000; Adah *et al.*, 2001; Aminu *et al.*, 2008; Pennap and Umoh 2010; Junaid *et al.*, 2011).

On the other hand, the prevalence of adenovirus infection (12.4%) in this study is comparable to the results of other studies where a prevalence of 14% was reported in Bangladesh (Kim *et al*., 1990), 13% in Mexico (Maldonado *et al.*, 1998), 16.7% in Lagos (Audu *et al.*, 2002), and 16.2% in Khartoum (Elhag *et al.*, 2013). In Africa and other parts of the world, lower prevalence of 3.8% in Jos, Nigeria (Nimzing *et al.,* 2000), 7.8% in Gaborone (Basu *et al.*, 2003), 5% in Northern France (Tran *et al.*, 2010), 3.3% in South Western Iran (Kajbaf *et al*., 2013), and 3.7% in Najra region, Saudi Arabia (AlAyed *et al.*, 2013) have been reported. However, a higher prevalence of 22.3% in Nigeria has been reported (Aminu *et al*., 2007).

The presence of adenoviruses in non-diarrhoeic children may probably reflect the facts that adenoviruses other than enteric adenoviruses (EAds) are often excreted in stool. Similar studies by Audu *et al,* (2002) and Aminu *et al,* (2007) have previously reported presence of adenoviruses in non-diarrhoeic children.

The observed differences in prevalence might be due to the differences in geographical location or alternatively it might reflect changing trends (CDC, 2008). The differences could also be due to difference in climatic conditions, environmental and socio-economic factors or cultural practices. Overcrowding, poverty and poor general sanitation, which are all factors enhancing the transmission of human rotavirus infection (Aminu *et al.*, 2008) might account for high level of rotavirus infection seen in other parts of the country.

The lower prevalence of rotavirus observed in this study might also be due to the long period of specimen storage, which might lead to disintegration of the viral particles, hence insufficient antigen in the specimen. Also the patients might be shedding rotavirus antigen lower than the detection limits of the test assay.

Rotavirus infection was observed to be slightly higher in males (5.5%) than in females (5.0%), hence there was no statistically significance difference in rotavirus infection between male and female children with diarrhoea in this study (P>0.05). This is similar to other studies in Nigeria where rotavirus was detected in slightly higher rates in male than in females (Aminu *et al.,* 2008, Pennap and Umoh 2010; Junaid *et al*., 2011). Similar studies in other countries such as in South Western Iran (Kajbaf *et al.,* 2013), and in Sudan (Magzoub *et al.*, 2013) have reported higher detection rates of rotavirus infection in males than in females. However, studies from Cameroon (Ndze *et al.,* 2012) reported higher detection rates in females (45.3%) than in males (40.8%). Conversely, adenovirus infection was observed to be slightly higher in females (13.2%) than in males (11.0%), hence there was no statistically significance difference in adenovirus infection between male and female children with diarrhoea in this study (P>0.05). This is similar to the findings of many studies (Audu *et al.,* 2002; Samarbaf-Zadeh *et al.,* 2010) where they found no any significant difference in infection between both sexes. The reason for the lack of statistically significance difference in detection rate between males and females may be explained by the fact that at younger age, both sexes have little or no major differences in their life style. Alternatively, the slight differences observed might be due to sex susceptibility or by chance, whether this difference is due to sex susceptibility or by chance is however questionable and needs further investigation.

In this study, 82.4% of the children positive for rotavirus were under two years of age, emphasizing the fact that rotavirus infection occurs early in life. This is in consistent with a number of similar studies in Nigeria (Audu *et al*., 2002; Aminu *et al*., 2008; Junaid *et al*., 2011; Anochie *et al*., 2013) and most parts of the world (Basu *et al*., 2003; Kargar *et al*., 2012; Almusawi *et al.*, 2013; Magzoub *et al.*, 2013; Karakus *et al*., 2014).

The highest prevalence of rotavirus infection occurred in the age-group 7-12 months (7.7%). This age-group distribution is comparable to previous reports by Junaid *et al,* (2011) and Kajbaf *et al,* (2013) where they reported higher prevalence in children 7-12 months old.The least prevalence of rotavirus infection was observed in the age-group 0-6 months. The low rate of infection in infants may be attributed to a higher rate of breast feeding thereby providing partial protection due to present of maternal antibodies in breast milk.

The detection of rotavirus mostly in children under 2 years in this study is in accordance with the assumption that in under-developed areas the early peak of rotavirus diarrhoea may result from early exposure to contaminated sources as well as over-crowded homes, more so, since almost all humans experience at least one rotavirus infection by 3 years of age and circulating rotavirus antibodies remain detectable indefinitely (Bernstein and Ward 2004). This may lead to protection against rotavirus infection and disease or at least milder forms of disease, which result in lower rate of rotavirus gastroenteritis in older children.

In an analysis of adenovirus infection by age, it was observed that (70.5%) of positive cases were in children under 2 years of age. This result is consistent with the findings of most studies conducted worldwide where children less than 2 years of age are more vulnerable to adenovirus infection (Basu *et al.*, 2003; Filho *et al*., 2007; Samarbaf-Zadeh *et al*., 2010). However, this result is in contrast to the findings of Aminu *et al,* (2007) where the detection of adenovirus infection was mostly in children older than 2 years of age.

The highest prevalence of adenovirus infection occurred in the age-group 0-6 months (20.0%). Our observation of a higher prevalence in children within the age brackets below 24 months of age and a lower prevalence in the age groups of 24-60 months of age may mean that the children are infected much earlier in life but go on to develop resistance to infection. In other words, this could be explained by the fact that older children acquire protective immunity during repeated exposures to the virus and therefore subsequent infections are mild or asymptomatic (White *et al.*, 2008).

Rotaviruses were detected in four out of the six hospitals while adenoviruses were detected in all the hospital studies and across all the 3 senatorial zones, there by indicating their wide distribution throughout the state. It is not surprising that adenoviruses were detected throughout the study area since they are associated with different clinical conditions. Similar to this study, variability of prevalence in study areas has been observed in many studies (Aminu *et al*., 2008; Farzaneh *et al.*, 2012; Ndze *et al.*, 2012; Kajbaf *et al*., 2013).

Similar studies have also reported variability of adenovirus prevalence in their respective study areas (Aminu *et al*., 2008).

In this study, rotavirus and adenovirus was significantly associated with dehydration. Viral infection rate was higher among children with mild dehydration. Previous studies by Aminu, (2006), Parashar *et al,* (2006) and Pennap and Umoh, (2010) have made similar observations.

Rotavirus and adenovirus shedding was highest when a combination of all the three symptoms (diarrhoea, fever and vomiting) occurred together and lower when two occurred together and lowest when diarrhoea occurred alone. These clinical features observed are the major symptoms that accompanied rotavirus diarrhoea. This is similar to the observations made by Pennap and Umoh (2010) and Junaid *et al*, (2011). Similar observations of these clinical features among adenovirus infected children have been made Audu *et al*, (2002) and Aminu *et al*, (2007). These clinical features observed are suggestive of the sign and symptoms cause by adenovirus associated diarrhoea.

As for the socio-demographic factors associated with diarrhoea in this study, the socio-economic status of the child`s parents was not significantly associated with prevalence of rotavirus and adenovirus infection in this study. Similar observations were reported by Pennap and Umoh (2010) and Aminu *et al.* (2008). However, rotavirus and adenovirus infection predominated in children from families with low socio-economic status with 5.5 and 12.8% prevalence respectively.

Rotavirus infection was significantly associated with source of drinking water. Most of the patients (8.7%) were found depending on public well as their source of water. This emphasize the fact that rotavirus is transmitted by faecal-oral means. Conversely, adenovirus infection was not associated with source of drinking water, however, most of the adenovirus infected children used river as their water source. The high mortality and morbidity associated with rotavirus diarrhoea, coupled with the fact that improvement in water supply, sanitation or hygiene has not had a significant impact on disease (Bernstein *et al*., 1995; WHO, 2005), has been the main reasons why the WHO and Global Alliance for Vaccine Initiative (GAVI) made the development of a vaccine against rotavirus a high priority. Furthermore, researchers have noted that rotavirus is resilient and highly contagious and therefore improvements in water and sanitation are unlikely to be affective prevention measures of rotavirus diseases, hence supporting advocacy for mass vaccination program (Huppertz *et al*., 2008).

Parashar *et al,* (2006) noted that while diarrhoeal disease incidence has reduced in recent years, due in part to improved hygiene practices and sanitation, the incidence of rotavirus infection continues to increase. They further opined that hygiene and water interventions are likely to be effective only on diarrhoea caused by bacteria and parasitic agents.

In this study, type of toilet, previous history of diarrhoea was not significantly associated with rotavirus and adenovirus infection (P>0.05). Similar studies in Nigeria and other parts of the world have reported similar observations (Pennap and Umoh 2010; Junaid *et al.*, 2011).

In this study, there was no significant relationship between mother's level of formal education and viral infection. However, the outcome of rotavirus infection was observed more in children whose mothers are illiterate (5.5%), AlAyed *et al,* (2013) from Saudi Arabia and Magzoub *et al,* (2013) have made similar observations. The outcome of adenovirus infection was observed more in children whose mothers have primary education (14.3%: 11/77).The prevalence of adenovirus infection was lowest (4.8%: 1/21) among children whose mother's level of education is tertiary The high prevalence of rotavirus and adenovirus infection among children whose mothers have little or no formal education may be explained by the fact that the more educated parents will have the skills, practice and knowledge to protect their children from likely exposure to viral enteric pathogens. It has been shown that the more educated mothers are, the more they knew about ways to take care of their children with diarrhoea and lower the prevalence. This study, along with others, has shown that diarrhoea is more common among children whose mothers have a low level of education (Nguyen *et al*., 2007; Moawed *et al*., 2000; Bani *et al*., 2002).

5.2 Electron Microscopy

The detection of rotavirus particle in this study is similar to the report of Aminu, (2006). Similar to the findings of our study, Aminu *et al*, (2007) detected only one adenovirus particle out of the ten examined by electron microscopy while Avery *et al*, (1992) did not detect any adenovirus particle from 66 diarrhoel stool specimens examined by electron microscopy.

The detection of rotavirus and adenovirus particles in just a few samples examined by electron microscopy in our study may attributable to insufficient amount of viral particle in stool as it has been shown that electron microscope detects greater than or equals to 10^6 particles per ml and that specimens containing viral particle below the detection threshold for electron microscope would not be detected (Moore *et al.*, 1998).

Paul and Hans (2003) opined that “several major pitfalls exist in the identification of viral agents by negative stain electron microscopy. First, the failure to detect and identify an agent does not mean that it is not there. Second, if you look long enough and hard enough you will eventually find something that resembles what you wish to find.”

5.3 Co-Infection of Rotavirus with Adenovirus

Co-infection of rotavirus with adenovirus in this study was observed in 0.6% of the diarrhoeic children. This result is comparable with the results of Nimzing *et al*, (2000) where a prevalence of 1.1% was reported. However, our result is lower than previous reports by Aminu *et al,* (2008) and Tran *et al,* (2010) where they both reported a prevalence of 3.2%. The dual infection observed in this study may be attributable to either a single virus is responsible for the diarrhoea or the two viruses act in synergy. More so, the multiple infections observed may be attributable to poor hygienic conditions and overcrowding peculiar to the study area. Poor hygienic condition has been suggested to contribute to multiple infections in developing countries (Guix *et al*., 2002).

5.4 Nutritional Status

The finding of this study reflects high prevalence of malnutrition among the children in the study area and is a matter of public health concern. Our results of 59.0, 47.0 and 46.3% prevalence of underweight, stunting and wasting among the children is comparable with the report of Nigeria Demographic and Health Survey 2013 by the National Population Commission were 46.0, 58.5 and 24.3% prevalence of underweight, stunting and wasting was reported in Katsina State, Nigeria. In this study, underweight was the most prevalent nutritional problem identified. This higher prevalence of underweight in this study as compared to other indicators of undernutrition may be explained by the fact that it reflects both past (chronic) and present (acute) under nutrition among the children. This finding is in agreement with the findings of Goon *et al,* (2011) in Makurdi where they found underweight among the children studied to be the most prevalent (52.7%) among other indicators. However, the findings is in contrast to the results of Nayera *et al,* (2012) in South Sinai, Egypt and Chesire *et al,* (2008) in Kenya where they stated that there is under nutrition among the children they studied with stunting being the most prevalent.

Analysis of the nutritional status by sex among the diarrhoeic children showed that girls were more underweight and wasted than boys while boys were more stunted than girls.

Generally, the prevalence of undernutrition was higher in girls than boys in the diarrhoeic children.

Analysis by age among the diarrhoeic population showed that children under 2 years old were more undernourished in this study. The proportion of underweight was highest among children age-group 0-6 months old, while stunting and wasting was highest in children 19-24 months old. The high proportion of underweight in children aged 0-6 months old may be attributed to low birth weight, lack of exclusive breast feeding, it has been shown that exclusive breastfeeding has a rate of only 17% in Nigeria (Victor *et al.*, 2013). High proportion of stunting and wasting in children aged 19-24 months old may be attributed to lack of adequate weaning practice, susceptibility to diarrhoea and other child killer diseases, lack of fully developed immune system.

The relatively high rate of undernutrition among the diarrhoeic as compared to the non-diarrhoeic children may be attributable to the fact that during diarrhoel episodes there is marked decline in dietary intake (due to anorexia, vomiting and with-holding of food), nutrient intake and absorption by about 30% or more. Each episode of diarrhoea can cause weight loss and growth faltering. In infants, there is marked decreased in breast-feeding. Similarly, the relatively high rate of undernutrition among the non-diarrhoeic children may be attributable to infection with other diseases that affects their nutrition because diarrhoea is just among other factors that affects nutrition.

CHAPTER SIX

6.0 CONCLUSIONS

In this study, rotaviruses and adenoviruses were found to be an important cause of diarrhoea in children 0-5 years old in Katsina State, Nigeria. However, the prevalence of rotavirus infection among the children appears to be relatively low while adenoviruses were shown to circulate at a higher frequency in association with gastroenteritis in children less than five years old. Our study provides evidence that adenoviruses can be a leading cause of viral gastroenteritis infection in children less than five years of age.

Rotavirus infection was more prevalent in children 7-12 months old while adenovirus virus was more prevalent in children 0-6 months old. Rotavirus and adenovirus detection was greatest when diarrhoea, vomiting and fever occurred together and lowest when diarrhoea occurred alone. Source of drinking water was the possible risk factor to rotavirus infection in this study.

The findings of this study showed that, there is high rate of undernutrition among the children in the study area especially among those with diarrhoea than in those without diarrhoea; hence provide evidence that diarrhoea is an important cause of undernutrition. Generally, high rate of undernutrition was observed in children within the age-group 0-24 months old in this study.

6.1 RECOMMENDATIONS

Extensive research on adenoviruses should be carried out because their role and position in viral gastroenteritis as shown by this study and many others, is becoming increasingly higher as compared to other viral agents of gastroenteritis.

Since the detection of rotavirus in this study was mostly in children 2 years and below, rotavirus vaccine should be included into the Expanded Program on Immunization (EPI) as this will greatly reduce rotavirus associated morbidity and mortality within this group.

Government at all levels should provide portable and safe drinking water.

Further studies should be carried to fully confirm the effect of diarrhoea in malnutrition.

National program for the prevention, control and management of diarrhoea and malnutrition in children should be established were emphasis on community education on environmental sanitation and personal hygienic practices, improved nutrition enlightenment, improved feeding approaches, proper child rearing, breast feeding and weaning practices should be made.

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APPENDIX 1: QUESTIONNAIRE

PREVALENCE OF ROTAVIRUSES AND ADENOVIRUSES ASSOCIATED WITH DIARRHOEA AND NUTRITIONAL STATUS OF CHILDREN AGED 0-5 YEARS OLD IN KATSINA STATE, NIGERIA.

DATA COLLECTION QUESTIONNAIRE

ELIGIBILITY: children of 0-5 years who have passed ≥ 3 loose/watery stools in the past 24 hours.

(A) BACKGROUND INFORMATION

LGA: ______________________Name/Address of Hospital_____________________

Name of child___________________ Sex: M/F_______________ Age (Months):_______

(B) ANTHROPOMETRIC INDICES

Weight (kg)……………………. Height (cm) ………………………..

(C) SOCIO-ECONOMIC DATA

1. Occupation of father:

Civil servant ☐ Trader ☐ Artisan ☐ Farmer ☐ Unemployed ☐

2. Occupation of mother:

Civil servant ☐ Trader ☐ Artisan ☐ Farmer ☐ Housewife ☐

3. Level of father`s formal education:

None ☐ Primary ☐ Secondary ☐ Tertiary ☐

4. Level of mother`s formal education:

None ☐ Primary ☐ Secondary ☐ Tertiary ☐

5. Main source of drinking water:

Pipe-borne ☐ Private well ☐ Public well ☐

Borehole ☐ River/Stream ☐ Other(s) (specify)_________________

6. Treatment of drinking water:

Boiling ☐ None/raw ☐ Filtering ☐ Other(s) (specify)_______

7. Main type of toilet:

Pit latrine ☐ Open-field ☐ Water closet ☐

Other(s) (specify)_________________________________

(D) TRADITIONAL MANAGEMENT OF DIARRHOEA

1. How do you know your child has diarrhoea ____________________________________

____

2. Where do you go for the treatment when you notice your child is sick______________?

3 (a). Have you used any drug to treat the child before coming to the hospital?

YES ☐ NO ☐

3(b). If YES, where did you obtain the drug? _____________________________________

4. What do you think is the cause of the diarrhoea ________________________________

5(a) Have you used ORS? YES ☐ NO ☐

5(b). If NO, why? ____

(E) ASSESSMENT OF CHILD FOR DIARRHOEA

(a). **MEDICAL HISTORY**

1. How many times has your child passed loose stools in the last 24 hours? _____________

2. For how long has the child passed loose stool? _________________________________

3. (a) Is the child vomiting? YES ☐ NO ☐

3. (b) If yes, how many times has he vomited in the last 24 hours?____________________

4. Has there been any blood in the stool? YES ☐ NO ☐

5. Did your child had diarrhoea in the last 3 months? YES ☐ NO ☐

(b). **PHYSICAL EXAMINATION OF THE CHILD**

1. What is the child general condition?

Well and alert ☐ Restless and irritable ☐ Lethargic/unconscious ☐

Malnourished ☐ Normal eyes ☐ Sunken eyes ☐

2. Does the child have fever? YES ☐ NO ☐

3. Degree of dehydration: None ☐ Mild ☐ Moderate ☐ Severe ☐

APPENDIX 2: Anthropometric measurements and Z-scores

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
1	Male	1096	36	10	No	79	-0.47	-4.61	-3.01
2	Male	731	24	10.5	No	86.5	-1.73	-0.21	-1.28
3	Male	1096	36	10	No	87	-2.51	-2.45	-3.01
4	Male	517	17	9.5	Yes	78	-0.72	-1.23	-1.1
5	Male	426	14	7	Yes	75	-3.81	-1.23	-3.29
6	Male	487	16	7	Yes	74	-3.56	-2.4	-3.61
7	Male	457	15	9	Yes	78	-1.39	-0.46	-1.23
8	Male	1096	36	14.5	No	97	-0.05	0.25	0.09
9	Male	913	30	12	No	89	-0.66	-0.86	-0.87
10	Male	670	22	8.5	Yes	80	-2.58	-2.06	-2.87
11	Male	731	24	12	No	89	-0.66	0.61	-0.11
12	Male	578	19	7.5	Yes	74	-2.69	-3.35	-3.49
13	Male	700	23	8	Yes	76	-2.4	-3.65	-3.49
14	Male	731	24	12.5	No	89	-0.15	0.61	0.25
15	Male	731	24	10.5	No	86	-1.59	-0.37	-1.28
16	Male	609	20	8.5	Yes	73	-0.82	-3.98	-2.57
17	Male	731	24	7.4	No	79	-4.19	-2.66	-4.19
18	Male	670	22	9	No	78	-1.55	-2.51	-2.36
19	Male	731	24	12	No	86	0.09	-0.37	-0.11
20	Male	670	22	9	Yes	87	-3.66	0.32	-2.36
21	Male	731	24	7.2	No	77	-4.09	-3.31	-4.38
22	Male	731	24	12.5	No	86	0.58	-0.37	0.25
23	Male	700	23	8.1	Yes	72	-1.12	-4.99	-3.39
24	Male	731	24	7.2	No	73	-3.13	-4.62	-4.38
25	Male	700	23	8	Yes	76	-2.4	-3.65	-3.49
26	Male	578	19	8	Yes	78	-2.9	-1.9	-2.96
27	Male	639	21	10.5	Yes	76	0.94	-3.18	-0.84
28	Male	731	24	12.5	No	84	1.08	-1.02	0.25
29	Male	670	22	7.2	Yes	76	-3.72	-3.43	-4.17
30	Male	183	6	4	Yes	57	-3.05	-4.97	-5.46
31	Male	152	5	6	Yes	60	0.02	-2.79	-1.99
32	Male	304	10	8	Yes	70	-0.63	-1.43	-1.24
33	Male	335	11	5.8	Yes	68	-3.98	-2.81	-4.21
34	Male	213	7	5.3	Yes	65	-3.95	-1.92	-3.91
35	Male	235	7.7	5	Yes	65	-4.61	-2.37	-4.54
36	Male	274	9	6	Yes	59	0.59	-5.78	-3.52
37	Male	365	12	9	Yes	76	-0.92	0.11	-0.63
38	Male	639	21	7.5	Yes	81	-4.3	-1.44	-3.75
39	Male	731	24	9	No	74	-0.58	-4.3	-2.64
40	Male	639	21	9	Yes	79	-1.62	-2.13	-2.21
41	Male	731	24	7	No	76	-4.2	-3.64	-4.57

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
42	Male	639	21	8	Yes	75	-2.14	-3.53	-3.24
43	Male	365	12	8	Yes	68	0.05	-3.26	-1.7
44	Male	1416	46.52	10	No	87	-2.51	-3.74	-3.76
45	Female	396	13	5.2	Yes	67	-4.2	-3.13	-4.57
46	Female	457	15	4.8	Yes	63	-3.67	-5.3	-5.37
47	Female	731	24	6.5	No	72	-3.39	-4.25	-4.62
48	Female	731	24	9.5	No	83	-1.59	-0.85	-1.57
49	Female	639	21	7	Yes	77	-3.57	-2.17	-3.69
50	Female	731	24	6.5	No	81	-5.3	-1.47	-4.62
51	Female	731	24	5.2	No	73	-5.82	-3.94	-5.96
52	Female	639	21	6	Yes	79	-5.52	-1.52	-4.78
53	Female	731	24	8.5	No	72	-0.27	-4.25	-2.54
54	Female	639	21	8	Yes	78	-2.23	-1.85	-2.58
55	Female	700	23	4.2	Yes	65	-5.64	-6.47	-6.91
56	Female	731	24	9.6	No	89	-3.02	1.01	-1.48
57	Female	731	24	9.5	No	79	-0.61	-2.09	-1.57
58	Female	639	21	8	Yes	73	-1.03	-3.48	-2.58
59	Female	731	24	7	No	75	-3.29	-3.33	-4.1
60	Female	731	24	5.5	No	69	-4.38	-5.18	-5.65
61	Female	731	24	6.5	No	75	-4.09	-3.33	-4.62
62	Female	731	24	8.5	No	81	-2.41	-1.47	-2.54
63	Female	791	26	6.5	No	83	-5.71	-1.33	-4.84
64	Female	852	28	8.5	No	86	-3.69	-0.9	-3.12
65	Female	883	29	9.5	No	82	-1.33	-2.27	-2.29
66	Female	335	11	5.5	Yes	68	-3.91	-1.9	-3.82
67	Female	304	10	6	Yes	68	-2.95	-1.4	-2.91
68	Female	244	8	6	Yes	70	-3.55	0.52	-2.36
69	Female	244	8	4.4	Yes	61	-3.78	-3.29	-4.68
70	Female	274	9	6	Yes	69	-3.26	-0.47	-2.65
71	Female	365	12	5.6	Yes	66	-3.07	-3.11	-3.88
72	Female	274	9	5.7	Yes	65	-2.48	-2.13	-3.09
73	Female	365	12	8.5	Yes	69	0.72	-1.94	-0.42
74	Female	304	10	6.5	No	68	-2.22	-1.12	-2.22
75	Female	609	20	8.5	Yes	80	-1.98	-0.9	-1.89
76	Female	457	15	9	Yes	79	-1.07	0.54	-0.53
77	Female	578	19	6.5	Yes	77	-4.36	-1.59	-3.95
78	Female	365	12	6.5	Yes	69	-2.31	-1.94	-2.69
79	Male	335	11	8	Yes	66	0.77	-3.67	-1.48
80	Male	731	24	9	No	78.7	-1.71	-2.76	-2.64
81	Male	1461	48	12.5	No	86.4	0.48	-4.04	-2.14
82	Male	1096	36	10	No	92	-3.73	-1.1	-3.01

APPENDIX 2: Anthropometric measurements and Z-scores continued.

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
83	Male	731	24	10	No	81.3	-0.98	-1.91	-1.71
84	Male	731	24	7	No	77.5	-4.51	-3.15	-4.57
85	Male	1461	48	9	No	91.4	-4.82	-2.84	-4.52
86	Male	1461	48	8	No	87.5	-5.28	-3.77	-5.2
87	Male	731	24	10	No	81.3	-0.98	-1.91	-1.71
88	Male	1461	48	10	No	83.8	-1.62	-4.66	-3.84
89	Male	213	7	5.5	Yes	66	-3.9	-1.46	-3.63
90	Male	639	21	9	Yes	83	-2.57	-0.74	-2.21
91	Male	609	20	13	Yes	78.7	2.78	-1.96	1.21
92	Male	274	9	8	Yes	61	2.82	-4.89	-0.98
93	Male	1461	48	13	No	95.3	-1.05	-1.91	-1.82
94	Male	731	24	8	No	78.2	-3.1	-2.92	-3.61
95	Male	1096	36	7	No	71.7	-3.11	-6.58	-5.39
96	Male	548	18	9.5	Yes	78.7	-0.88	-1.32	-1.27
97	Male	487	16	9	Yes	70.5	0.64	-3.76	-1.41
98	Male	1096	36	8	No	86	-4.93	-2.72	-4.6
99	Male	1461	48	16	No	96.5	1.25	-1.63	-0.17
100	Male	1096	36	7	No	83.8	-5.79	-3.31	-5.39
101	Male	1096	36	8	No	91.4	-6.05	-1.26	-4.6
102	Male	548	18	7	Yes	73.7	-3.48	-3.17	-3.89
103	Male	731	24	9.5	No	80	-1.32	-2.33	-2.16
104	Male	1461	48	12	No	90.2	-0.95	-3.13	-2.47
105	Male	731	24	8	No	81.3	-3.79	-1.91	-3.61
106	Male	761	25	9	No	72.4	-0.14	-5	-2.76
107	Male	1096	36	7	No	83.5	-5.72	-3.4	-5.39
108	Male	396	13	10	Yes	73.7	0.95	-1.33	0.11
109	Male	30	0.99	3	Yes	50.6	-1.63	-2.09	-2.81
110	Male	91	3	4	Yes	57.2	-3.19	-2.06	-3.74
111	Male	365	12	6	Yes	76	-5.67	0.11	-4.14
112	Male	731	24	9	No	78.4	-1.64	-2.86	-2.64
113	Male	1096	36	13	No	96.6	-1.33	0.14	-0.81
114	Male	1491	49	14.5	No	99.1	-0.51	-1.13	-1.02
115	Male	609	20	11	Yes	71.1	2.77	-4.66	-0.28
116	Male	731	24	14	No	81	3.03	-2.01	1.24
117	Male	304	10	3.8	Yes	66	-7.5	-3.18	-6.58
118	Male	1461	48	14	No	89.9	1.04	-3.2	-1.23
119	Male	731	24	7	No	78.7	-4.74	-2.76	-4.57
120	Male	548	18	8	Yes	76.2	-2.46	-2.25	-2.8
121	Male	274	9	5	No	67.3	-5.58	-1.77	-4.84
122	Male	548	18	7	Yes	76.2	-4.09	-2.25	-3.89
123	Male	731	24	9	No	83.8	-2.99	-1.09	-2.64

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
124	Male	731	24	7	No	78.6	-4.72	-2.79	-4.57
125	Male	517	17	9	Yes	71.7	0.27	-3.61	-1.58
126	Male	365	12	6	Yes	53	4.54	-9.57	-4.14
127	Male	731	24	8	No	74.9	-2.3	-4	-3.61
128	Female	1461	48	11	Yes	99.1	-3.42	-1.01	-2.91
129	Female	244	8	2.7	Yes	73.7	-10.02	2.08	-7.14
130	Female	1096	36	7	No	83.8	-5.18	-2.96	-5.19
131	Female	61	2	3	Yes	50.6	-1.67	-3.18	-3.91
132	Female	731	24	10	No	78.7	0.03	-2.18	-1.14
133	Female	700	23	7	Yes	71.1	-2.07	-4.54	-3.97
134	Female	91	3	4	Yes	58.5	-3.61	-0.61	-2.97
135	Female	244	8	8	Yes	62.5	2.19	-2.65	0.05
136	Female	731	24	12.5	No	73.7	3.42	-3.73	0.68
137	Female	731	24	10	No	78.7	0.03	-2.18	-1.14
138	Female	61	2	6	Yes	60	0.23	1.43	1.21
139	Female	1096	36	7	No	83.5	-5.12	-3.03	-5.19
140	Female	822	27	13	No	78.7	2.82	-2.84	0.57
141	Female	152	5	5	Yes	58.5	-1.03	-2.49	-2.64
142	Female	731	24	7	No	75	-3.29	-3.33	-4.1
143	Female	304	10	6	Yes	63.5	-1.29	-3.23	-2.91
144	Female	1461	48	16	No	109.5	-1.53	1.57	-0.03
145	Female	1461	48	15	No	91.5	1.55	-2.61	-0.5
146	Female	548	18	10	Yes	71.1	1.88	-3.31	-0.19
147	Female	396	13	6	Yes	61	-0.23	-5.41	-3.55
148	Female	1461	48	10	No	86.4	-1.87	-3.79	-3.61
149	Female	731	24	8	No	78.6	-2.54	-2.21	-3.07
150	Female	304	10	6	Yes	63.5	-1.29	-3.23	-2.91
151	Female	731	24	10	No	86.4	-1.87	0.21	-1.14
152	Female	822	27	12	No	81.3	1.45	-2.07	-0.07
153	Female	1461	48	11	No	99.1	-3.56	-0.84	-2.91
154	Female	1096	36	12	No	87.5	-0.02	-1.98	-1.14
155	Female	30	1	2	Yes	45.5	-2.8	-4.16	-4.68
156	Female	1096	36	9	No	81.3	-1.8	-3.61	-3.51
157	Male	578	19	9.5	Yes	72.5	0.68	-3.9	-1.43
158	Male	244	8	4.7	Yes	63	-4.51	-3.45	-5.03
159	Male	365	12	8	Yes	65	1.15	-4.52	-1.7
160	Male	426	14	5.4	Yes	67	-4.46	-4.46	-5.16
161	Male	365	12	9	Yes	81	-2.07	2.21	-0.63
162	Male	365	12	8	Yes	67.5	0.23	-3.47	-1.7
163	Male	304	10	8	Yes	68	0.05	-2.3	-1.24
164	Male	1461	48	10.5	No	96	-3.94	-1.75	-3.5

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
165	Male	1461	48	14	No	106	-2.47	0.64	-1.23
166	Male	731	24	12	No	84	0.59	-1.02	-0.11
167	Male	731	24	9	No	82	-2.49	-1.68	-2.64
168	Male	426	14	5.4	Yes	67	-4.46	-4.46	-5.16
169	Male	1826	60	10	No	98	-4.86	-2.58	-4.4
170	Male	365	12	6	Yes	68	-3.58	-3.26	-4.14
171	Male	731	24	6.6	No	76	-4.84	-3.64	-4.95
172	Male	213	7	6.5	Yes	65	-1.38	-1.92	-2.21
173	Male	122	4	5.5	Yes	62	-2.13	-0.91	-2.1
174	Male	365	12	9	Yes	75	-0.66	-0.31	-0.63
175	Male	183	6	6.5	Yes	62	-0.05	-2.64	-1.8
176	Male	183	6	5.4	Yes	65	-3.73	-1.23	-3.4
177	Male	883	29	8	No	80	-3.5	-3.34	-4.1
178	Male	1157	38	15	No	103	-0.95	1.48	0.18
179	Male	1826	60	14	No	98.6	-0.83	-2.45	-2.04
180	Male	1461	48	17	No	108	-0.55	1.11	0.31
181	Male	670	22	9	Yes	80	-1.84	-2.06	-2.36
182	Male	1096	36	12	No	97	-2.46	0.25	-1.48
183	Male	731	24	9	No	79	-1.78	-2.66	-2.64
184	Male	1461	48	14.5	No	88	1.88	-3.65	-0.95
185	Male	365	12	9	Yes	78	-1.39	0.95	-0.63
186	Female	365	12	8	Yes	68	0.35	-2.33	-0.92
187	Female	1096	36	14	No	98	-0.52	0.77	0.08
188	Female	335	11	7	Yes	68	-1.13	-1.9	-1.83
189	Female	731	24	8.5	No	86	-3.69	0.08	-2.54
190	Female	1826	60	20	No	115	-0.34	1.17	0.62
191	Female	731	24	12	No	81	1.51	-1.47	0.35
192	Female	517	17	8	Yes	82	-3.21	0.82	-1.88
193	Female	578	19	9	Yes	88	-3.36	2.13	-1.23
194	Female	731	24	9	No	76.6	-0.69	-2.83	-2.04
195	Female	822	27	8	No	79	-2.64	-2.75	-3.48
196	Female	244	8	7	Yes	64	0.24	-2.02	-1.06
197	Female	731	24	9	No	82	-1.98	-1.16	-2.04
198	Female	1096	36	12	No	96	-1.89	0.25	-1.14
199	Female	1309	43	12.5	No	98	-1.83	-0.41	-1.48
200	Female	731	24	6	No	72	-4.25	-4.25	-5.13
201	Female	122	4	5	Yes	57.7	-0.58	-2.04	-2.03
202	Female	731	24	5.5	No	75	-5.71	-3.33	-5.65
203	Female	1096	36	12	No	96	-1.89	0.25	-1.14
204	Female	183	6	6	Yes	65	-1.88	-0.33	-1.62
205	Female	1461	48	15.5	No	100.5	0.09	-0.52	-0.26

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
206	Female	639	21	10	Yes	82	-0.57	-0.54	-0.67
207	Female	791	26	8	No	79	-2.64	-2.53	-3.35
208	Female	1461	48	17	No	111.5	-1.31	2.04	0.4
209	Female	304	10	7	Yes	71	-2.04	-0.19	-1.59
210	Female	365	12	9	Yes	72	0.54	-0.78	0.05
211	Female	517	17	9	Yes	86	-2.86	2.23	-0.88
212	Female	731	24	6.4	No	75	-4.25	-3.33	-4.72
213	Female	639	21	9	Yes	88	-3.36	1.42	-1.56
214	Female	578	19	6.5	Yes	76	-4.16	-1.93	-3.95
215	Female	731	24	6	No	65	-2.14	-6.42	-5.13
216	Female	1096	36	14	No	88	1.55	-1.85	0.08
217	Female	457	15	6.2	Yes	68.5	-2.72	-3.29	-3.66
218	Female	365	12	9	Yes	73.6	0.14	-0.16	0.05
219	Female	1096	36	13	No	89	0.53	-1.59	-0.5
220	Female	548	18	9.7	Yes	86	-1.94	1.82	-0.44
221	Male	1096	36	9.2	No	84.6	-2.93	-3.1	-3.65
222	Male	731	24	9	No	78.5	-1.66	-2.82	-2.64
223	Male	731	24	6.8	No	78.5	-5.01	-2.82	-4.76
224	Male	1096	36	12.5	No	88	0.09	-2.18	-1.14
225	Male	731	24	9	No	78.5	-1.66	-2.82	-2.64
226	Male	335	11	6.2	Yes	68	-3.18	-2.81	-3.7
227	Male	731	24	6	No	73.5	-5.3	-4.46	-5.53
228	Male	731	24	6.2	No	68	-3.42	-6.26	-5.34
229	Female	244	8	5.6	Yes	58	0.49	-4.55	-2.95
230	Female	1096	36	7.5	No	74	-2.22	-5.53	-4.77
231	Female	487	16	6.8	Yes	68	-1.47	-3.8	-3.11
232	Male	213	7	6.9	Yes	61	1.15	-3.76	-1.67
233	Male	731	24	9.3	No	86	-3.18	-0.37	-2.35
234	Female	731	24	8	No	73.5	-1.33	-3.79	-3.07
235	Female	731	24	7.2	No	76	-3.19	-3.02	-3.89
236	Female	670	22	6	Yes	69	-3.26	-5	-4.9
237	Female	731	24	8	No	79	-2.64	-2.09	-3.07
238	Female	731	24	7	No	71	-2.24	-4.56	-4.1
239	Female	548	18	6.9	Yes	74	-3.04	-2.31	-3.34
240	Female	639	21	7	Yes	82	-4.65	-0.54	-3.69
241	Female	1096	36	7.9	No	81	-3.29	-3.69	-4.43
242	Female	883	29	9.8	No	88	-2.52	-0.55	-2.02
243	Female	578	19	7	Yes	71	-2.04	-3.62	-3.38
244	Female	731	24	6.9	No	73.5	-3.09	-3.79	-4.2
245	Female	731	24	5.2	No	71	-5.4	-4.56	-5.96
246	Female	609	20	10	Yes	86	-1.58	1.09	-0.51

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
247	Female	731	24	7.5	No	89	-5.57	1.01	-3.59
248	Male	365	12	6.5	Yes	82	-5.98	2.64	-3.52
249	Female	731	24	8	No	76	-1.93	-3.02	-3.07
250	Male	609	20	10.5	Yes	80	0.06	-1.5	-0.69
251	Male	1461	48	15	No	104	-1.17	0.16	-0.68
252	Male	1096	36	9	No	96	-5.63	-0.02	-3.81
253	Male	457	15	9.5	Yes	78	-0.72	-0.46	-0.75
254	Female	1096	36	7.9	No	88	-4.89	-1.85	-4.43
255	Female	731	24	7.2	No	86	-5.37	0.08	-3.89
256	Female	1096	36	7.2	No	86	-5.37	-2.38	-5.02
257	Female	578	19	7	Yes	76	-3.35	-1.93	-3.38
258	Female	731	24	6.9	No	73.5	-3.09	-3.79	-4.2
259	Female	517	17	5.3	Yes	66	-3.68	-4.79	-5.05
260	Female	548	18	6.9	Yes	68	-1.3	-4.38	-3.34
261	Female	731	24	7	No	76	-3.51	-3.02	-4.1
262	Female	1096	36	7	No	85	-5.43	-2.64	-5.19
263	Female	731	24	7	No	72	-2.52	-4.25	-4.1
264	Female	731	24	6.2	No	78	-5.16	-2.4	-4.93
265	Female	1096	36	7.9	No	89	-5.08	-1.59	-4.43
266	Female	304	10	3.2	Yes	68	-8.33	-1.4	-6.74
267	Female	731	24	7.9	No	82	-3.53	-1.16	-3.17
268	Female	365	12	6.5	Yes	73	-3.47	-0.39	-2.69
269	Female	487	16	6.3	Yes	76	-4.48	-0.93	-3.71
270	Female	944	31	10.6	No	82	-0.08	-2.64	-1.61
271	Female	731	24	8	No	73	-1.21	-3.94	-3.07
272	Female	487	16	6.3	Yes	76	-4.48	-0.93	-3.71
273	Female	1461	48	14	No	98	-0.52	-1.1	-1.02
274	Female	365	12	4.8	Yes	76	-6.88	0.77	-4.92
275	Female	487	16	9.5	Yes	78	-0.24	-0.22	-0.26
276	Female	731	24	7.5	No	79	-3.4	-2.09	-3.59
395	Male	365	12	4.5	Yes	78	-8.37	0.95	-5.98
396	Male	246	8.07	4.2	Yes	68	-7.17	-1.23	-5.72
279	Male	1461	48	6	No	81	-6.66	-5.32	-6.56
280	Male	731	24	10	No	83	-1.4	-1.35	-1.71
281	Male	365	12	8	Yes	72	-1.27	-1.57	-1.7
282	Male	639	21	8.5	Yes	87	-4.33	0.65	-2.72
283	Male	548	18	6	Yes	70	-4.22	-4.55	-4.97
284	Male	274	9	4.8	Yes	65	-5.05	-3.11	-5.11
285	Male	609	20	6.5	Yes	78	-5.25	-2.21	-4.66
286	Male	731	24	6.3	No	78	-5.68	-2.99	-5.24
287	Male	335	11	3.5	Yes	79	-10.04	1.91	-7.1

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
288	Male	152	5	5.5	Yes	60	-1.04	-2.79	-2.75
289	Male	578	19	10.5	Yes	80	0.06	-1.17	-0.53
290	Male	183	6	6	Yes	65	-2.41	-1.23	-2.51
291	Male	457	15	10	Yes	74	0.87	-2.04	-0.28
292	Male	487	16	9.5	Yes	77	-0.5	-1.24	-0.92
293	Male	1096	36	12.5	No	88	0.09	-2.18	-1.14
294	Male	426	14	8.6	Yes	75	-1.23	-1.23	-1.45
295	Male	731	24	11.5	No	82	0.56	-1.68	-0.48
296	Male	426	14	7	Yes	75	-3.81	-1.23	-3.29
297	Male	1461	48	10.1	No	89	-2.91	-3.42	-3.77
298	Male	335	11	8.5	Yes	72	-0.52	-1.09	-0.93
299	Male	578	19	10.8	Yes	81	0.18	-0.81	-0.28
300	Male	304	10	7.1	Yes	87	-6.21	6.01	-2.32
301	Male	639	21	9.8	Yes	89	-3.13	1.35	-1.46
302	Male	274	9	8.3	Yes	69	0.15	-1.32	-0.64
303	Male	213	7	8	Yes	65	1.15	-1.92	-0.33
304	Male	1461	48	16	No	112	-2.14	2.07	-0.17
305	Male	91	3	4	Yes	55	-1.53	-3.13	-3.74
306	Male	183	6	4.2	Yes	62	-5.28	-2.64	-5.16
307	Male	304	10	4.7	Yes	70	-6.67	-1.43	-5.42
308	Male	365	12	4	Yes	76	-8.92	0.11	-6.59
309	Male	183	6	4.7	Yes	67	-5.9	-0.3	-4.43
310	Male	731	24	10.4	No	79	0.01	-2.66	-1.37
311	Male	700	23	5.4	Yes	72	-5.85	-4.99	-6.04
312	Male	274	9	5.9	Yes	63	-1.72	-4	-3.65
313	Male	335	11	6.5	Yes	69	-2.94	-2.38	-3.33
314	Male	152	5	5.5	Yes	68	-4.58	1	-2.75
315	Male	670	22	9.5	Yes	82	-1.62	-1.38	-1.88
316	Male	731	24	7.2	No	86	-6.01	-0.37	-4.38
317	Male	1461	48	20.2	No	103	2.51	-0.08	1.64
318	Male	517	17	10	Yes	80	-0.53	-0.47	-0.63
319	Male	365	12	2.8	Yes	56	-6.14	-8.31	-8.06
320	Male	700	23	8.5	Yes	82	-3.06	-1.65	-3
321	Male	578	19	10	Yes	75	0.61	-2.99	-0.97
322	Female	335	11	5.8	Yes	68	-3.34	-1.9	-3.42
323	Male	548	18	9.5	No	75	-0.18	-2.43	-1.27
324	Male	1096	36	12.5	No	85	0.83	-2.99	-1.14
325	Male	1461	48	12.5	No	91.5	-0.72	-2.82	-2.14
326	Male	213	7	5.8	Yes	66	-3.26	-1.46	-3.2
327	Male	1461	48	12.5	No	92	-0.83	-2.7	-2.14
328	Male	1461	48	16	No	96.5	1.25	-1.63	-0.17

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
329	Male	7	0.23	2.8	Yes	48	-0.58	-1.64	-1.47
330	Male	1096	36	11.8	No	91.5	-1.46	-1.24	-1.62
331	Male	487	16	8.4	Yes	75	-1.52	-2.02	-2.03
332	Male	731	24	9	No	79	-1.78	-2.66	-2.64
333	Female	731	24	10	No	82	-0.74	-1.16	-1.14
334	Female	1096	36	11.8	No	89	-0.56	-1.59	-1.27
335	Female	1461	48	12.5	No	94	-0.99	-2.03	-1.89
336	Female	822	27	12	No	81.3	1.45	-2.07	-0.07
337	Female	396	13	7	Yes	68	-1.13	-2.75	-2.27
338	Female	1461	48	15	No	91.4	1.57	-2.63	-0.5
339	Female	304	10	6.5	Yes	66	-1.31	-2.22	-2.22
340	Male	731	24	10.2	No	86	-1.97	-0.37	-1.54
341	Male	609	20	8.5	Yes	73	-0.82	-3.98	-2.57
342	Male	731	24	10.6	No	80	0.02	-2.33	-1.2
343	Female	852	28	9.6	No	82	-1.21	-2.07	-2.06
344	Female	274	9	5.7	Yes	65	-2.48	-2.13	-3.09
345	Female	365	12	6.5	Yes	69	-2.31	-1.94	-2.69
346	Female	578	19	6.5	Yes	77	-4.36	-1.59	-3.95
347	Female	791	26	8.5	Yes	83	-2.75	-1.54	-2.84
348	Female	639	21	8	Yes	78	-2.23	-1.85	-2.58
349	Female	731	24	9.5	Yes	81	-0.92	-1.68	-1.57
350	Male	639	21	8	Yes	75	-2.14	-3.53	-3.24
351	Male	639	21	8.8	Yes	87	-3.93	0.65	-2.41
352	Male	731	24	9.8	Yes	80	-0.78	-2.56	-1.89
353	Male	274	9	6.5	Yes	65	-1.38	-3.11	-2.85
354	Male	731	24	10	No	86	-2.23	-0.37	-1.71
355	Male	731	24	9	No	83	-2.76	-1.35	-2.64
356	Male	1096	36	14.5	No	97	-0.05	0.25	0.09
357	Female	122	4	5	Yes	67.7	-4.79	2.58	-2.03
358	Female	731	24	8.5	No	85	-3.45	-0.23	-2.54
359	Male	426	14	9.4	Yes	87	-3.13	3.61	-0.65
360	Male	304	10	6.5	Yes	68	-2.57	-2.3	-3.1
361	Male	1461	48	14	No	102	-1.59	-0.32	-1.23
362	Male	731	24	8.5	No	84	-3.74	-1.02	-3.13
363	Male	426	14	8.4	Yes	77	-2.03	-0.42	-1.67
364	Male	122	4	5.5	Yes	62	-2.13	-0.91	-2.1
365	Male	731	24	8.5	No	86	-4.26	-0.37	-3.13
366	Female	731	24	12.5	No	81	1.94	-1.47	0.68
367	Female	244	8	5	Yes	64	-3.61	-2.02	-3.82
368	Female	791	26	8	No	89	-4.96	0.47	-3.35
369	Female	1461	48	17	No	111.5	-1.31	2.04	0.4

APPENDIX 2: Anthropometric measurements and Z-scores continued…

ID	Sex	Age (d)	Age (m)	Weight (kg)	Recumbent	Height (cm)	WHZ	HAZ	WAZ
370	Female	517	17	8	Yes	78	-2.23	-0.58	-1.88
371	Female	578	19	9	Yes	79	-1.07	-0.92	-1.23
372	Male	731	24	10	No	83	-1.4	-1.35	-1.71
373	Male	274	9	5.8	Yes	65	-2.85	-3.11	-3.78
374	Male	91	3	4	Yes	56	-2.29	-2.64	-3.74
375	Male	335	11	6.5	Yes	68	-2.57	-2.81	-3.33
376	Male	670	22	9.5	Yes	82	-1.62	-1.38	-1.88
377	Female	731	24	8.2	No	84	-3.6	-0.54	-2.86
378	Female	731	24	9.8	No	80	-0.49	-1.78	-1.31
379	Female	731	24	6.9	No	82	-4.94	-1.16	-4.2
380	Female	517	17	11	Yes	86	-0.47	2.23	0.75
381	Male	517	17	10	Yes	78	-0.1	-1.23	-0.63
382	Male	639	21	9.8	Yes	89	-3.13	1.35	-1.46
383	Male	731	24	10.4	No	79	0.01	-2.66	-1.37
384	Female	548	18	6.9	Yes	78	-3.94	-0.93	-3.34
385	Male	731	24	10.9	No	83.5	-0.45	-1.19	-0.95
386	Male	731	24	8	No	79	-3.28	-2.66	-3.61
387	Male	670	22	8.5	Yes	79	-2.35	-2.41	-2.87
388	Female	731	24	10.8	No	78	1.03	-2.4	-0.5
389	Male	731	24	9.8	No	86	-2.49	-0.37	-1.89
390	Male	213	7	6.9	Yes	61	1.15	-3.76	-1.67
391	Male	1096	36	10.7	No	88	-1.89	-2.18	-2.44
392	Male	731	24	8.2	No	73.5	-1.61	-4.46	-3.42
393	Female	944	31	10.6	No	82	-0.08	-2.64	-1.61
394	Male	304	10	6.2	Yes	68	-3.18	-2.3	-3.49
395	Female	487	16	9.3	Yes	76	-0.04	-0.93	-0.44
396	Female	21	0.69	3	Yes	45	2.11	-3.88	-1.7
397	Female	1461	48	13	No	96	-0.96	-1.56	-1.58
398	Female	609	20	9	Yes	78	-0.85	-1.56	-1.4
399	Male	8	0.26	3	Yes	45	2.17	-3.3	-1.09
400	Male	244	8	6.5	Yes	64	-0.96	-3	-2.56

KEY: WHZ= Weight for-Height Z-Score

HAZ= Height for-Age Z-Score

WAZ= Weight for-Age Z-Score

APPENDIX 3: Graphs of Z-scores of children in relation to WHO standard curves

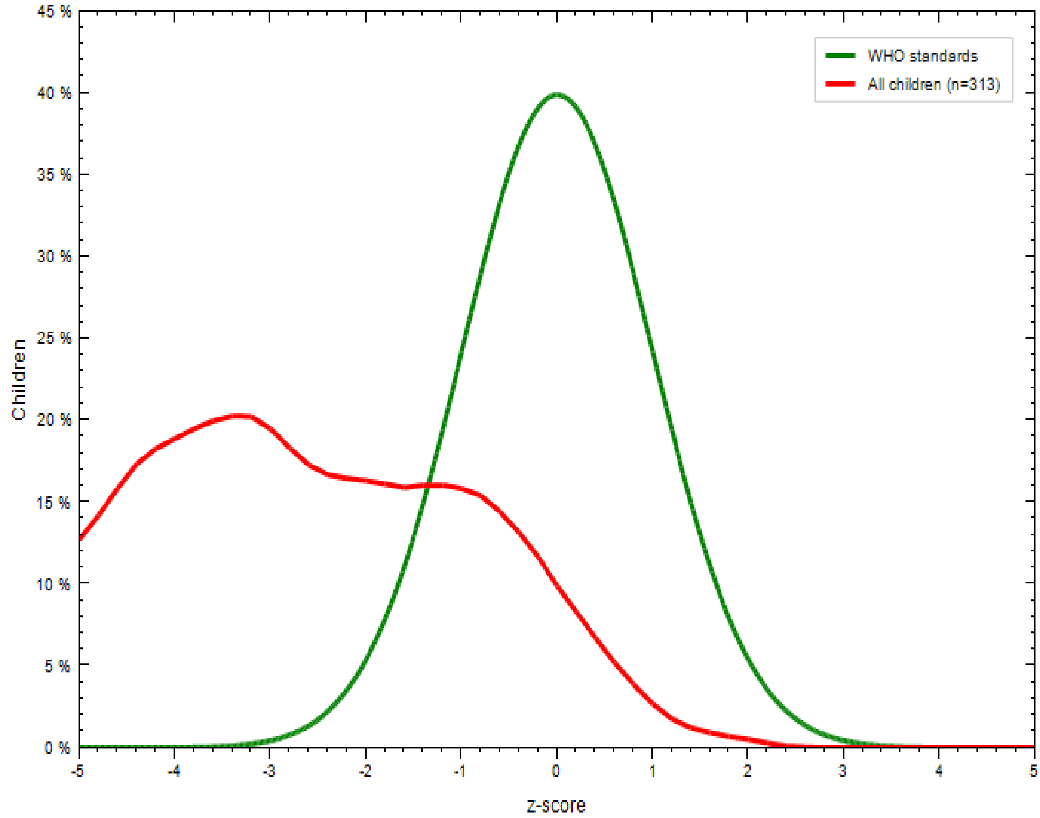

Figure 4.6a: WAZ of the diarrhoeic children in relation to WHO standard curve.

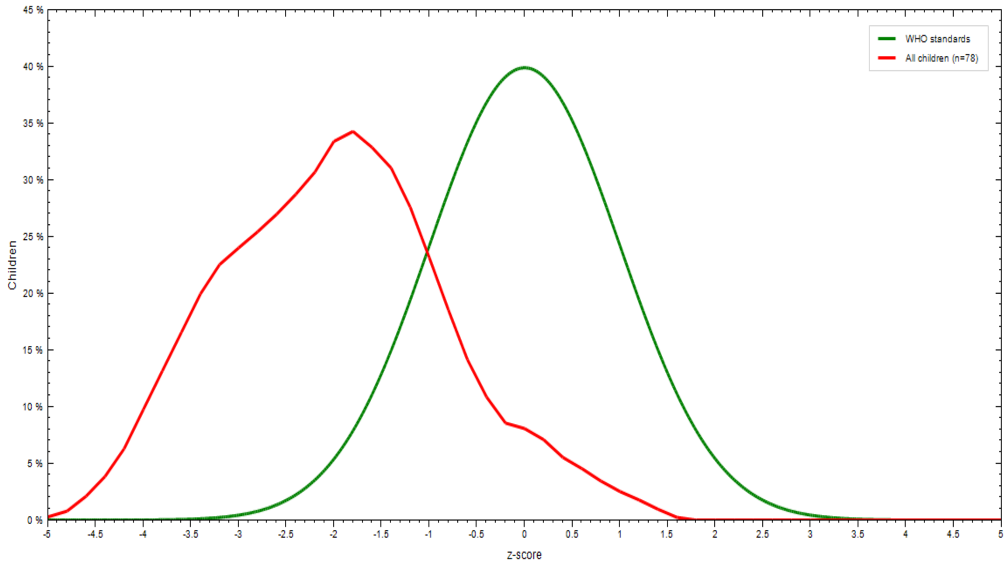

Figure 4.6b: WAZ of the non-diarrhoiec children in relation WHO standard curve.

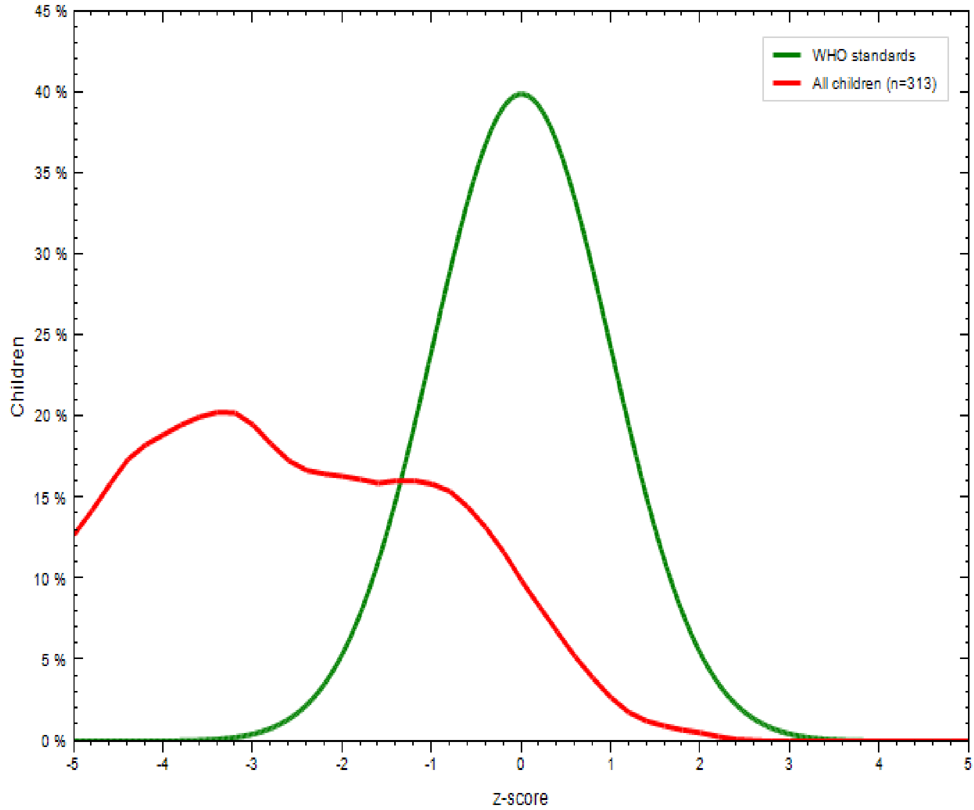

Figure 4.7a: HAZ of the diarrhoeic children in relation to WHO standard curve.

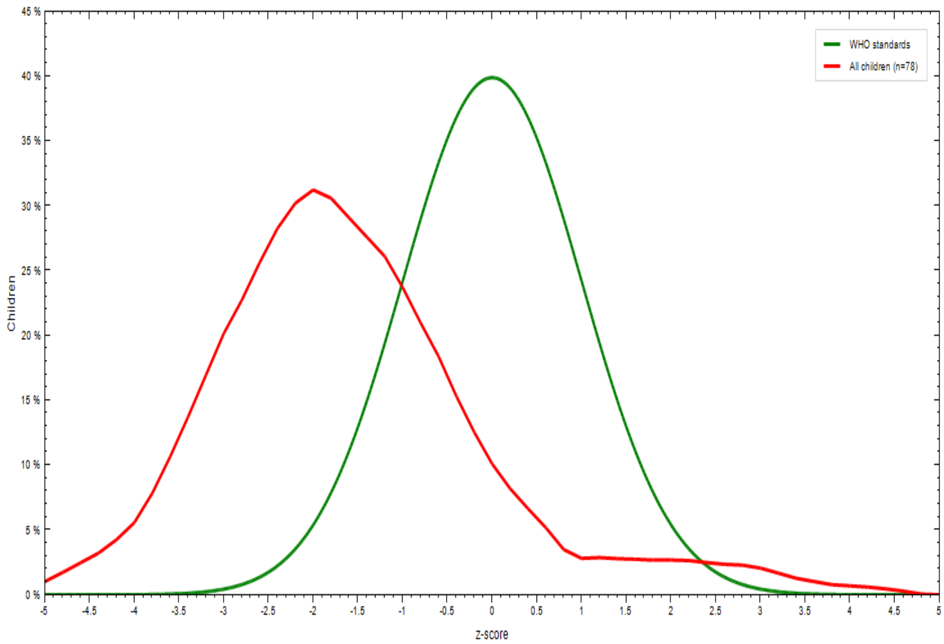

Figure 4.7b: HAZ of the non-diarrhoiec children in relation to WHO standard curve.

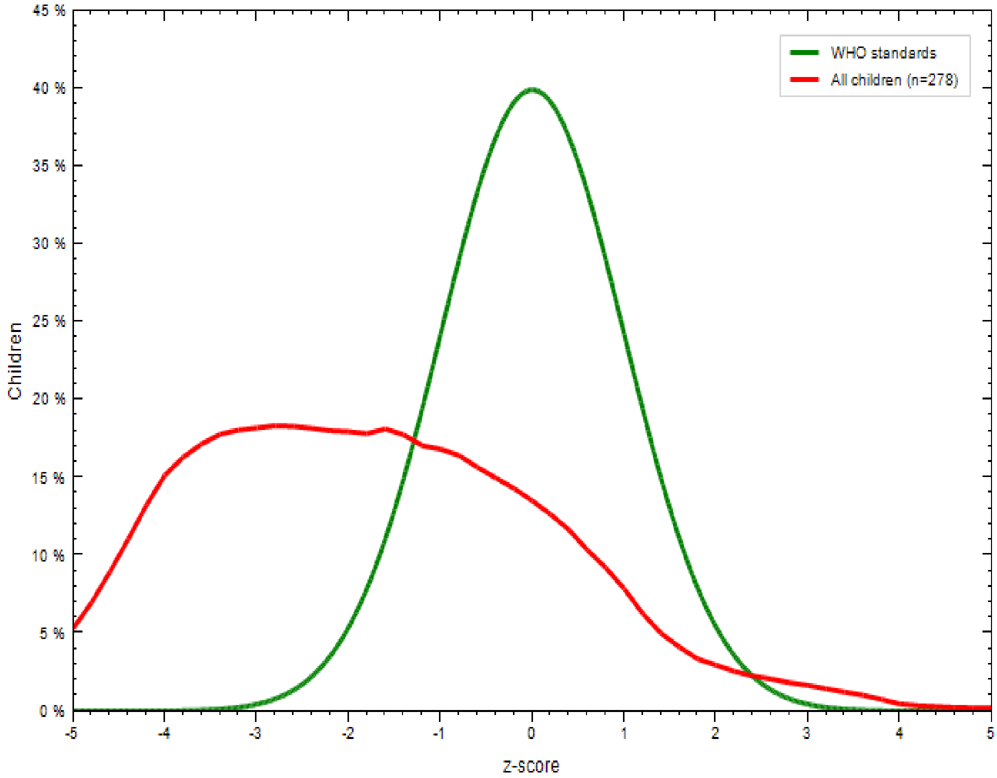

Figure 4.8a: WHZ of the diarrhoeic children in relation to WHO standard curve.

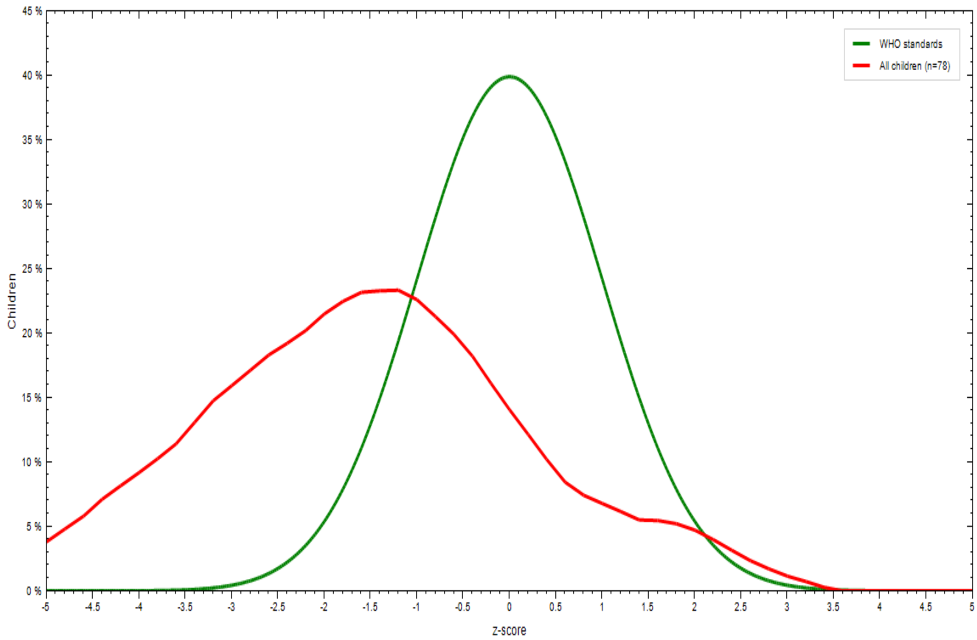

Figure 4.8b: WHZ of the non-diarrhoeic children in relation to WHO standard curve.

APPENDIX 4: INFORMED CONSENT FORM (ICF)

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

INFORMED CONSENT FORM (ICF)

***Serial No.:* …………. *Hospital No.:* …………………… *Age:* …………….. *Phone No.:* ……….……………………….**

This Informed Consent Form is basically for children 0-5 years attending some selected Hospitals in Katsina State. We are inviting your child/ward to participate in this research work titled "Prevalence of some diarrhoea associated enteric viruses and the nutritional status of children aged 0-5 years old in Katsina State, Nigeria. The research will involve collection of stool samples of participants. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I ………………………………father/mother of ……………………………………………….. do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that stool sample will be taken for the test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant …………………………………………………….…
Signature/right thumb print of participant ………………………………. Date …………………….

Statement by Witness:

I have witnessed the accurate reading of the consent form and detail explanation of the study to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness…………………………………………………………
Signature of witness……………………………………………….…. Date ……………….

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability.

I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher ……………………………………………………….

Signature ………………………………………………………………...   Date. ………………

APPENDIX 5: RESEARCH ETHICAL APPROVAL

MINISTRY OF HEALTH
KATSINA STATE

TEL" Hon. Commissioner 065-434537(DL).
434518(DL)

State secretariat Complex,
I.B.B. Way Dandagoro
P.M.B.2075,Katsina

Permanent Secretary 065-35554

Our Ref

17th June, 2013

Mukhtar G. Lawal,
Dept of Microbiology,
A.B.U. ZARIA.

APPROVAL OF THE DIARRHEA RESEARCH

I have been directed to convey to you the approval of the honorable commissioner on your research proposal "Epidemiology of enteric viruses associated with diarrhea in children 0-5 Years old in Katsina state". That was after the state ethics subcommittee had gone through the proposal and recommended it to the state ORAC which deliberated on it and finally recommended it for approval by the commissioner.

Kindly adhere to all principles guiding ethical conduct of research in Nigeria and we wish to inform you that ORAC will as usual require your logistic support in monitoring this activity.

We also would like to share the findings.

Please accept the Hon. Commissioner's highest regards.

Dr Idriss A. H

For
Hon Commissioner

"Home of Heritage and Hospital"

APPENDIX 6

Table 4.4: Distribution of Rotavirus and Adenovirus Infection by Hospital in Children 0-5 Years Old in Katsina State, Nigeria

	Rotavirus		**Adenovirus**		
Hospital	**Positive (%)**	**Negative**	**Positive (%)**	**Negative**	**Total**
GH Dutsin-ma	5 (10.9)	41	7 (15.2)	39	46
TUY-MCH Katsina	4 (6.3)	59	3 (4.8)	60	63
GH Baure	0 (0.0)	45	8 (17.8)	37	45
GH Daura	2 (4.3)	45	3 (6.4)	44	47
GH Funtua	6 (10.7)	50	5 (8.9)	51	56
MCH Malumfashi	0 (0.0)	65	14 (21.5)	51	65
Total	**17 (5.3)**	**305**	**40 (12.4)**	**278**	**322**

Key:
GH= General Hospital
TUY-MCH= TuraiUmaruYardua- Maternal and Children Hospital
MCH= Maternal and Children Hospital

APPENDIX 7

Table 4.5: Distribution of Rotavirus and Adenovirus Infection by Senatorial Zone in Diarrhoeic Children 0-5 Years Old in Katsina State, Nigeria

	Rotavirus		**Adenovirus**		
Senatorial Zone	**Positive (%)**	**Negative**	**Positive (%)**	**Negative**	**Total**
Katsina South	6 (5.0)	115	19 (15.7)	102	121
Katsina Central	9 (8.3)	100	10 (9.2)	99	109
Katsina North	2 (2.2)	90	11 (12.0)	81	92
Total	**17 (5.3)**	**305**	**40 (12.4)**	**282**	**322**

$^{RV}\chi^2$= 3.542, df= 2, p= 0.170163; $^{AdV}\chi^2$= 0.0049, df= 1, P= 0.94395

Key: RV= Rotavirus; AdV= Adenovirus