1. INTRODUCTION

1.1 Background:

Diarrheal disease has been recognized in humans since antiquity. Until the early 1970s, a bacterial, viral, or parasitic etiology of diarrheal disease in children could be detected in fewer than 30% of cases. In 1973, Bishop and colleagues observed a virus particle in the intestinal tissue of children with diarrhea by using electron micrography. This virus was subsequently called “rotavirus” because of its similarity in appearance to a wheel (Rota is Latin for wheel). By 1980, rotavirus was recognized as the most common cause of severe gastroenteritis in infants and young children in the United States. It is now known that infection with rotavirus is nearly universal, with almost all children infected by 5 years of age. Rotavirus is responsible for 20–60 deaths per year in the United States and up to 500,000 deaths from diarrhea worldwide (Fischer et al; 2007). The group A rotavirus is the most important etiological agent that causes diarrhea in newborn humans and animals. They belong to the Reoviridae family and have a genome consisting of 11 segments of double-stranded RNA enclosed in a triple-layered capsid. Rotaviruses are classified in six groups (A to G) based on the VP6 capsid protein or on the migration pattern of genomic segments in polyacrylamide gel. Groups A, B, and C were found either in humans or in animals, while groups D to G were found only in animals (Estes et al; 2001). Rotavirus group A has represented the most ordinary cause of worldwide childhood acute diarrhea. in developed countries, it was estimated that gastroenteritis associated to these etiological agents has been responsible for 600000 to 870000 death / year, which means 20 to 25 % of death due to diarrheic disease, as well as 6% of global mortality among children below five years old (Linhares; 2000).
Children between the ages of 6 and 24 months are at greatest risk for developing severe disease from rotavirus infection. Rotavirus infection in the bowel is the most common cause of severe diarrhea and causes the death of about 600,000 children worldwide annually. The highest prevalence of the disease is experienced in temperate climates during the cooler months of the year (Barnett; 1982). In tropical climates rotavirus infection can occur year round (Kapikian and Chanock; 1996). The age groups most susceptible to the disease are that of infants and children and geriatric patients (Barnett; 1982). This rate has not declined between 1993 and 2002 (Charles et al; 2006). In adults rotavirus infection effect is usually mild. Rotavirus is transmitted by the fecal-oral route, via contact with contaminated hands, surfaces and objects. Studying molecular and pathophysiological changes in diseases are useful to understand and interpret possible therapeutically targets (Surendran, 2005; Surendran and Kumaresan 2007; Surendran et al; 2007). In other diarrheal disease, important of hygiene and sanitation may reduce the incidence, but these measures are unlikely sufficient for rotavirus control. Vaccine is only control measure likely to have a significant impact on the incidence of severely dehydrating rotavirus disease (Dennhey; 2008). In adult, symptomatic rotavirus infection are relatively rare, but can cause health problems and outbreaks in the elderly and in immune compromised individuals (Anderson and Weber; 2004; Kirk et al; 2010).

Rotavirus C has been associated with rare and sporadic cases of diarrhoea in children, and small outbreaks have occurred in families (Desselberger et al; 2001).
1.2 Rationale:

There is an increase in morbidity and mortality rates among children below five years of age with diarrheal disease annually. Rotaviruses play a major causative agent especially in developing countries including Sudan; however the clinical significance of rotavirus species is not fully understood. The majority of the research done in Sudan on rotavirus strains was on animals (Osman; 2004) and there are limited number of studies in human rotaviruses (Mukhtar; 2006, Hemidan et al; 2011 and Abdalla; 2011). For these reasons and because of the clinical importance of the disease we found it highly important to study the frequencies of rotaviruses in children with non-specific diarrhoea presenting to many teaching hospitals in Khartoum State, Sudan.

1.3 Objectives:

1.3.1 General objective:

To identify rotavirus antigen by ELISA in stool of children with diarrhea.

1.3.2 Specific objective:

- To determine the association between rotavirus associated diarrhoea and child age, sex and symptoms (fever, vomiting). -To determine the frequency of rotaviruses associated diarrhoea of children.
2. LITERATURE REVIEW

2.1 history of rotavirus:

D.r Ruth bishop is credited as the discoverer of the rotavirus in human. She isolated the virus in cell taken from the intestine of kids with severe gastrointestinal disorder, according to the national institute of health. The Australian coined the term ‘duovirus’, because the virus was found in apportion of the intestine called duodenum. In 1974, a year after bishop’s discovery, Irish Dr.Thomas Hennery Flewett suggested the name we know the virus by today. Flewett noted that the virus looked like circle, so he called ‘rotavirus’; in a nod to the Latin word rota for wheel (CDC; 2009). In 1976, related viruses were described in several other species of animals. These viruses all causing acute gastroenteritis, were recognised as a collective pathogen affecting human and animal’s worldwide (Flewett and Wood; 1978). Dr. Richard, of what is now part of Cincinnati Children Hospital Medical Centre, began the journey to develop a vaccine in the early 1980. He was later joined by Dr. David by the mid 1980; the doctor began to experiment with a vaccine in adult volunteers, attempting to isolate the smallest dose that would cause rotavirus infection. The first studies with children came a few years later, in 1988 the vaccine administered to more than 200 children (Vesikari et al; 1985).

2.2 Geographical distribution of rotavirus:

Rotavirus infection has been reported throughout the world. Studies between 1986 and 2006 showed that more than 51 rotavirus genotypes were found in Brazil. Approximately 43 of genotypes were that of P [8] G1, followed by P [8] G9 (22) and P [4] G2 (7). In Kenya, the genotype G1 was mainly observed up to the year 2002. Then G9 has emerged as the most predominant genotype and followed by a
less frequent genotype G8 (Kiulia et al.; 2008). In the United States, the G9 genotype was detected in a 1995–1996 outbreak (Ramachandran et al.; 1996). In Australia, the overall G9 detection rate increased up to 29 % in 2001 (Kirkwood et al.; 2003). In Japan, G9 was mainly reported in 1998–1999 (Zhou et al.; 2000). In India, G9 strains were detected and were usually found in combination with the P[11] or P[6] genotypes at a detection rate of about 20 % (Das et al.; 1994). While genotypes G1P8, G2P4, G3P8, and G4P8 were also seen among Indian children (33 %), strains of P6 (G1P6, G2P6, G3P6, G4P6, and G9P6), which primarily infect asymptomatic newborns but are rare in children with diarrhea were common in India (43 %) (Ramachandran et al.; 1996). The P [8] G9 was found in New Delhi in late 1998 (Jain et al.; 2001). In Europe G1–G4 and G9 were the most prevalent genotypes identified: Genotype G1 was identified in Spain, Sweden, and the United Kingdom; G9 in Italy, France, and Belgium; and genotype G4 in Germany. Only the G4 and G9 genotypes were identified in all areas (Banyai et al.; 2004; Damme et al.; 2007).

2.3 Taxonomy:

According to the International Committee on Taxonomy of Viruses (ICTV) Rotaviruses belongs to the family Reoviridae, subfamily Sedoreoviridae and genus Rotavirus where the genuses Rotavirus include five different species, Rotavirus A-E ((ICTV), .The current classification of rotaviruses was established by Matthijnsen. The previous way of classifying group A rotavirus by its serological aspects was gradually replaced when sequencing became a more popular way of analyzing viruses. The sequencing made it possible to compare the genomics of the virus on nucleotide level and a classification system that compared the genome of the virus in the genes VP4 and VP7 was established. In 2008 the classification system was elongated to include all 11 of the rotavirus gene segments. This made it possible to have a classification where the whole genome of the virus was
considered. The classification system is as follows: Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx and are used for the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/NSP6 genes respectively.

A Rotavirus Classification Working Group was established to ensure the accuracy, maintain, and evaluate and develop the new classification system. The group receives nucleotide sequences from potential new rotavirus genotypes a performs their own phylogenetic analysis of the virus (Matthijnssens et al, 2011).

2.4 Host spectrum:
Rotaviruses have a large host range and are able to infect a wide array of mammals and birds. The virus transmits through a fecal-oral route and calves are most often infected by contact with other calves, primarily or secondary through objects, feed and water. It has been proposed that calves can also be infected by virus shed by the dam at birth. The infected calves shed virus through the feces from the second day of infection and the shedding may last for 7-8 days. The virus primarily affects neonatal individuals, and calves more than 3 months of age are usually not affected (Dhama et al., 2009). Rotaviruses were long thought to be host specific but several different studies have shown rotavirus to have a cross species potential. In vivo tests trying to infect mice with both a strain of simian rotavirus as well as bovine rotavirus and recombinants of the two showed that some of the recombinants were able to infect and replicate in the mice several times better than the original viruses. Some genes were shown to have a greater effect on the ability of the virus to infect and replicate. Also showed that a triple reassortant virus found in Korea containing several human and porcine-like genes were able to infect calves and cause severe diarrhea. Here the genes VP4 and NSP1 where of porcine origin while the VP7 was of bovine origin (G6P) (Feng et al; 2011).
2.5 Morphology:
Rotaviruses have a “wheel-like” appearance which explains the origin of its name. The virion consists of a triple layer capsid covering a genome of double stranded RNA. The mature infectious virion has a diameter of 100 nm. The envelope is lipid-free and consists of three concentric layers of protein. The different layers are made up of three of the 13 proteins that the rotavirus genome encodes. VP2 forms the innermost layer and surrounds the viral dsRNA. The middle layer is composed of VP6 and the outermost layer of VP4 and VP7. The middle and outermost layer have 132 large channels that link the outside of the virion to the VP2 layer.

VP7 makes up the base of the outermost layer while VP4 forms spike like extensions extending out of the virion.

VP4 also extends in through the two outer layers, and possibly also have some interaction with the VP6 layer. Both have important roles in the infectivity of the virion. Infectivity is quickly lost in the presence of disinfectants like chlorine and 95% ethanol. These remove the outer shell and thus make the virus unable to infect cells. The virus is stable and infectious in pH range between 3-9 and may under the right concentration of calcium chloride stay infectious for months at 4°C and even up to 20°C (International Committee on Taxonomy of Viruses & King; 2012).

2.6 Genetic structures:
The genome of rotavirus consists of 11 segments of dsRNA averaging 18,550 bp with segments at a varying size between 663 and 3302 bp. All segments share a short common pattern of nucleic acid at the 5’ and 3’ terminals, 10 and 8 nucleotides respectively. Within these there are another common pattern of 30-40 nucleotides that are segment specific. The 11 segments of dsRNA encode 13 different proteins ranging from VP1-VP7 and NSP1-NSP6 where segment 9 encodes for 2 different types of VP7. Of the proteins six have been found to be
structural. Three are associated with the dsRNA, VP1, VP3 and VP2. VP1 and VP3 are directly associated with the dsRNA and make up complexes that link it to the core shell consisting of VP2. The protein VP4 is spike like and extends through the 2 outer shell layers and about 20 nm further outside the virion. Cleavage of VP4 by trypsin stabilizes the spikes by forming VP5 and VP8 which improves the infectivity of the virus. VP8 takes on a crystallized form and have hemagglutination activity. The protein VP7 makes up the outermost shell together with VP4. Less is known about the functions of the six nonstructural proteins, NSP1-NSP6. NSP1 is the largest of the rotavirus proteins and have been shown to bind both zinc and the 5’-end of ssRNA. It serves as a component in the early stages of replication, more specifically the genome segment selection. NSP2 have both ssRNA and dsRNA activity and have a direct role in viral replication but the exact role has yet not been discovered. NMSP3 shuts of the protein 8). Synthesis of the infected cell and promotes viral translation by helping viral mRNA circulate in the cytoplasm. It has also been reported that NSP4 have an endotoxin like effect that causes calcium to be let out of the ER. A product from cleaving of NSP4 is secreted from infected cells and binds to receptors initiating a pathway which result in calcium release from the cell storages. NSP5 have ssRNA and dsRNA binding activity but the effect of this protein is unknown. AndNSP6 has undefined function (International Committee on Taxonomy of virus & king, 2012).

2.7 Replication:
Rotavirus has a cycle of replication at 10-12 hours at 37°C. Little is known about the early steps of replication. VP4 is known to attach to an unknown receptor on the host cell. There are 2 proposed ways of entry into the cell. One is by endocytosis and the other by direct entry by the virus. When the virion enters the cell it loses its outermost shell and the double layered transcriptionally active
particle is set free into the cytoplasm. MRNA 2 transcripts, the full length of the different segments, are produced by DLP3 (International Committee on Taxonomy of Viruses. & King, 2012) associated enzymes from the dsRNA minus strand. The mRNA serves two purposes. They are used in the synthesis of viral proteins and control expression of the individual genes making some genes more transcriptionally active than other. The other purpose is serving as template for genome replication. Minus strand synthesis is accomplished after assembly of all the necessary mRNA and takes place in an intermediate viral core. The intermediate core then transfers to the inside of the endoplasmic reticulum by actions of NSP4. The virion enters the ER by budding in and receiving a temporary envelope. Inside the temporary envelopes lost and VP7 and VP4 form the outermost shell of the virion (International Committee on Taxonomy of Viruses. & King, 2012).

2.8 Seasonality:
All the studies demonstrated circulation of rotavirus year-round, and no clear relationship between the timing of the peak in rotavirus activity with either season (cooler vs. warmer months) or ioditude was observed between countries. The strong winter peak was seen primarily in the Americas, though cities which cover various climate. However in tropics the pattern is less defined and autumn/spring peak are more common (Cook et al; 1990). A recent review considered the tropical region specifically and associated monthly disease incidence with meteorological variables for the same month. The analysis found that a 1 degree centigrade increase in mean temperature resulted in 10% decrease in rotavirus incidence and a 1 cm increase in mean monthly rainfall was associated with a 1% decrease in rotavirus incidence (Levey et al; 2009). Recent data suggest that the seasonality of rotavirus could be changed by the introduction of rotavirus vaccines (Hull et al; 2011).
2.9 Transmission:
Rotavirus is transmitted by the faecal-oral route, via contact with contaminated hand, surface and object, and possibly by the respiratory route. The faeces of an infected person can contain more than 10 trillion infectious particles per gram. Fewer than 100 of these are required to transmit infection to another (Butz et al; 1993).

2.9.1 Fecal transmission:
Rotaviruses multiply in the small intestine of an infected person; the most common mode of transmission is through faecal contact. The virus is transmitted from the hands. After contact with infected faeces, to the mouth. Only small numbers of the virus are needed to cause infection. Infected people can contaminate their hand with faeces after a bowel movement, or infant care taker can contaminate their hand with faeces after disappear change. Hand washing does not always kill rotavirus. Rotavirus can live on the hand for 4 hours and contaminate object for several days (Siberry et al; 2006).

Endemic rotavirus disease is caused primarily by person to person transmission. The principal means of transmission is by the faecal-oral route. Rotavirus is excreted in stool at high level that reaches $10^9$ infectious particles/milliter stools. Transmission of as few as 10 infectious particles can result in infectious. Outbreaks due to contamination of municipal water supplies and food borne transmission have been reported but appear to be rare (Moselio et al, 2007).

2.9.2 Respiratory transmission:
There is some evidence that rotavirus may be transmitted through the air, according to Centers for Disease Control and Prevention. This is evidenced by the finding of rotavirus present in the lung sputum of some infected people. Speculation exists that rotavirus may spread by the respiratory route. Almost one-half of the children with rotavirus
diarrhea tested in the days before symptoms appeared were already shedding virus, and high rates of asymptomatic shedding of rotavirus have also been reported in young children. Presymptomatic shedding and the high rate of asymptomatic rotavirus infection may be important factors in the introduction and transmission of rotavirus (Pickering et al; 1988).

2.10 pathogenesis:

At least three factors are thought to play a role in the pathogenesis of rotavirus include diarrhoea; loss of brush border enzyme, the direct effect of the rotavirus enterotoxin NSP4, and activation of the enteric nervous system. Acute rotavirus infection is associated with decreased level of intestinal brush border enzyme such as maltase, sucrose and lactase leading to malabsorption of d-xylose and lactose in the sitting acute infection (Bishop et al; 1973). Osmotic diarrhoea likely occur as a resulting of villous epithelial cell destruction with resulting brush border enzyme deficiency and complex sugar malabsorption. The rotavirus protein NSP4 has been shown to have direct toxic effect on the gastrointestinal mucosa and antibody to this protein may be associated with protection from rotavirus induced diarrhoea. However, sequence variation in the gene for NSP4 do not always correlate with differences in virulence among strain with the clinical severity of infection. In normal hosts, infections rarely occur in another organ system, although extra intestinal infection has been seen in immunocompromised hosts (Gilger et al; 1992).

2.11 Rotavirus-mediated diarrhoea:

Rotavirus primarily infects small intestinal villus cells and cause watery diarrhoea without any significant intestinal inflammation (Pesavento et al; 2006). NSP4 has suggested playing a critical role in fluid and electrolyte secretion (Ball et al; 1996). Interestingly, NSP4 is absent in the mature infective virion and synthesised in infected villus enterocytes. NSP4 and virus particles are released through the apical
membrane of polarized epithelial cells by a non-classical secretory pathway (Greenberg and Estes; 2009). However, NPS4 is also released from the basolateral side of infected enterocytes, although the role of basolateral-released NPS4 in diarrhoea is not clearly understood (Boshuizen et al; 2004). In contrast to classical secretory diarrhea, the viral enterotoxin, NPS4, induces diarrhea subsequent to maldigestion of carbohydrates concomitant with decrease water absorption, increased calcium mobilization and a relatively mild chloride secretory component. Maldigestion of carbohydrates has been suggested as a major mechanism underlying the pathophysiology of rotavirus-induced diarrhea. Rotavirus infection Caco-2 cells decreases sucrose-isomaltase activity and apical expression in the absence of enterocytes destruction, suggesting the involvement of trafficking mechanism (Jourdan et al, 1998). In addition, NPS4 applied exogenously is known to induce calcium release from intracellular stores and plasmalemmal calcium influx through a phospholipase C-dependent mechanism (PLC) (Greenberg and Estes, 2009). This NPS4 mediated calcium mobilization can support diarrhea by influencing calcium dependent epithelial processes such as ion transport, barrier function or cytoskeletal regulation. Indeed, rotavirus has been demonstrated to increase paracellular permeability in Caco-2 cells (Dickman et al; 2000). In addition, NPS4-mediated calcium mobilization may trigger the release of amines/peptides as well as the release of cytokines, prostaglandins and reactive oxygen species, which can alone or collectively activate the enteric nervous system (Lorr and Vasseur; 2007). Intracellular NSP4 however is also known to increase intracellular calcium levels through a PLC-independent mechanism (Berkova et al; 2003). Recent studies demonstrate that intracellular NSP4 causes action reorganization in a calcium-dependent manner through decreased phosphorylation of the actin remodelling protein cofilin (Berkova et al; 2007). Modulation of subcortical actin dynamics and dysregulation of cofilin influences membrane
trafficking events and ion transport processes (Desmarais et al; 2004). The chloride secretory component underlying the pathogenesis of rotavirus-associated diarrhea is complex, comprised of both pro-and anti-secretory components. Unlike the purely secretory diarrhea caused by cholera toxin, rotavirus infection only moderately increases luminal chloride concentration (Lorrot and Vasser; 2007). An increase in luminal chloride concentrations could be a consequence (Lorrot and Vasser; 2007). Of decreased absorption and/or increased secretion (Ball et al; 1996).

2.12 Damage and clinical finding:

Rotavirus produces a spectrum of disease ranging from asymptotic infection to mild diarrhea, to severe diarrhea with potentially fatal dehydration. Severe gastroenteritis generally occurs in children between 6 and 24 month. Rotavirus infection typically has a 2-day incubation period. Vomiting often precedes the onset of gastroenteritis by 2 or 3 days. Watery diarrhea may last 3-8 days who become symptomatic. Fever and abdominal cramps are common. Red blood cells or leukocytes are generally not found in the stool of patients with rotavirus gastroenteritis. Morphological changes have been identified from biopsies of the mucosa of the proximal of infant and children with rotavirus gastroenteritis; these include shorting and atrophy of the villi, denuded villi and mononuclear cell infiltration of the lamina propria. Viral invasion of the epithelial cells of the small intestine result in destruction of the mature absorptive cells which are then replaced with young, virus free cells. This process results in diarrhea for at least two reasons. First, immature replacement cells have a reduced ability to absorb salt and water. Second, immature cells have a reduced ability to produce disaccharides which result in malabsorption of carbohydrate. The severity of rotavirus–induced diarrhoea is proportional to the extent of mucosal damage in the small intestine. In infant who
are under 6 months of age, rotavirus infection is less common, except for premature neonates who may acquire the infection during outbreaks in neonatal

2.13 Complications:
Rotavirus infection in infants and young children can lead to severe diarrhea, dehydration, electrolyte imbalance, and metabolic acidosis. Children who are immunocompromised because of congenital immunodeficiency or because of bone marrow or solid organ transplantation may experience severe or prolonged rotavirus gastroenteritis and may have evidence of abnormalities in multiple organ systems, particularly the kidney and liver (Baker and Long; 2009).

2.14 Immunity:
Local immunity in the gut lumen is also important in man, although the protective mechanism involved are poorly understood. There have been many reports of human antibodies to rotavirus in different specimens. Most have reported serum or fecal antibodies, with fewer reporting intestinal, salivary or colostrums antibody in levels. However, it is the levels in these latter fluids that are more likely to be important in protection against disease. Since natural immunity does not prevent asymptomatic or mild re-infection, in order to develop an effective immunization strategy it is necessary to know what immunological markers indicate protection against disease in infants and young children. Attempts have been made to relate local intestinal antirotavirus antibodies to serum, fecal or salivary immune responses so as to use one of these antibodies as a marker of intestinal anti-rotavirus immunity, but none has proved ideal. When studying protection against re-infection, homotypic protection is probably of longer duration than heterotypic, but the incidence of symptomatic re-infection may be influenced by the variety of rotavirus strains circulating.
Serum anti-rotavirus IgG antibodies against VP6, as determined for instance by indirect ELISA, indicate previous exposure but not protective immunity, whereas
IgA antibodies against VP6 in secretions reflect ability to neutralize virus and mucosal immunity and resistance to re-infection. Antibodies against VP4 and V7, as determined either by neutralizing antibodies in a plaque reduction assay or by VP4- or VP7-specific blocking ELISA, also indicate protective immunity. P7 is highly immunogenic and mostly induces serotype-specific but also cross-reactive antibodies. As indirect ELISA tests are technically easier to perform than neutralization tests or epitope-blocking assays, it is not surprising that much more is known about antibodies directed against VP6 than against VP4 or VP7 (Mittian et al, 1994).

2.14.1 Breast milk and breast milk antibodies:
If the presence of antibody locally in the small intestine at the time of exposure to rotavirus is important in preventing infection or disease, or in preventing both infection and disease, the presence of anti-rotavirus IgA and neutralizing antibodies in colostrums and breast milk should also be important. However, breast-feeding does not provide total protection against rotavirus infection, and its role in protecting against disease is uncertain. Human neonates are, therefore, different from newborn animals, where the value of colostrums is known. However, colostrums from cow’s hyperimmunized with human rotavirus have been found to shorten rotavirus excretion in infants (Hilpert; 1987). Confounding factors which are not considered in all breast-feeding studies include social class, smoking and parity. Breastfeeding perhaps postpones rather than prevents life threatening rotavirus diarrhea. In a study of infants in rural Bangladesh, breast-feeding gave no overall protection during the first 2 years of life, although exclusive breast-feeding in the first year did protect against severe rotavirus diarrhea (Clemen et al; 1993). The differences reported may not wholly result from specific immunity acquired passively via colostrums or breast milk, but from the presence of trypsin inhibitors in breast milk, or a qualitative or quantitative lack of
the intestinal enzymes required to activate rotavirus infectivity or the different VP4 of neonatal rotavirus Gastric acidity and digestive enzymes have been shown to reduce rotavirus neutralizing activity of bovine colostrums immunoglobulin. Mucin prevents experimental disease in mice, and its presence in human milk may inhibit rotavirus replication. IgA anti-rotavirus antibodies have been reported variously as present in all milk specimens up to 1 week postpartum except in women with selective IgA deficiency (Mclean and Holmes; 1980).

2.14.2 Mucosal antibodies following natural infection:

At all mucosal surfaces, IgA are the predominant immunoglobulin isotype. Passive antibodies from breast milk may be a source of error in these specimens, especially saliva. Anti-rotavirus IgA or IgM responses, or both, have been detected in 47-73% of salivary samples after natural infection, where as copro IgA detection in convalescence is more with higher levels reported in prolonged disease or in those excreting virus (Riepenhoff; 1981). However, four infections (i.e., three-infections) may need to occur before 100% copro IgA conversion. Copro anti-rotavirus IgG was generally present in a minority or none. In uninfected, breast-fed infants whose mothers had IgA and in their milk, there was a good correlation With the presence of these antibodies in the infants' faeces, even though these antibodies were found in only a few samples of the infants' duodenal fluid; 40 the Explanation for this finding is uncertain. Antibodies in intestinal secretions have been measured in few studies of natural infection. Antirotavirus IgM is normally present after 7 days, but its presence is only short-live. Variable anti rotavirus IgG responses in duodenal fluid at 1 month, ranging from none to 63% ' have been reported. These may reflect passive transfer from serum when the serum antirotaviruss IgG level is high. The importance of local intestinal immunity is supported by prophylactic and by therapeutic studies. Giving immunoglobulin orally to children hospitalized because of rotavirus gastroenteritis has been
associated with faster recovery, and has protected low birth-weight infants from contracting rotavirus diarrhea (Barnes; 1994).

2.14.3 Serum antibodies following naturally-acquired infections in children
In neonates and infants, anti-rotavirus IgG and neutralizing antibodies may reflect passive immunity obtained via the placenta rather than a response to infection. After primary infection, few neonates develop IgM, whereas older infants do however, when re-infections were studied in some of the same children, few produced IgM. Anti-rotavirus IgA detection in children has varied from none in neonates or few to most or all. When definite re-infections were studied, the anti-rotavirus IgA response rate was variable (Coulson et al; 1990).

2.15 laboratory diagnosis:
2.15.1 specimen:
diagnosis of rotavirus infection is a on the identification of rotavirus in faeces or suspension of rectal swab collected early in the illness. The stool samples must be collected using sterile wide mouth universal containers which should be covered and labelled accordingly.

Techniques for rotavirus detection include:
-Cell culture
-Electron microscopy (EM).
-Immunochromatography test (ICT).
-Poly acryl amide gel (PAGE).
-Real time-PCR (RT-PCR).
-Latex aagglutination.
-Enzyme linked immunosorbent assay (ELISA)
2.15.2 Cell culture:

Rotavirus has been cultured in MA104 and primary African green monkey cell culture in roller bottle after trypsin pre-treatment (Scherlock et al., 1989). This not practical method for routine diagnostic use as it is technical demanding, time consuming and expensive and difficulty in growing human rotaviruses in cell culture has now been largely overcome (Kapilian et al, 1996). A Variety of cell lines have been tested for rotavirus isolation from clinical samples, include MDBK, PK-15, BSC-1, LLC-MK2, CV-1, MA-104 and HR-29 (Specter et al; 2000).

2.15.3 Electron microscopy:

Electron microscopy it was original method used to detect rotavirus and it is sensitive and specific diagnostic tool due to the high viral load in acute disease and characteristic morphology of the organism (Kapikian and Chanock; 1996). Electron microscopy has advantages of being able to detect non rotavirus causes of diarrhea or infection by strains of rotavirus not detected in the antigen assays (Doane and Anderson; 1987). In addition, EM requires an expensive instrument and highly trained personnel and cannot distinguish between rotaviruses of different groups (WHO; 2009). Electron microscopy observation is usually classified into ultrathin-section EM and immune EM. Ultrathin-section EM can be used to observe the viral morphology and its genesis process and can be stored for long period but usually this process it complex and time consuming. In this type using negative staining, fast and simple EM can be accomplished, with characteristic of high resolution and image clarity. However, negative-staining EM usually has sensitivity and requires large numbers of virus particles, leading to a limited range of applications. In addition, it is difficult to identify the virus serotypes using this method. The immune electron microscope approach can be
used for highly accurate and sensitive detection, but the operation is complicated and the cost is high (Zhang and Wang; 2011).

2.15.4 Poly acryl amide gel:
Because of the large quantities of rotavirus present in stool samples from children with gastroenteritis, the viral nucleic acid segments can be visualized directly after extraction from virus particles, by electrophoresis on acryl amide gels, and staining with ethidium bromide or silver nitrate. After electrophoresis, human rotavirus Groups A, B, and C have distinct patterns of gene-segment distribution, designated electropherotypes. The results of electropherotyping correlate with the presence of viruses of a specific group as shown by using other methods. Thus, the presence of distinct electropherotypes patterns has long been considered diagnostic for the presence of individual rotaviruses of Groups A, B, and C (Steele et al; 2004). For Group A rotaviruses, most samples that are positive for rotavirus by EIA will be positive for the characteristic pattern of rotavirus RNA segments after electrophoresis and silver staining. In some cases, silver nitrate staining of viral nucleic acid has roughly the same sensitivity as EIA methods (Herring et al; 1982). Consequently, the PAGE method has sometimes been used to diagnose Group A rotavirus infections for surveillance studies. However, this method is very labor intensive and time consuming.

2.15.5 Real time-PCR:
A variety of sensitive conventional or real-time reverse-transcription polymerase chain reaction (RT-PCR) methods have been developed based on primers specific for several different rotavirus genes (Widlé et al; 1992). These methods have been particularly useful in detecting rotavirus in extra-intestinal tissues, in studies of the duration of viral shedding in stool and the correlation between disease severity and virus load (Richardson et al; 1998). RT-PCR is also useful for verifying that RNA extracts contain intact rotavirus RNA. However, because it is relatively expensive
and labor intensive and detects low copy numbers of rotavirus RNA, RT-PCR is not suitable for use in routine rotavirus detection studies.

2.15.6 Enzyme immunoassay:
Of the various commercial ELISA kits available, Dakopatts kit, developed in Denmark was originally formulated for use in developed countries. This kit was sensitive, specific and could identify 98 per cent of positive specimens. The kit was modified at the request of WHO so that it was suitable for distribution to laboratories in developing countries. It was evaluated by WHO in six diagnostic laboratories in different countries. The sensitivity of the modified Dakopatts kit recorded by the WHO collaborating centre in England was 100%. However, the high cost of the kit has hampered research on rotaviruses in developing countries. There is no commercially available Indian kit for rotavirus diagnosis. In 1993, we developed an indigenous ELISA for rotavirus diagnosis. The test was compared with Dakopatts kit because the latter had been reported To be superior to other polyclonal ELISA kit and Flewitt had suggested that the kit was suitable for rotavirus diagnosis in etiological studies conducted by Laboratories in developing countries. The NIV routine ELISA was 100% specific and sensitive. The test compared very well with the Dakopatts kit. The only drawback of the NIV routine ELISA is, the test requires at least 6 h against Dakopatts test that needs 4 h. The objective of the present study was to develop a rapid diagnostic test for rotavirus infection (Kelkar and Zade; 2004). The most appropriate antigen detection format for large-scale surveillance studies is an ELISA that uses rotavirus–specific antibodies to capture antigen on to well of plastic plates. The antigen is then detected in colorimetric reaction using a second rotavirus-specific antibody coupled to a detector enzyme. The optical density results can be easily recorded with a standard plate reader, permitting analysis of results with standard computer programs (WHO; 2009). Neutralization assay have
been used to determine antibody response to specific rotavirus serotype. Alternatively, epitope-blocking assay utilizing monoclonal antibodies can be measure antibody responses to specific epitopes, such as those that are G type specific (Spector et al; 2000).

2.16 prevention and control:
The strategies for rotavirus control include identifying the target population for rotavirus vaccination, educating parents on how to identify and recognize the sign of dehydration and also to know that rotavirus infection in children is unavoidable and should be looked out for. However, the significant higher prevalence in children attending day care emphasizes the need to pay attention to the role of child care as an important factor in the epidemiology of rotavirus gastroenteritis (Surajudeen et al; 2011). Adherence to universal precaution for infection control, such as hand washing and barrier method (gloves, gown) are important for minimizing disease spread (Moselio et al; 2007).

2.16 Treatment of the disease:
Intake of fluid is important to avoid oral dehydration. In healthy subjects the disease lasts only a few days because of immune system. Antibiotics are administered intravenously. Electrolyte solution is administered into the vein of dehydrated patients (Dennehy; 2008). Several potential new approaches to prevention and treatment of viral gastroenteritis are being considered. Since mucosal surfaces may contain only small concentrations of secretory IgA antibodies, oral administration of gamma globulin preparations containing high titers of antibody against enteric viruses may increase antiviral activity. A second approaches involves the use of protease inhibitors because rotaviruses require protolytic activity to efficiently penetrate host cell (Moselio et al; 2007).
2.18 rotavirus vaccine:
A vaccine to prevent rotavirus gastroenteritis was first licensed in 1998 but was withdrawn in 1999 because of its association with an uncommon type of bowel obstruction called ‘Intussusceptions’. In 2006, the U.S. food and drug administration (FDA) approved a new rotavirus vaccine called RotaTeq by Merck. In 2008 FDA approved a second rotavirus vaccine called Rotarix by GlaxoSmithKline. Both vaccines are live attenuated (weakened) viral vaccine. The babies will get either 2 or 3 doses, depending on which vaccine is used. Doses of rotavirus vaccine are recommended at this age:

- First Dose: 2 month of age.
- Second Dose: 4 month of age.
- Third Dose: 6 month of age.

Rotavirus vaccine is liquid that is swallowed, not a shot. Rotavirus vaccine may safely be given at the same time as other vaccine. The vaccine is very good to preventing diarrhoea and vomiting caused by rotavirus. Almost babies who get rotavirus vaccine will be protected from severe diarrhoea and most of these babies will not get rotavirus diarrhoea at all. The vaccine will not prevent diarrhoea or vomiting caused by other germ. Some babies should not get this vaccine the reasons of these cases may be; allergic reaction to dose of rotavirus vaccine should not get another dose or babies with severe combined immunodeficiency (SCID) or babies who are mildly ill can probably get the vaccine today or babies who have had a type of bowel blockage (intussusceptions) should not rotavirus vaccine.

2.18.1 Risks of vaccine reaction:

With vaccine, like any medicine, there is a chance of side effects. There are usually mild and go away on their own. Serious side effect are also possible, but
are every rare. Most babies who get rotavirus vaccine do not have any problem with it but some problem have been associated with rotavirus vaccine. These problem may be mild which include babies might become irritable, or have mild temporary diarrhoea or vomiting. And the problem may be serious which include intussusceptions (CDC; 2013).

2.19 Global surveillance of rotavirus:

As rotavirus vaccination continues to increase in prevalence worldwide, global surveillance of rotavirus has become an important tool. Surveillance is used to describe serotype distributions in different countries and their regions’ identify and predict the development of emerging strains’ monitor the impact vaccines by identifying successes and gaps, and identify the causes of diarrhoea other than rotavirus. In 2002, the WHO and CDC developed a generic protocol for the standardized surveillance of rotavirus, with a focus on severe diarrhoea requiring hospitalization among children less than 5 years of age (WHO, 2002; Widdowason et al; 2009) this protocol was first implementation in Asia (Bresee et al., 2004). With the support of the rotavirus vaccine program (RVP), the implementation of this protocol was expanded to include the Americas, the eastern Mediterranean region, Eastern Europe, and sub-Saharan Africa (Widdowson et al; 2009). In recent years, these systems have generated data from 196 sites in 59 countries throughout the world (WHO; 2010). This regional surveillance model has led to improved data standardization, increased visibility and perceived validity of and improved sustainability of the surveillance platforms.

The results of the global surveillance program using the common generic protocol demonstrate that, among children hospitalization for severe diarrhoea in different regions of the world, 39% (regional median) test positive for rotavirus.
Although the percentage of diarrhoea cases that are positive for rotavirus ranges from 20-73% in individual countries, the predominant role of rotavirus as a cause of severe diarrhoea is consistent across all regions. Rotavirus surveillance also generates valuable data on the circulating rotavirus strains. These data are vital to improving vaccine development, tracking emergent types, and helping to assess vaccine effectiveness and changes in strain diversity after vaccine are introduced (Widdowson et al; 2009).

2.20 Rotavirus in Sudan:

In Sudan, rotavirus has been one of the most causative agents of diarrhoea among children. Rotavirus A well know as the leading cause of diarrhoea in young children worldwide. It was estimated to account for 41% of hospitalized cases of acute gastrointestinal among children in sub-Saharan Africa (Magzoub; 2013). The prevalence of rotavirus infection in children presented with diarrhoea in Khartoum teaching hospital to be 24.6% (Mukhtar and Selma; 2006). The world Health Organization studies rotavirus in Sudan during 2009 and reported that rotavirus causes approximately 42% of childhood diarrhoea in Sudan hospitals (WHO; 2010).
3. MATERIALS AND METHODS

3.1 Study design:

Descriptive study, to detect rotavirus antigens in stool of children with non specific diarrhoea in Khartoum State, Sudan.

3.2 Study area:

The study was carried out in Mohammed Alamin Hamid children hospital in Omdurman, Khartoum State, Sudan.

3.3 Study population:

Both boys and girls below five years of age suffering from non specific diarrhoea were included in the study.

3.4 Study duration:

The study was conducted from January 2014 to September 2014.

3.5 Inclusion criteria:

Both boys and girls below five years of age suffering from non specific diarrhoea live in Khartoum State and admitted to Mohammed Alamin Hamid children hospital.

3.6 Sample size:

A total of 100 diarrheic stool sample (n=100) were collected in sterile stool containers, and screened for the presence of rotavirus antigen using ELISA. All samples were stored at -20 C° until used.
3.7 Materials:

stool containers, tubes, tube rack, pester pipettes, multichannel pipettes, microwells plate, ELISA device, and reader.

3.8 Sample processing:

All the patients stool specimens collected were screened for the presence of rotavirus antigens using the commercially available ELISA kits obtained from Plasmatic Laboratory Products, UK.

3.9 Data collection:

Data were collected using structured interviewing questionnaire designed to collect the information from the parents of the children (Appendix 1).

3.10 Ethical consideration:

Ethical approval for this study was obtained from Research and Ethical Committee at the College of Graduate Studies, Sudan University of Science and Technology. All samples were taken ethically after informing the children parents about the study purpose and importance of the research. Informed consent was obtained from the parents of children before collection of the specimens.

3.11 Experimental work:

3.11.1 Collection of specimens:

All stool samples were carefully collected in sterile labelled containers directly from children below five years of age and transported immediately to laboratory using ice box.
3.11.2 Enzyme linked immunosorbent assay (ELISA):

3.11.2.1 Principle:

The prospect rotavirus test utilizes a polyclonal antibody in a solid phase sandwich enzyme immunoassay to detect group specific antigen present in group A rotaviruses. Break-apart microwells are coated with a rotavirus specific polyclonal antibody. Fecal suspension or control sample is added to the microwells and incubated simultaneously with a rotavirus specific polyclonal antibody conjugated to horseradish peroxidase. Rotavirus antigen presents in the sample is captured between antibody on solid phase and the enzyme conjugated antibody. After 60 minutes incubation at room temperature, the microwells are washed with working strength wash buffer to remove excess specimen and any unbound enzyme labelled antibody. Achromogen is added to the microwells and incubated for 10 minutes at room temperature. The presence of specifically bound enzyme labelled antibody in the microwells results in colour change, which is stopped by the addition of sulphuric acid. Colour intensity significantly above background levels is indicative of the presence of rotavirus antigen in the specimen or control.

3.11.2.2 Collection of faecal specimens:

Faecal specimens should be collected as soon as possible following the onset of symptoms. Faecal specimens for direct testing were collected into containers that do not contain media, preservatives, animal sera, metal ions, oxidising agents or detergents, as all of these additives may interfere with the prospect rotavirus test. Specimen may be stored for 8 days at 2-8 C° prior to testing. For long term storage of faecal specimens, store at -20 degree centigrade.
3.11.2.3 Procedure:

Before use, reagents were brought to room temperature 20-25 °C and mixed gently. The unused reagents were returned to the refrigerator after use. Firstly open foil pouch was opened the required number of microplate strips were removed and placed into a microplate strip holder. Two well was used for the negative control and two well for the positive control. Two drops (100 microliter) of each diluted specimen, negative control or positive control was added to the separate microwells, after addition of specimens and controls, 100 microliter was added of conjugate to each microwell, covered the plate and incubated the microwells at room temperature for 60 minute. The plate should be inverted and tapped on absorbent paper to remove the last traces of wash buffer, 100 microliter of substrate to each microwell, cover the plate and incubate the microwells at room temperature for 10 minutes, The substrate reaction was stopped by adding 100 microliter of stop solution sulphuric acid to each microwell, mixing of the microwells before reading the result. The coloured product was stable for up to 30 minutes after addition of stop solution and read by reader at 450 nm.

3.12 Spectrophotometric determination:

The negative control value, or mean of the negative control value, should be less than 0.150 absorbance units.

The positive control value must be greater than 0.500 absorbance units.

Interpret the test results:

positive: Clinical sample absorbance value > the cut-off value.

Negative: Clinical sample absorbance value < the cut-off value.
Equivocal: Clinical sample absorbance value within 0.010 absorbance units of the cut-off value. These samples should be retested or the patients resample.

2.13 Data analysis:

The data was analyzed using SPSS computer program (software version 11.5). Frequencies, Chi-square test values were calculated.

Calculate the cut-off value by adding 0.200 absorbance unit to negative control value, or mean value when more than one negative control is included.
4. RESULTS

The patients included in this study were 55 (55%) males and 45 females (45%). They were distributed into 4 age groups as follows; 36 patients (36%) less than one year, 29 patients (29%) between 13-24 months, 26 patients (26%) between 25-36 months and 9 patients (9%) between 37-48 months of age.

4.1 Detection of rotavirus antigen in the diarrhea stool tested by sandwich ELISA

Table 4.1 demonstrates that out of the 100 stool specimens examined 44 were shown positive (44%) for rotavirus antigen, while 56 subject were found negative (56%).

4.2 The effect of sex of children on rotavirus infection

Table 4.2 illustrates that out of 55 male subjects 24 were found positive for rotavirus antigen (24%), while 20 out of the female subjects were shown rotavirus antigen positive (20%), showing no significant difference ($P=0.16$).

4.3 The effect of age of children on rotavirus infection

Table 4.3 revealed that highest cases for rotavirus infection were (36%) among children less than one year of age, but less than four year of age had the lowest (9%) rotaviral infection. These finding showed no significant difference ($P=0.31$).

4.4 Clinical presentation of children with or without rotavirus infection among the diarrheal cases

Most of non-specific diarrhea in children is usually associated with fever vomiting as well known clinical symptoms. Table 4.4 illustrates that there were high frequency of positive subjects among patients with fever (33%) and vomiting (30%); however, the frequency of positive cases decreased in patients without
fever and vomiting (11.4% and 14.5% respectively). Furthermore, among rotavirus negative case there were 39.5% with fever and 22.9% with vomiting. These results showed no significant difference

4.5 Detection of frequency of positive and negative results for Rotavirus according to duration of diarrhea.

Table 4.5 shows that most of the rotavirus infection occurred in the duration 1-3 days diarrhea.
Table 4.1 Detection of rotavirus antigen from faecal sample of children examined by sandwich ELISA and 100 sample.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>44</td>
<td>44%</td>
</tr>
<tr>
<td>Negative</td>
<td>56</td>
<td>56%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 4.2 The effect of sex of children on rotavirus infection.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Subject examined</th>
<th>Rotavirus Antigen</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>Male</td>
<td>57</td>
<td>24</td>
<td>24%</td>
<td>33</td>
</tr>
<tr>
<td>Female</td>
<td>43</td>
<td>20</td>
<td>20%</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>44</td>
<td>44%</td>
<td>56</td>
</tr>
</tbody>
</table>

Person Chi-square =1.976
P value =0.161
Table 4.3 The effect of age of children on rotavirus infection using sandwich ELIS

<table>
<thead>
<tr>
<th>Age of children(months)</th>
<th>Subject examined</th>
<th>Rotavirus Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Less than 12</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>13-24</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>25-36</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>37-48</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>44</td>
</tr>
</tbody>
</table>

Person Chi-square = 4.426
P value = 0.351

Table 4.4 Clinical presentation of children with and without rotavirus among the diarrheal cases.

<table>
<thead>
<tr>
<th>Clinical Presentation</th>
<th>Number and percentage of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rotavirus positive (n=44)</td>
</tr>
<tr>
<td>Fever</td>
<td>33</td>
</tr>
<tr>
<td>No fever</td>
<td>11</td>
</tr>
<tr>
<td>Vomiting</td>
<td>30</td>
</tr>
<tr>
<td>No vomiting</td>
<td>14</td>
</tr>
</tbody>
</table>

For fever Person Chi-square = 0.168 ; P value = 0.682
For vomiting Person Chi-square = 0.379 ; P value = 0.54
Table 4.5 frequency of positive and negative results for Rotavirus according to duration of diarrhea.

<table>
<thead>
<tr>
<th>Frequency of diarrhea (days)</th>
<th>Subject examined</th>
<th>Rotavirus Antigen</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>58</td>
<td>33</td>
<td>33%</td>
<td>25</td>
</tr>
<tr>
<td>More than 3</td>
<td>42</td>
<td>11</td>
<td>11%</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>44</td>
<td>44%</td>
<td>52</td>
</tr>
</tbody>
</table>

Person Chi-square = 2.526

P value = 0.471
5.1 DISCUSSION

Diarrheal diseases remain the second most common cause of death among children younger than 5 years of age. This study was conducted on rotavirus antigens, the main causative agents of acute gastroenteritis in both developed and developing countries. The frequency of rotavirus antigens detected in this study among children less than 5 years of age was (44%) it is higher than those previously reported in different parts of Sudan by Hind Ibrahim (2013) in Gaffer Ibnoaf Specialized Children, (Elhag and Wafa; 2009) in Khartoum State, (Abdalla; 2011) in Omdurman Paediatric Hospital (2005%) and (Bonkoungou, 2010) in Burkina Faso. However, the results obtained in this study were similar to those reported by (WHO; 2010) in Sudan. The low or high rates of rotavirus infections observed by different investigators could be attributed to several factors including, the study population, the diagnostic techniques used, the incidence rate of the virus in different environments, the living conditions and standards of the study group, the season during which the study was conducted and different subgroups and serotypes of rotaviruses prevalent in the area of the study (Desselberger and Gray; 2009). During this study, although males admission to hospitals was observed to be higher than females, but they showed no differences in their response to the disease this was previous reported by Newman et al, (1999). The finding that most of rotavirus-infected children with diarrhea were less than one year of age (36), this group was the main target of rotavirus infection, this is agreement with many recent studies reported by (Bonkoungou et al; 2010) in Burkina Faso and (Moyo et al; 2011) in Tanzania. This is clearly indicated that, most of the non-specific diarrhea among infants and children in Africa was mainly due to rotavirus infection. Similar to many previous studies done in Africa and other parts of the world, this study observed that the main apparent clinical
symptoms of rotavirus infection among children less than five years of age were fever (33%) and vomiting (30%). However, there was no significant difference between rotavirus-positive and rotavirus-negative children for both fever (\( P = 0.683 \)) and vomiting (\( P = 0.54 \)), an indication that both fever and vomiting are considered the major clinical symptoms of any type of diarrhea in children less than 5 years of age (Bonkoungou et al; 2010). Furthermore, the rates of asymptomatic rotaviral infections among infants and children were observed to vary from 2-4% in some African countries to as high as 36% in other countries (Omoigberale et al; 1996), indicating that the socioeconomic and environmental conditions are major predisposing factors for this viral infection. The high prevalence of asymptomatic rotaviral infection among infants and children reported in many Africans countries supports the possibility of the presence of a potential reservoir for continuous rotavirus transmission (Omoigberale et al, 1996; Bonkoungou et al, 2012; Moyo et al, 2011; Soltani et al, 2012). These observations are strongly supported by fact that rotavirus shedding begins before symptoms develop, and children may be shedding the virus for up to 2-3 days before they exhibit symptoms (Pickering et al; 1988). In addition the rotavirus can remain viable for hours on human hands and for days in vomits (Harrison; 1998) which account for the high rates of spread among the hospitalized children and those in the day-care centres (Florence et al; 2012). In this study, while the duration of diarrhea was shown 1-2 days among the majority of infected children (35%), 3-4 days duration was reported among 20% of them. It is estimated that more than 80% of all rotavirus-related death occur in resource-limited countries in south Asia sub-Saharan Africa (Parashar et al; 2009). Nearly every child is infected with rotavirus by 5 years of age, irrespective of location eg; urban or rural area or socioeconomic status (Bilcke et al; 2009). Because improvement in housing, water supply, sanitation, personal hygiene, food quality, nutrition and
maternal education do not appear to reduce the overall incidence of rotavirus infections, non-faecal routes of infection may play a role in transmission (Chanran et al; 2010). Consequently, vaccines are the most effective public health intervention for the control of rotavirus disease (Patel and Parashar; 2011). Furthermore, continuous rotavirus surveillance generates valuable data on the circulating rotavirus strains. These data are vital to improving vaccine effectiveness and changes in strain diversity after vaccines are introduced (Widdowason et al; 2009).
5.2 CONCLUSION

Diarrheal diseases are one of the major causes of mortality among children less than five years of age. Infection with group A are common among worldwide. In this study the incidence of rotavirus among children was reported 44% in this group. This virus infection was not related to age, sex and symptoms (fever, vomiting and fluid loss).

5.3 RECOMMENDATIONS

1- Rotavirus diagnosis should be requested by the physician as a routine test for all patients with diarrhoea especially children below five years of age.

2- Further investigations are needed to provide a more accurate picture of epidemiology of rotavirus disease and also its serotypes in Jos. This is highly needed to design an effective vaccination against rotavirus in future.

3- Improvement of rotavirus vaccines and development of alternative vaccines should continue and then administered to children in developing countries To Lower the incidence rate.

4- Laboratory workers should assay for rotavirus in diarrhea cases when bacterial and parasitic agent assays all show negative results by advanced techniques like PCR.

5- To understand the reasons for the observed different findings of rotavirus infection and the seasonal pattern, conducting studies covering a longer period of Time is essential.
REFERENCES


53- Kelkar SD and Zade JK (2004). Group B rotaviruses similar to strain CAL-1, have been cirulating in Western India since 1993. Epidemiol Infect. 132 (4):745-749.


Appendix 1

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Serological Detection of Rotavirus Antigen in Stool of Children with Diarrhea in Khartoum

1- Date: ........................................................................................

2- Number: ........................................................................................

3- Name: ............................................................................................

4- Age: less than 1 year  1-3 years  3-5 years

5- Sex: male  Female

6- Residence: ........................................................................................

7- Personal hygiene: bad  good

8- Clinical information:
   A- Fever: a- Yes  b- No
   B- Vomiting: a- Yes  b- No
   C- Duration of diarrhea: a- 1-2 days  b- 3-4

9- Other diseases: ..................................................................................

Signature: ..........................
Appendix 2

ELISA machine
Appendix 3

Reader
Appendix 4

Result after addition of substrate
Appendix 5

Result