Sudan University of Science and Technology
College of Graduated studies

Seropravelance of Rotavirus among diarrheal children at Algedaref Pediatric Hospital in Agadaref State, Sudan

الكشف المصلي لفيروس الروتا لدى الأطفال المصابين بالإسهال في مستشفى القضارف للأطفال ، القضارف ، السودان

A thesis submitted in partial fulfillment for the requirement of M. Sc degree in Medical Laboratory Science (Microbiology)

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2016
الآية

قال تعالى:

(إِنَّمَا أَمْرُهُ إِذَا أَرَادَ شَيْئًا أَنْ يُقُولَ لِهِ كُنْ فَيَكُونُ)

سورة يس الآية (لدك)
DEDICATION

I would like to dedicate this simple work to

My mother and father who teaching me the meaning of life

To my brothers

Mohamed Ali, Omer and Mosab

To my sisters

Alshimaa, Alkansaa and Doaa

To my friends who share me the roads of life

To my mother Nemat Okasha who teaching me the meaning of kindness and tenderness
ACKNOWLEDGMENTS

Thanks to ALMIGHTY ALLAH for giving me knowledge, health and patience to complete this work.
My gratitude must be extended to my supervisor Prof. Yousif Fadlalah Hamedenil for his supervision.
Thanks must also go to the staff member of Microbiology Department, Algdaref Pediatric Hospital for the technical help who provided me during collection of specimens.
Thanks also extend to Mr. Mugahid Abd elgani (lab person for WHO rotavirus program) for technical help and for the supply me with some of the laboratory materials.
Thanks must go to the patients and parents for their understanding and cooperation during the study period.
ABSTRACT

This study was conducted for serological detection of rotavirus antigens in stool of vaccinated children with diarrhea in Algdaref State during the period from September to December 2015. Stool specimens were collected from 92 children less than three years of age suffering from diarrhea in Algdaref Pediatric hospital. All the samples were tested for the presence of rotavirus antigen using rapid immunoassay and ELISA technique. The results obtained were processed and statistically analyzed using person chi-square test. The rotavirus antigens were detected in 19 samples (20.7%) out the 92 samples examined by rapid immunoassay and 58 samples (63%) out the 92 samples examined by ELISA. Most of positive cases were in children less than one year of age (60.3%).

The study showed that, there was no significant difference (p=value 0.186) between males children (62.1%) and females (37.9%) contracting the disease. Most of rotavirus infection was reported among children who were suffering from vomition (81%) and fever (67.2%).

The study showed that infection by rotavirus was not affected by age (p=value 0.419), type of feeding (p=value 0.063) and clinical presentation (vomiting and fever).
ملخص الأطروحة

أُجريت هذه الدراسة بغرض الكشف عن مستضدات فيروس الروتا في عينات البراز للأطفال المصابين بالإسهال في ولاية القضارف خلال الفترة من سبتمبر إلى ديسمبر 2015.

جُمعت 92 عينة براز من الأطفال دون سن الثالثة والذين يعانون من الإسهال في مستشفى القضارف للأطفال. تم اختبار كل هذه العينات باستخدام تقنية الكشف المناعي السريع وتقنية الإليزا للكشف عن وجود مستضدات فيروس الروتا. النتائج المتحصل عليها تم تحليلها إحصائيا.

مستضدات فيروس الروتا وجدت في 19 عينة (20.7%) من إجمالي العينات المختبرة بالتقنية. الكشف المناعي السريع و 58 عينة (63% من إجمالي العينات) بـ 60.3% من عمر سنة.

أوضح النتائج أنه لا يوجد فرق بين الذكور والإناث في معدل الإصابة بالمرض. أظهرت الدراسة أن الإصابة بفيروس الروتا لا تتاثر بالعمر. نوع التغذية ولا الأعراض السريرية (القيء والحمى).
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<td>CDC</td>
<td>Centre for Disease Control and prevention</td>
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<td>CNS</td>
<td>Cenreal Nervous System</td>
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<td>EIA</td>
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CHAPTER ONE

INTRODUCTION AND OBJECTIVES
1. Introduction

1.1. Background

Diarrheal diseases are one of the major causes of morbidity and mortality among children less than five years of age. The World Health Organization attributed a worldwide estimate of 17% mortality due to diarrhea in children younger than 5 years of age, 40% of them were in Africa (Bryce et al., 2005).

Rota viral gastroenteritis is a serious public health problem in both developed and developing countries. The disease associated with the deaths of more than 600,000 children per year worldwide, with the majority of deaths occurring in Africa and Asia. Yearly death tolls are high in India (146,000), Nigeria (47,500), China (41,000), Pakistan (36,500), Congo (29,000), and Ethiopia (29,000) Bangladesh has the highest per capita death rate from the disease. Although mortality rates in the United States are much lower (20–60 deaths/year).

Rota viral gastroenteritis has been shown to cause approximately 40% of all outpatient visits for acute gastroenteritis in infants and young children to pediatric primary care practices. Furthermore, between 30% and 50% of all hospitalizations for gastroenteritis among United State children aged <5 years (Bernstein, 2009).

In Sudan, rotavirus has been one of the important causative agents of diarrhea among children. Rotavirus A is well known as the leading cause of diarrhea in young children worldwide. It was estimated to account for 41% of hospitalized cases of acute gastroenteritis among children in Sub-Saharan Africa (Magzoub et al., 2013).

Due to the antigenic and genomic diversity rotavirus has been classified into 7 groups (A, B, C, D, E, F and G) and 4 subgroups within group A (Surendran, 2008).
Group A viruses are the major cause of rotavirus diarrhea in the U.S. and Groups B and C can cause gastroenteritis in adult (Parashar et al., 2003; 2006).

Rotavirus A is the most frequent cause of diarrhea in under-five year old children worldwide where it is estimated to cause 114 million cases of gastroenteritis resulting in 527,000 deaths annually (Magzoub et al., 2013).

Epidemiological knowledge concerning rotaviruses among infants and children is critical for the development of effective measures, including vaccines. There is incidence of rotavirus infection among vaccinated children with Rotarix vaccine against rotavirus infection. The use of universal vaccine (multiple serotypes) is the most important preventive strategy (Ahmed et al., 2015).

1.2 Rational

There is an increase in morbidity and mortality rates among children below five year of age with diarrheal disease annually. Rotavirus plays a major causative agent especially in developing countries including Sudan. The majority of the research done in Sudan on rotavirus strains was on animals (Osman, 2004). There are limited numbers of studies in human rotaviruses (Ahmed et al., 2015 and Magzoub et al., 2013).

For these reason and because of the clinical importance of the disease it is highly important to study the frequencies of rotavirus among children with non-specific diarrhea presenting at many hospitals in Sudan especially developing states such as Al gadaref State.
1.3 Objectives

1.3.1 General objectives

- To detect the prevalence of rotavirus antigen among vaccinated children in Algardaf State.

1.3.2 Specific objectives

- To estimate the rotavirus antigen in stool specimens among vaccinated children.
- To compare between rapid immunoassay and ELISA technique for detection of rotavirus.
CHAPTER TWO

LITREATURE REVIEW
2. Literature review

2.1. Introduction
The human intestinal rotaviruses were first found in 1973 by electron microscopic examination of duodenal biopsy from infant with diarrhea (Ryan and Ray, 2010).

2.2 Classification
The rotaviruses belong to the family *Reoviridae*, which currently consists of nine genera; *orthoreoviruses* and *rotaviruses* affect mainly vertebrates, *orbiviruses* and *coltiviruses* are found in vertebrates and insects, while the remaining genera contain viruses of insect and plants (Vanregenmortel *et al.*, 2000).

2.3 Structure
Rotaviruses are naked icosahedral capids, spherical particles 65 to 75 nm in diameter (smaller forms have also been described) with a genome containing 11 segments of double-stranded RNA, an RNA-dependant RNA polymerase, and a double-shelled outer capsid; two segments encode proteins of the outer capsid (VP4 and VP7), which are target of neutralizing antibodies (Ryan and Ray, 2010). The name rotavirus comes from the characteristic wheel like appearance of the virus when viewed by electron microscopy (the name rotavirus is derived from the Latin *rota*, meaning "wheel") (Surendran, 2008).

2.4 Antigenic types
Rotaviruses have three important antigenic specificities: group, sub group and serotype. Group A rotaviruses are major pathogen in humans and animals. Ten serotype of human group A rotaviruses are defined by neutralization of one (VP7) of the two outer capsid proteins. Of the non-group A rotaviruses (groups B through G) only groups B and C have been detected in humans; they are not important cause of disease in infant and young children(Singh, 2010). The group A was further classified using the
glycoprotein VP7 defining G types, and the protease-sensitive protein VP4 defining P types. The P-type is indicated by a number for the P-serotype and by a number in square brackets for the corresponding P genotype. G-serotypes are similarly numbered but the G-genotype number is the same as the G-serotype. Approximately 14 G types and 20 P types have been reported, of which approximately 10 G types and 11 P types are identified in humans (Surendran, 2008).

2.5 Multiplication

The virus enters the cell by endocytosis or by direct membrane penetration if activated by protease. Replication occurs in the cytoplasm. Removal of the outer shell of the capsid in lysosomes activates the RNA polymerase. Newly assembled sub viral particles acquire the outer capsid proteins by budding through the endoplasmic reticulum, and are released by cell lysis (Singh, 2010). The rotaviruses use negative-sense RNA strategy for transcription and replication. RNA-dependant RNA polymerase direct the synthesis of early and late mRNAs followed by genome replication by using the negative-strand RNA of double-stranded RNA genome. Early proteins are made that are required for virus replication, whereas late proteins are mainly the structural proteins (Ryan and Ray, 2010).

2.6 Transmission and Epidemiology

Rotavirus is highly contagious. Transmission by the fecal-oral route is most frequent, although respiratory transmission may also occur (Glass et al., 2006). Although good hygiene measures can help prevent spread of the disease, the robustness of rotavirus and the low infectious dose (10–100 virus particles), makes standard sanitary measures to halt transmission of the virus relatively ineffective (Gray, 2011). Rotavirus infections in humans cause gastroenteritis that usually lasts from three to eight days (Glass et al., 2006). The proportion of patients
hospitalized with gastroenteritis who had confirmed rotavirus infection ranged from 25% during the off season to more than 70% during peak season (Staat et al., 2002). In the United States, annual epidemics begin in the Southwest during November and December, progressing north and east and reaching the Northeast by April or May (Bernstein et al., 2004). A similar pattern has been identified in Europe, with the seasonal peak beginning in Spain in January, spreading to northern countries by March (Bernstein et al., 2004). Seasonality is less marked closer to the equator but the disease is more pronounced during drier and cooler months. The reason for this seasonality remains unknown (Bernstein et al., 2004 and Dennehy, 2008). Recent data from the Centers for Disease Control and Prevention suggest that the seasonality of rotavirus could be changed by the introduction of rotavirus vaccines. Rotavirus is characterized by substantial genetic diversity, as evidenced by the presence of multiple serotypes. The most common circulating strains associated with rotavirus gastroenteritis worldwide are serotype G1 through G4 and G9. These strains are responsible for 95% of pediatric rotavirus diarrhea worldwide (CDC, 2007). G1 is particularly prevalent in North America, Australia, and Europe (70% of infections) but less so in South America, Asia, and Africa (20%–30%) (Santos and Hosino, 2005 and Griffin et al., 2000). In addition, G9 has emerged in recent years as an important strain, with the highest rates in South America and Australia. Other serotypes continue to emerge including G5, G8 and G12 strains (Santos and Hosino, 2005).

2.7 Pathogenesis

RV infects cells of the small intestine and produces a number of non-structural (NS) proteins enhancing viral replication, disarming the natural immune response, and manipulating cellular signaling pathways (Hu et al., 2012).
Among the NS proteins an enterotoxin, NSP4, destroys intestinal mucosa cells resulting in severe watery diarrhea (Ward et al., 1996). After an incubation period of usually <48 h, clinical symptoms include acute onset of vomiting and fever followed by watery diarrhea (Davidson et al., 1975; Kapikinan et al., 1983). The disease spectrum ranges from a mild, short-lasting course to severe and dehydrating diarrhea that may lead in rare circumstances to complications such as seizures, CNS involvement, or even death (Johansen et al., 2008).

2.8 Clinical feature
In rotavirus infected patients, diarrhea and vomiting is more common and prolonged than in pediatric gastroenteritis caused by other pathogens, and fever is reported in 30–70% of children (Gimenez et al., 2010). Gastrointestinal symptoms resolve usually in <7 days, and at 1-month follow-up 88% of children have returned to their usual health status (Rodriguez et al., 1987).

2.9 Complications
Rotavirus infection in infant 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).

2.10 Immunity
Patients with rotavirus infection respond with production of type-specific humeral antibodies that appear to last for years, perhaps lifetime. In addition, type –specific secretary(s IgA) antibodies are produced in the intestinal tract and their presence seem to correlate best with immunity to
reinfection. Breast feeding also seem to play protective role against rotavirus disease in young infants (Rayan and Ray, 2010).

2.11 laboratory diagnosis

Laboratory confirmation of RV infection can be obtained by detection of the virus, viral antigen or viral RNA in stool samples. The most commonly used method is antigen detection by one of several commercially available enzyme immunoassays (EIA), which are easy to perform and provide a low detection limit. Other techniques include real-time PCR and electron microscopy (Stockman et al., 2008).

2.11.1 Collection of samples

Stool specimens should be collected as soon as possible following the onset of symptoms. Peak excretion of rotavirus in faeces from patients with gastroenteritis is reported to occur 3-5 days after the onset of symptoms (Flewett and Wood, 1978).

2.11.2 Technique for rotavirus detection

2.11.2.1 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 strains of virus not detected in the antigen assay (Doane and Anderson, 1987).

2.11.2.2 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 is not practical method for routine diagnostic use as it is technical demanding, time consuming and expensive and difficulty in
growing human rotavirus in cell culture has now been largely overcome (Kapikian and Chanock, 1996).

A variety of cell lines have been tested for rotavirus isolation from clinical samples, include MDBK, PK-15, LLC-MK2, CV-1, MA-104 and HR-29 (Specter et al., 2000).

2.11.2.3 Enzyme-Linked Immunosorbent Assay

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 second rotavirus-specific antibody coupled to a detector enzyme. The optical density results can be easily recorded with a standard plate reader, then 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 (Specter et al, 2000).

2.11.2.4 Rapid detection of rotavirus antigen

The recent advent of antigen detection method based on immunological techniques using polyclonal or monoclonal antibodies has gained the attention of researchers. Direct detection of anigen in stool samples by rapid one-step assay is an-inexpensive, easy to handle and sensitive test with no need of invasive procedure and speacialized insterumentation( Cukor, 1984).

2.11.2.5 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 (Widle 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 correlation between disease severity and virus load (Richardson et al., 1998).

2.12 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 (Surajudeen et al., 2011).

2.13 Treatment
Treatment of rotavirus is supportive and primarily aimed at the replacement of fluid and electrolyte losses (Bernstein et al., 2004). Rehydration can be accomplished using the World Health Organization formulation or any of a number of commercial formulations. Studies have shown that these formulations are effective for children who are mildly to moderately dehydrated (WHO, 2006 and Bass et al., 2007). Intravenous fluids should be used for those with severe diarrhea, intractable vomiting, altered consciousness, or if the child cannot or will not drink. Nutritional therapy is also important and can reduce the morbidity and mortality of rotaviral gastroenteritis. Early initiation of refeeding is important because oral rehydration therapy is low in calories (Bass et al., 2007).

Several potential new approaches to prevention and treatment of viral gastroenteritis are being considered. Since mucosal surface may contain only small concentration of secretary 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).
2.14. The rotavirus vaccination

There are two rotavirus vaccines authorized for use by the European Medicines Agency, Rotarix® (manufactured by GSK) and RotaTeq® (manufactured by Sanofi Pasteur MSD). Both are highly effective at preventing rotavirus infection in infants. However, the vaccines are not known to be interchangeable and a course of vaccine started with one product should be completed with the same vaccine to achieve full protection (De Vos et al., 2004).

Rotarix® is the vaccine offered as part of the UK national childhood immunization program. It is a live attenuated vaccine derived from a virus initially isolated from a 15-month-old child and then attenuated by serial cell culture passage (De Vos et al., 2004).

In clinical trials Rotarix® has been shown to protect against gastroenteritis due to rotavirus serotypes G1P [8], G2P [4], G3P [8], G4P [8], and G9P [8]; some efficacy against uncommon rotavirus genotypes G8P [4] and G12P [6] has also been demonstrated. The vaccine is over 85% effective at protecting against severe rotavirus gastroenteritis in the first two years of life. The effectiveness of the vaccine in protecting against any rotavirus infection varies between the serotypes listed. (Salinas et al., 2005; Vesikari et al., 2007; Soares et al., 2012).

The licensed vaccine is prepared as a lyophilized powder to be reconstituted with 1 ml of solvent. Each 1.5 ml vaccine dose contains \( \geq 10^6 \) CCID\(_{50}\) (cell culture infectious dose 50%) of the parent RotaVirus strain. Two oral vaccine doses, given at least 4 weeks apart, are necessary for a complete vaccination series. The first dose should be administered as soon as possible after the age of 6 weeks. The vaccination series should be completed preferably before 16 weeks of age, and not later than 24 weeks of age (Koch et al., 2013).
RotaTeq® is a live, oral rotavirus vaccine that contains five reassortant rotaviruses developed from human and bovine parent strains. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, G4) from the human parental strain and the attachment protein from the bovine rotavirus strain. The fifth reassortant virus expresses the attachment protein P1 from the human rotavirus parental strain and the outer capsid protein (G6) from the bovine rotavirus parental strain. The parental bovine rotavirus strain WC3 (G6, P7) was isolated in 1981 from a calf with diarrhea and passaged in African green monkey cells (Clark et al., 1986). The reassortants are propagated in Vero monkey kidney cells (Heaton and Ciarlet, 2007).

The licensed vaccine is a ready-to-use 2 ml solution that contains ≥2.0–2.8×10⁶ infectious units (IU) per individual dose, depending on reassortant. The vaccination course consists of 3 doses. The first dose may be administered from the age of 6 weeks and not later than after the age of 12 weeks. Doses should be given at least 4 weeks apart. The vaccination series of three doses should be completed preferably by the age of 20–22 weeks, but not later than the age of 32 weeks (Koch et al., 2013).
CHAPTER THREE

MATERIALS AND METHODS
3. Materials and Methods

3.1 Study design
This was cross sectional descriptive study.

3.2 Study area
This study was conducted at Al gadaref Pediatric Hospital in Al gadaref State, Sudan.

3.3 Study population
Both vaccinated boys and girls below three years suffering from non specific diarrhea were included in the study.

3.4 Study duration
Study was carried out during 3 month period between September and December 2015.

3.5 Ethical consideration
Approval to conduct this study was obtained from the College of Graduate Studies, Sudan University of Science and Technology. Permission to carry out the study was taken from Department of Microbiology Laboratory at Al gadaref Pediatric Hospital. After explaining the study and its goals, a verbal consent was taken from the parents of children before collection of the specimens.

3.6 Sampling and sample size
A total of ninety-two diarrheic stool sample (n=92), tested by a rapid immunoassay and confirmed by indirect enzyme- linked immunosorbent assay (ELISA) for the presence of rotavirus antigen, were enrolled in the study. All samples were collected in sterile stool containers and stored at -20 C° until used.
3.7 Data collection
Personal and clinical data were collected using structured interviewing questionnaire designed to collect the information from the parents of the children (Appendix 1).

3.8 Laboratory methods

3.8.1 Detection of rotavirus antigens by rapid immunoassay

3.8.1.1 Principle of the test
The ImmunoCard STAT! Rotavirus assay detects the presence of rotavirus antigen in stool. Patient specimen is diluted 1:15 in sample diluent. The suspension is mixed and 150 µl is added to the sample part of the device. The sample mobilizes gold particles coated with monoclonal antibody to rotavirus and migrates along the membrane through the test (polyclonal anti-rotavirus antibody) and control zones. After ten minutes, the test and control zones are observed for the presence of red-purple lines across the membrane surface. If rotavirus is present in the sample, a complex is formed between the capture antibody and the monoclonal antibody-gold conjugate which can be seen visually as red-purple line in the test zone. No red-purple line in the test zone indicates a negative result. The control line serves as a procedural control to assure that the sample has migrated the appropriate distance along the membrane.

3.8.1.2 Sample dilution
Three hundred and fifty micro liters of sample diluents were added to each 12×75 mm test tube for each specimen to be tested, 25µl of liquid or semi-solid stools were drew using transfer pipette and were dispensed the stools into the sample diluents in appropriate 12×75mm tube. For solid stools 2mm of diameter portion of stools were transferred into the sample diluents in the appropriate 12×75mm tube using a wooden applicator stick.
3.8.1.3 Procedure of the test
ImmunoCard STAT! Rotavirus devices were removed from their pouches and labeled appropriately.
Each diluted specimen was vortex for ten seconds, and then 150µl of diluted samples were added to sample port and incubated for ten minute at room temperature, after incubation period the control and test zones were visually read for the presence and absence of red/purple line.

3.8.2 Detection of rotavirus antigens by ELISA
3.8.2.1 Principle of ELISA
The prospect rotavirus test utilizes 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 present in the sample is captured between antibody on the 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 labeled antibody. Chromogen is added to the microwell and incubates for 10 minutes at room temperature. The presence of specifically bound enzyme labeled antibody in the microwells results in a colour change, which is stopped by the addition of acid. Colour intensity significantly above background level is indicative of the presence of rotavirus antigen in the specimen or control

3.8.2.2 Preparation of 10% suspension of stool
Approximately 0.1g of solid feaces (small pea-sized portion) or approximately 100µl of liquid feaces was added to 1ml of sample diluents at suitable labeled container.
3.8.2.3 ELISA procedure

Before use all reagents and specimens were brought to room temperature 20-25 C° and mixed gently.

Firstly foil pouch was opened and the total number of 96 microplate stripes was removed and placed into microplate stripe holder.

Two well was used for the negative control and two wells for the positive control.

100µl of each diluted specimens, negative control or positive control was added to the corresponding separated microwells.

Then 100µl of conjugate was added to the all microplates, the plate was covered and incubated at room temperature for 60 minutes.

All contents of the wells were shaked out and each well was washed five times by completely filled with diluted wash buffer.

100µl of substrate was added to each microwell; the plate was covered and incubated at room temperature for 10 minutes.

The substrate reaction was stopped by added of 100µl of stop solution (0.46 mol/L sulphuric acid) to each microwell.

The microwells were thoroughly mixed before read spectrophotometrically using ELISA reader at wave length 450nm.

3.9 Interpretation of the results

After the cut-off value had been calculated according the formula in the pamphlet clinical samples absorbance value greater than the cut of value were considered positive while Clinical samples absorbance value less than the cut of value were considered negative.

3.10 Data analysis

The data obtained were analyzed and presented using Statistical Package for Social Science (SPSS) computer software version 16 for windows. Significance of differences was determined using Chi-square test. Statistical significance was set at P< 0.05
CHAPTER FOUR

RESULTS
4. Result

The children included in this study were 61/92 (66.3%) males and 31/92 (33.7%) females. They were distributed into two groups according to the age as follows: 54/92 patients (58.7%) between 1-12 months, 38/92 patients (41.3%) between 13-36 months of age.

4.1 Detection of rotavirus antigen among children diarrheal stools tested by rapid immunoassay

A total of 92 stool specimens from pediatric diarrheal patients were collected and examined by The ImmunoCard STAT! for the presence of rotavirus antigen. Rotavirus was detected in 19/92 patients (20.7%) while 73/92 patients (79%) were rotavirus negative (Fig 1).

Fig 1: Detection of rotavirus antigen among children diarrheal stools tested by rapid immunoassay
4.2 Detection of rotavirus antigen among children diarrheal stools tested by sandwich ELISA

A total of 92 stool specimens from pediatric diarrheal patients were collected and examined by sandwich ELISA for the presence of rotavirus antigen. Rotavirus was detected in 58 patients (63%) while 34 patients (37%) were rotavirus negative (Fig 2).

![Pie chart showing the detection of rotavirus antigen among children diarrheal stools tested by sandwich ELISA](image)

**Fig 2:** Detection of rotavirus antigen among children diarrheal stools tested by sandwich ELISA
4.3 Distribution of rotavirus in pediatric diarrhea tested by ELISA according to age groups

The highest positive cases for rotavirus infection were 35 patients (60.3%) among the age group 1-12 months, 23 patients (39.7%) among the age group 13-36 months and 0% among 37-60 months with no significant difference (P > 0.05) between the age groups examined (Fig 3).

Fig 3: Distribution of rotavirus in pediatric diarrhea according to age groups
4.4 Distribution of rotavirus in pediatric diarrhea tested by ELISA according to gender

The results demonstrate that the highest positive cases of rotavirus infection were 36/92 patients (62.1%) among males and 22/92 patients (37.9%) among females with no significance difference (P > 0.05) between the gender types examined (Table 1).

**Table 1: The effect of gender of children on rotavirus infection**

<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>Rotavirus infection</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. positive</td>
<td>Percentage%</td>
<td>Percentage</td>
<td>from total%</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>61</td>
<td>36</td>
<td>59%</td>
<td>62.1%</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>31</td>
<td>22</td>
<td>71%</td>
<td>37.9%</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>92</td>
<td>58</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Person Chi-square = 1.260; P value = 0.186 (P>0.05).
4.5 Effect of type of feeding on rotavirus infection

Rotavirus positive cases revealed that there was no significant difference (p > 0.05) between incidence of rotavirus infection among breast feeding 13.8% of cases, breast and bottle feeding 48.3% of cases, bottle feeding 24.1% and others 13.8% (Table 2).

<table>
<thead>
<tr>
<th>Number examined</th>
<th>Rotavirus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
</tr>
<tr>
<td>Breast</td>
<td>15</td>
</tr>
<tr>
<td>Breast and bottle</td>
<td>50</td>
</tr>
<tr>
<td>Bottle</td>
<td>19</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 2: The effect of type of feeding on rotavirus infection

Person Chi-square = 7.285; P value = 0.063 (P>0.05).
4.6 Clinical presentation of children with and without rotavirus among the diarrheal cases

Rotavirus positive cases revealed that there were 67.2% with fever and 81% with vomiting, however among rotavirus negative cases there were 70.6% with fever and 76.5% with vomiting (Table 3).

Table 3: Clinical presentation of children with and without rotavirus among the diarrheal cases

Person Chi-square = 0.111; P value = 0.463 (P>0.05) and Person Chi-square = 0.272; P value = 0.395 (P>0.05).

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Number and percentage of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus positive (n=58)</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Fever</td>
<td>39 67.2%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>47 81%</td>
</tr>
</tbody>
</table>
4.7 Comparisons of rapid immunoassay and sandwich ELISA results for rotavirus

A total number of 92 diarrheal specimens were tested by both rapid immunoassay and ELISA for the presence of rotavirus, 19 samples were positive by both rapid immunoassay and ELISA and 39 samples were positive by ELISA only (Table 4).

**Table 4: Comparisons of rapid immunoassay and sandwich ELISA results for rotavirus**

<table>
<thead>
<tr>
<th>Rapid immunoassay results</th>
<th>ELISA results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>No. negative</td>
</tr>
<tr>
<td>Rapid immunoassay</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>No. negative</td>
<td>39</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>34</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

DISCUSSION
5.1 Discussion

The frequency of rotavirus antigens detected in this study among children less than 5 years of age (63%) were higher than those previously reported in different parts of Sudan by WHO (2010) in Sudan (42%). The results were also higher than those recently reported by Bonkoungou et al. (2010) in Burkina Faso (33.8%), Soltani et al. (2012) in Tunisia (30%), Florence et al. (2012) in Cameroon (28.7%), Anupam et al. (2010) in India (50%), and Mehmet et al. (2011) in Turkey (52.7%).

The low or high rates of rotavirus infection observed in this study and by other investigators could attributed to several factors including, the study population, the season during which study conducted, living conditions, the diagnostic techniques used in investigations, the incidence rate of the virus in different environments 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 (62.1%) was observed to be higher than females (37.9%), but they showed no differences in their response to the disease this was previous reported by Elnagi and Hassan (2013).

The finding that most of rotavirus-infected children with diarrhea were less than one year of age (60.3%) was in agreement with many recent studies reported by Elnagi and Hassan (2013) in Khartoum State, Sudan, Bonkoungou et al. (2010) in Burkina Faso, all in conformance that children less than one year of age were the main target of rotavirus infection, this could be explained by the declining immunity incurred through breast feeding that protect the infants below 6 months of age and
starting “oral phase” of the normal developmental milestones in children when babies put almost everything into mouth (Kurugol et al., 2003).

In this study it was found that rotavirus incidence occurred mostly among breast and bottle-fed (48.3 %) than bottle-fed (24.1%), breast-fed (13.8%) and others (13.8%) as reported in previous study conducted in Burkina Faso by Bonkoungou et al (2010) and Ahmed et al (2015) in Omdurman, Sudan. This high rates of rotavirus infections through breast and bottle-fed could be due to the declining of maternal antibodies or it could be due to the poor hygienic environment. The low rates of rotavirus infections through other feeding could be due to acquired protective immunity during previous exposures to rotavirus.

In this study the clinical symptoms, fever and vomiting were 67.2%, and 81% of infected children with rotavirus respectively. Vomiting is more common than other as in previous studies by (Ahmed et al, 2015) in Omdurman, Sudan and (Moyo et al., 2007) in Dar essalaam, Tanzania, this might be due to high stimulation of enteric nervous system by NSP4 Cunliffe and Nakagomi (2007).
5.2 Conclusion
The results conducted by this study indicate that gastroenteritis caused by rotavirus in the Sudan is an important health problem, particularly among children less than 3 years of age. There is high incidence of rotavirus infection among vaccinated children below three years of age. The rotavirus infection was not related to sex, age, type of feeding and symptoms (fever and vomiting).

5.3 Recommendation
1. Rotavirus detection should be carried out by ELISA for more accurate results.
2. More attention for rotavirus detection must be on children of less than one year old.
3. Rotavirus diagnosis should be requested by the physician as a routine test for all children with diarrhea especially who’s below five years of age.
4. Further studies covering a longer period of time and larger population are essential.
References


Appendices
Appendix (1)
Sudan University of Science and Technology
Collage of graduate studies
Microbiology department

Questionnaire

Title: frequency of rotavirus antigens among vaccinated children below three year with diarrhea in

By: Alromissa Abd Allah Abd Alati
Supervisor: Prof. Yosif Fadl Allah Ahamed

1. Date: ……………………………………
2. Name: …………………………………..
3. Sex: ……………………………………
   Male [ ] female [ ]
4. Age:
   1-12 months [ ] 13-36 months [ ] 37-60 months [ ]

5. Type of feeding:
   Breast feeding [ ] Breast and bottle feeding [ ]
   Bottle feeding [ ] others [ ]

6. Clinical presentation:
   a. Fever:
      Yes [ ] NO [ ]
   b. Vomiting:
      Yes [ ] NO [ ]

Signature:…………………..
Appendix (2)

Reagents and material of Immuno Card STAT! Rotavirus:
Appendix (3)

Result of immune card assay

(Two red lines) positive control  (Two red lines) positive result

(One red line) negative result
Appendix (3)

Kit content of ProSpecT rotavirus microplate assay
Appendix (4)

ELISA machines

ELISA washer

ELISA reader
Appendix (5)

ELISA results