# Addis Ababa University

# School of Graduate Studies


## Prevalence of Rota virus and Associated Risk factors among under five Children with Gastroenteritis in Selected Health Facilities of Addis Ababa, Ethiopia

**By**

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A thesis submitted to the School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the Degree of Masters in Clinical Laboratory Science (Specialty in Diagnostic and Public Health Microbiology)

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## List of Acronyms and Abbreviation

| | |
|---|---|
| AAU | Addis Ababa University |
| AsV | Astro virus |
| CDC | Center of Disease Control and Prevention |
| C.I | Confidence Interval |
| CLSI | Clinical Laboratory Standard Institute |
| COR | Crude odds ratio |
| dsRNA | Double Stranded Ribonuclic acid |
| DRERC | Department Research and Ethical Review Committee |
| Eads | Enteric Adeno viruses |
| EPHI | Ethiopian Public Health Institute |
| EIA | Enzyme Immuno-assay |
| ELISA | Enzyme linked immuno-sorbent assay |
| FMOH | Federal Ministry of Health, Ethiopia |
| GE | Gastroenteritis |
| Lab | Laboratory |
| MDG | Millennium Development Goal |
| NGO | Nongovernmental Organization |
| NSP | Nonstructural Protein |
| OD | Optical density |
| OPD | Outpatient department |
| PCR | Polymerase Chain Reaction |
| PI | Principal Investigator |
| RT-PCR | Reverse Transcriptase PCR |
| RV | Rota virus |
| SD | Standard deviation |
| SERO | Scientific and Ethical Review Office |
| SOP | Standard Operational Procedure |
| VP | Viral protein |
| WHO | World Health Organization |
| WGO | World Gastroenterology Organization |

**Operational Definitions**

**Acute Diarrhoea:-** is three or more loose or watery stools per day or a definite decrease in consistency and increase in frequency based upon an individual baseline as noted by caregiver.

**Acute Gastroenteritis**: It is interchangeably used with acute diarrhea and it shows the inflammation of the intestinal tract during infection by enteropathogens

**Care giver or Guardian**: - be a mother or father or anyone else responsible for keeping care of the child in close.

**Children**: a child with acute diarrhea whose age is less than five years

**Abstract**

**Title: -** Prevalence of Rota Virus and Associated Risk factors among Under Five Children with Gastroenteritis in selected health facilities of Addis Ababa, Ethiopia

**Background**: - Rota virus, the most common cause of severe gastroenteritis, is responsible for 2 million hospitalizations and 600,000 deaths among children under five years worldwide annually. More than 85% of these deaths occur in low resource countries. Ethiopia is one of the five countries with the greatest rotavirus burden worldwide and accounts for 6% of all rotavirus deaths globally.

**Objective**: - The main aim of this study was to determine the prevalence of rota virus and associated risk factors among under five children with gastroenteritis in Addis Ababa, Ethiopia.

**Methods**: - A cross-sectional, hospital based prospective study was conducted from January – March 2014. A total of 246 children with gastroenteritis were consecutively selected. Appropriate amount of stool specimen was collected and investigated for rota virus by using EIA method. Care givers were interviewed using structured and pre-validated questionnaire. Data was entered in to Epi Info version 3.5.4 and analyzed using SPSS version 20 software.

**Result**: - Among a total of 246 children, 127(51.6%) were males and the mean age of children was 28.5 months. One hundred thirty nine (56.5%) feed on solid food; however, 4.1% were on exclusive breast feeding. Regarding the clinical features, 54.1% had vomiting, 66.3% abdominal pain, and 19.1% were with tenesmus. Most of, 98% used piped water and 98.4% had good hand washing practice. Eighty five (34.6%) children were infected by rota virus, 31.3% by bacteria, and 26.4% by protozoa parasite. The most frequently isolated entro-bacterial pathogen was *E. coli*, and *Enteamoaba* was the most common parasite identified by direct microscopy. Rota virus infection was not significantly associated with any potential factors. Therefore, there was no an independent predictor of rota virus infection.

**Conclusion**: - The prevalence of rota virus was high among under five children with acute gastroenteritis in selected health facilities of Addis Ababa Ethiopia. Therefore early age vaccination and good hygiene keeping should be considered and be expanded as one important means of prevention for rota virus diarrhea. Large scale survey should be conducted in all regions to know the actual rota virus diarrhea burden and vaccine efficacy throughout the country.

# 1. Introduction

## 1.1 Background

Diarrhoea is the second most frequent cause of death among young children globally. Infectious diarrhea can be caused by a wide range of virus, bacteria and parasites. In both developed and developing countries, viruses are the leading cause of acute diarrhea. Among all viral diarrheal agents, rota virus is responsible for up to 84% of the cases (1).

Rota virus is a non-enveloped double stranded ribonucleic acid (dsRNA) genome virus belonging to the *Reoviridae* family. The genome has eleven distinct dsRNA segments that code for six structural viral proteins (VP1-4, VP6 and VP7) and six nonstructural proteins (NSP1-NSP6). The virus is stable in the environment (2).

Rotaviruses are divided into seven groups (A-G) based on VP6. Group A, B, and C rotaviruses have been found to infect humans and the rest found only in animals. Rotavirus group A causes more than 90% of rotavirus infections in humans (2, 3).

There are different strains/serotypes of rota virus A based on VP7 (G-protein) and VP4 (P-protein) proteins that pass separately to the offspring. Various combinations occur in any one strain and re-assortment is possible in double infection. VP7 and VP4 are responsible for evoking neutralizing antibodies (2, 3, 4).

Rotavirus is the most common cause of severe diarrhoea often with vomiting in infants and children responsible for an estimated 111 million diarrhea episodes, 25 million outpatient visits and 2 million hospitalizations among children under 5 years worldwide annually. By the age of five years, nearly every child has been infected at least once and reinfection is mild (5).

Symptoms of rota virus disease often start within 1-3 days of infection with vomiting followed by 4-8 days of profuse diarrhoea. Rota virus replicates in the cytoplasm of mature enterocytes lining the tips of intestinal villi, preventing uptake of nutrients and causing severe diarrhea that can be fatal if left untreated. Rotavirus sheds in high concentration in the stool and spreads by fecal-oral route before two days and after ten days of onset (6).

The most widely available method for confirmation of rotavirus infection is detection of rotavirus antigen in stool specimen by enzyme immunoassay (EIA). Electron microscopy, polymerase chain reaction (PCR), nucleic acid hybridization, sequence analysis, and culture

are mainly for confirmation. Reverse transcription (RT)-PCR can detect and identify all species and serotypes of human rotavirus (7).

Rota virus diarrhea can be prevented by vaccine and hygiene. Two safe and effective, oral attenuated live virus vaccines are available for children against rotavirus group A: Rotatrix (one strain: G1P8, two dose schedule) and RotaTeq (five strains, G1-4and P8 in one with three dose schedule). The treatment is non-specific, no anti-viral drug. Low-osmolarity oral rehydration salt and Zink supplementation are recommended for prevention of dehydration (8).

## 1.2 Statement of the Problem

According to the World Gastroenterology Organization (WGO) 2012 report, there are 2 billion acute diarrhea episodes and 1.9 million deaths among children under five globally.. Gastroenteritis, the second leading cause of death, contribute 18% of all under 5 deaths, and cause malnutrition, diminished growth, and impaired cognitive development especially in children of resource-limited countries. Seventy eight percent child deaths from diarrhea occur in the African and South-East Asian regions (9). Eighty percent of these deaths are in children below 2 years of age and more than 50 % of these cases are in South Asia and sub-Saharan Africa (10).

In developing countries, diarrhea is responsible for 25-30% of deaths among children younger than five years of age (13, 14). Sub-Saharan Africa remains the most affected region by diarrheal mortality and morbidity of children. Approximately 40% of childhood deaths from diarrhea worldwide were occurred in Sub-Saharan Africa by the year 2000, although only 19% of the world's under five population lives in this region (15).

Diarrhea is also the major cause of infant and child mortality and morbidity in Ethiopia. About 39 million episodes of diarrhea per year were estimated to occur; out of which 230,000 deaths occur in children below the age of five years. The median incidence of diarrhea is estimated to be five episodes/ child/year (16). According to World Bank report of 2011, child mortality rate of Ethiopia was 77 per 1,000 births and approximately one of every five deaths every year is due to diarrheal disease (17).

Rotavirus infection is the leading cause of severe acute gastroenteritis in young children worldwide, responsible for two million severe illnesses in infants and young children. Rotavirus diarrhea is responsible for up to 50% of hospitalizations due to severe diarrhea among under five children worldwide. Globally, rotavirus is estimated to cause 527,000 deaths in children annually approximately 85% of these deaths occur in low resource countries in South Asia and sub-Saharan Africa (7, 10, 11, 12).

Ethiopia is a country where rota takes the lives of more than 28,000 under five children each year, being one of the five countries with the greatest rotavirus burden worldwide and accounts for 6% of all rotavirus related deaths globally. Rota virus is responsible for an estimated 28% of all under-five diarrhea hospitalizations in Ethiopia (12, 18, 19).

Rotavirus disease is more severe than diarrhea caused by other enteric pathogens with symptoms including an average of 6 episodes per day, severe dehydration, vomiting and fever. Even though severe rotavirus diarrhea is a major public health problem in sub-Saharan Africa including Ethiopia; there is limited regional and country specific data on the disease burden (20).

Even though some studies have been conducted in Ethiopia to assess prevalence of rota virus, to the best search there is no study about rota virus prevalence and associated risk factors in Addis Ababa, Ethiopia. Therefore, this study aimed to fill this gap and generate base line survey data on prevalence and risk factors of rota virus diarrhea.

## 1.3 **Significance of the Study**

The present study will give basic information on the risk factors for rota virus infection in addition to determining the rota virus induced diarrhea burden. The study will be help full for health practitioners to consider rota virus in pediatric gastroenteritis management before using toxic antibiotics. Accumulating data on the prevalence of rota virus diarrhea and risk factors among under five children is important for developing effective preventive mechanisms and control measures to reduce the amount of rota virus associated diarrhea and minimize the spread.

## 2. Literature Review

Worldwide, diarrhea is a major public health problem and common cause of morbidity and mortality in children. Rotavirus constitutes the single most important cause of acute dehydrating viral gastroenteritis in young children in both developed and developing countries. The result of a study conducted in USA among 349 rota infected and 1242 control under five children to determine risk factors associated with rota virus hospitalization showed that breast feeding has protective effect for infants under 6 months of age and day care attendance, extra under two children in the house, maternal age less than 25 year and less than a high school education have a significant risk (21).

Another study in USA to see the prevalence of viral diarrheal etiology and association with gastroenteritis among 480 hospitalized children, Astrovirus was detected in 5.2% of all children compared to 6.8% with rotavirus and 0.8% with enteric adenoviruses by antibody based ELISA method (22).

In a study conducted in Brazil to study the epidemiology of rotavirus and estimate rotavirus-associated morbidity and mortality in children $\leq$ 5 years of age before 2004 vaccine introduction in Brazil by reviewing studies from 1999-2006, Rotavirus infection was implicated to cause an estimated 3,525,053 episodes of diarrhea, 655,853 visits to outpatient healthcare facilities, 92,453 hospitalizations and 850 deaths in Brazil $\leq$ 5 children (23).

In a study conducted at Northern Italy to determine rotavirus acute gastroenteritis and identify the circulating G and P genotypes among 521 under five children, 34.9% children were rotavirus-positive and 67.6% were under two years of age, and 13.2% were under six months. G1and P8 were the predominant genotypes identified (24).

In a study conducted in Northern Frence, 973 stool specimens were collected from under five children hospitalized for GE and tested for rotavirus, norovirus, adenovirus and astrovirus and the overall rates of prevalence were 21%, 13%, 5% and 1.8% respectively (25).

A study carried out in India to determine the prevalence of rotavirus infection in 250 children up to two years presenting with diarrhea and to discover factors associated with rotavirus infection, rota infection was confirmed from 24% children with diarrhea, 78.3% of children with rota viral diarrhea were in the age group of 6–15 months and there was a significant association between type of feeding and rotavirus diarrhea with reduced prevalence while on

exclusive breast-feeding. Though only 10.4% of children with rotavirus diarrhea had severe dehydration, 61.5% of children with severe dehydration were rotavirus positive but only10.4% children with rota diarrhea had severe dehydration (26).

In a study to determine the prevalence of rotavirus in stool samples by EIA among 184 children under 7 years hospitalized with acute gastroenteritis in Imam Sajjad Hospital of Yasuj, Iran, 28.3% were positive for rota virus and there was significant relationship between the seasonal distribution and virus detection, the highest (48.1%) incidence of rotavirus in autumn and the lowest (5.8%) in spring (27).

A study carried out in Israel to compare the demographic, clinical and laboratory characteristics of patients with rotavirus gastroenteritis to those with other causes among 533 under 18 years GE patients; 37.9% were positive and compared to patients with rotavirus-negative patients, rotavirus-positive gastroenteritis had a higher incidence of vomiting, lethargy and dehydration and the duration of hospitalization was higher in patients with rotavirus (28).

A research carried out in Riyadh, Saudi-Arabia to assess the epidemiology of rotavirus causing pediatric diarrhea among 1,007 hospitalized under five children, rotavirus was detected in 65.5% stool samples, children aged 1 year or less had more infection with rotavirus 81% than those who are over 1 year of age (19%), infections occur throughout the year with no clear significant seasonal peaks and the difference between males (57.5%, 380/660) and females (42.4%, 280/660) in terms of rotavirus positivity was statistically significant (29).

Another study conducted in Makkah and Jeddah cities of Saudi Arabia to describe the frequency of viral, bacterial and parasitic pathogens among 270 under five children with diarrhea, the total number of entero-pathogen positive samples were 106 (39%) and of this, 90 (33%) had viral etiology, of which Rotavirus group A was found in the majority of cases 60 (22%) followed by 20 (7%) Adenovirus and 10 (4%) Astrovirus (30).

In a retrospective study carried out in Cathay General Hospital, Taiwan among 238 rota positive under 15 children to determine the clinical characteristics of rota virus gastroenteritis, the most common symptom was diarrhea (98.7%), the peak month was May (20.4%) and all patients were discharged alive without complication (31).

In a study conducted in Southern Jordan to determine the incidence and clinical features of rota virus infection among 148 under five children hospitalized due diarrhea, 40% of stool samples

were rota virus positive by ELISA and there was higher rate of fever among rota positive cases. 96.6% of rota positive cases were among under two children (32).

In a study carried out in Nepal to determine rotavirus disease burden and distribution of genotypes in 1,768 under five hospital admitted children with acute watery diarrhea from October 2005 to November 2008, rota virus stool antigen was detected in 36.6% children and G12 and P[8] were the most common single genotypes sequenced (33).

In a rota virus surveillance initiated by WHO Regional Office for Africa in 8 selected African countries (Ghana, Kenya, Uganda, Zambia, Cameroon, Tanzania, Zimbabwe and Ethiopia) from June 2006 - December 2008, 5,461 stool samples were collected from under five children with diarrhea, 2,200 (40%) were positive for rotavirus. Ninety percent of all rotavirus hospitalizations occurred among children aged 3–12 months (20).

In a study conducted in Tamale of Northern Ghana to see epidemiological, clinical and microbiological characteristics of acute childhood diarrhoea by using stool specimens from 243 acute diarrhoea and 124 control children by RT-PCR assays, the most common pathogens in patients were rotavirus (55%), adenovirus (28%) and norovirus (10%) followed by intestinal parasites (5%) and bacteria (5%). Rotavirus was the only pathogen found significantly more frequently in patients than in controls and was associated with young age, fever and watery stools (34).

In a study aimed to determine the prevalence of group A rotavirus in children below 5 years with diarrhea in Northern Cameroon, 390 stool samples were collected during 2010 and 2011. 42.8% children had group A rotavirus infection by PCR and significantly higher among hospitalized (49%) than outpatient children (31%). The most affected children were below 24 months of age (44.7%), whereas the age group 49-60 months were the least affected (25%) and the prevalence of rota was 44.6% in urban and 28.9% in rural (35).

In a study conducted at Benin City of Nigeria to determine the prevalence of rotavirus and distribution of genotypes before vaccine introduction among 470 children under five years with diarrhea, 13.8% of the stools tested were positive for rotavirus antigen using ELISA and the most common single genotype (54.4%) was G1 (36).

In a study carried out to investigate the etiology of diarrhoea among 283 under five children with acute diarrhea and 60 healthy controls in Ouagadougou, Burkina Faso, at least one

pathogen was detected in 64% of patients and in 8% of controls. Rotavirus was found in 30% of the patients. Rota virus was detected mainly in children under 2 years and mostly during the cool dry season (37).

In a study to describe the prevalence of three enteric viruses, namely rotavirus (RV), adenovirus (Ad) and astrovirus (AsV), as agents of diarrhoea in and around Gaborone, Botswana, shedding of RV was detected in 9.2%, Ad in 7.8% and AsV in 2.7% by ELISA and an increased rota virus infection rate was noted in the autumn-winter season (13).

In a study carried out to estimate the proportion of rotavirus GE and identify rota diarrhea determinants at ten Tunisian hospitals, May 2009 - October 2010, 550 stool specimens were collected from children under five 5 years. Rota virus was detected in 27.3% of children and infants under 2 years of age were the most frequently affected (91.6%). The most dominant rotavirus genotype was G3P[8] accounted for 40.4% of the cases and rotavirus was significantly associated with the diarrhea episodes, vomiting, fever and dehydration (38).

In a cohort study conducted on 348 Egyptian children (birth-2 year) to determine the circulating rota virus genotype, 40% of children had rotavirus-associated diarrhea at least once by their second birthday and the most prevalent genotype was G2 (32%). Rotavirus episodes were significantly associated with fever and vomiting (39).

In another study conducted in Delta, Egypt to estimate the disease burden among 92 under five children complaining of acute diarrhea, 44.6% children had rota virus antigen by EIA and 91.1% of the positive cases of rota were under two years of age with highest prevalence in children 7-12 months. Breast feeding children were at greater risk of rota diarrhea and vomiting and severe dehydration were significantly associated with rota virus diarrhea (40).

In a study of Dares Salaam, Tanzania to see the etiological agents of diarrhoea among hospitalized under five children, at least one diarrheal pathogen was detected in 67.1% of the cases, and mixed infections were detected in 20.7% of cases. Overall, bacteria and viruses contributed equally accounting for 33.2% and 32.2% of all the cases, respectively, while parasites were detected in 19.2% patients (41).

In a study conducted to determine the prevalence of rota virus and associated risk factors among children five years with acute watery diarrhea attend in Mwanza, Tanzania, 20.7% children were found to have rotavirus infection by latex agglutination test. 84% of children with

rotavirus infection were aged below two years. Living next door to a child with diarrhea was significantly associated with rotavirus infection (42).

In a study carried out in Uganda, the prevalence of rotavirus infection was 45.4% among under five children and was significantly associated with a higher education (above secondary) level of the mother, dehydration and breastfeeding. There was no significant association between rotavirus infection and nutritional status, HIV status and attendance of day care or school (43).

In a hospital based surveillance of rota virus infection conducted from June 2009- May 2011 before vaccine introduction in Sudan, rotavirus was detected in 36% of 10,953 under five children hospitalized for gastroenteritis. 61% of the rotavirus hospitalizations occurred before one year of age and 91% occurred before 2 years of age (44).

In a cross-sectional study carried out in Jima Hospital, Ethiopia, to reveal the prevalence of rotavirus infection among 154 infants and young children, rotavirus was detected in 26.6 % of fecal specimens and 90.2% (37/41) occurred in children under 2 years. The highest rate of rotavirus antigen detection was observed among the 7-12 months of age group (34%) (45).

A study to see the epidemiology of Rotavirus and Norovirus in Awassa, Southern Ethiopia from 200 under five children with diarrhea 2008-2009, the prevalence of rota virus was 22% and the genotyping showed G3P[6] (48%, globally uncommon strain), G1P[8] (27%) and G2P[4] (7%) being the strains most commonly identified (46).

Data from hospital based surveillance of rota virus gastroenteritis among children less than five years from 2007-2011 in Addis Ababa, Ethiopia showed that rotavirus was prevalent in 20% of children enrolled from 1,749 diarrheal samples collected in the five year period (47).

## 3. Objective of the Study

### 3.1 General Objective

- To determine the prevalence of rotavirus and identify the possible risk factors among children under five years with gastroenteritis in selected health facilities of Addis Ababa, Ethiopia.

### 3.2 Specific Objectives

- To determine the prevalence of rota virus in under five children
- To identify the possible risk factors associated with rotavirus infection

## 4. Methods and Materials

### 4.1 Study Design and Period

Institution based cross-sectional prospective study was conducted from January – March 2014 on children with acute gastroenteritis (GE) at outpatient departments (OPDs) of selected health facilities in Addis Ababa, Ethiopia.

### 4.2 Study Area

The study was conducted in Selam, Kolfe, Semen and Addis Ketema Health Centers of Addis Ababa, Ethiopia. Sites are selected intentionally (conveniently) to get more severe non-referred acute diarrhea cases from front line patient servers (health centers). Beyond this other sites had been rejected because already participated in other virological surveillance activities or the nurses are too busy for taking data by questionnaire and nearest sites are selected to minimize transportation time and cost.

Addis Ababa is the capital city of Ethiopia and center of African Union. There are 15 public and 23 private hospitals. Of which (5) Federal Ministry of Health, (5) Addis Ababa Regional Health Bureau, (2) Non-Governmental Organization, (3) Defense Public Hospital, and the rest 23 are private hospitals and 52 health centers. Based on 2007 census results Addis Ababa has a total population of 2,738,248, consisting of 1,304,518 male and 1,433,730 female, and from which 195,932 are children under five years (48).

### 4.3 Source and Study population

All children with GE who visited health facility in Addis Ababa, seeking medical service primarily for their GE illness in the study period were the source population and those children with under five years of age and visited the selected health facilities were the study population.

### 4.4 Eligibility Criteria

#### 4.4.1 Inclusion Criteria

- Children visiting health facility for GE case
- Onset of GE within the last ten days
- Children under the age of five years
- Caregiver agreed to participate and give complete data
- Children who provided stool.

### 4.4.2 Exclusion Criteria

- Children took antibiotics within 14 days
- Children vaccinated for rota in the last three weeks
- Inadequate volume, formed and semi-formed stool

## 4.5 Sample Size Determination and Sampling Technique

A total of 246 children were enrolled based on single proportion sample size determination formula with 95% confidence interval, using consecutive sampling technique and 20% previous prevalence of rotavirus in Addis Ababa, Ethiopia (47). This sample size was also used to enroll care givers of children. The number of participants were selected proportionally to the number of children in each site.

$N= (z\alpha/2)^2 p(1-P)/d^2$

N = Number of children to be enrolled in the study,

z = standard normal distribution curve value for 95% confidence level ($z\alpha/2=1.96$),

p = Prevalence of rota virus in previous study,

d=margin of error taken as 5%.

## 4.6 Study Variables

### 4.6. 1 Dependent Variable

Prevalence of rota virus diarrhea

### 4.6.2 Independent variables

Sex, age, day care attendance, feeding practice, vaccination and clinical status of children, age, sex, level of education, occupation, monthly income, marital status, type of housing, hand washing practice, water source of care giver, latrine availability and waste disposal practice at home.

## 4.7 Data Collection and Specimen Transportation

Clear orientation was given for data collectors on the sample collection tools, purpose of the study and how to approach care givers. The parent/caregiver of any child who satisfies the study inclusion criteria were asked for consent to participate in the study. Consented care givers were interviewed using structured, pre-tested and local language (Amharic) translated

questionnaire designed to obtain basic socio-demographic characteristics of care giver and child, history of illness, clinical information and stool specimen of child were provided.

Fecal specimen of around 3 ml volume per child was collected in clean, labeled screw capped container and transported to Virology Research Laboratory (lab), Ethiopian Public Health Institute (EPHI) by cold-chain system.

### 4.8 Laboratory Investigation

All 246 stool samples were tested for rotavirus antigen using ProSpecT Rotavirus EIA kit specific to group A rota viruses, 100% sensitivity and 99.2% specificity for rota virus antigens in stool, with R240396 batch and 1155455 lot numbers (Oxoid Ltd Company, UK). Once collected, part of sample was analyzed microscopically in saline wet mount by laboratory professionals at each site for parasite identification and stool transported to EPHI and inoculated on MacConkey and Salmonella-Shigella agar (SSA) for isolation of bacterial enteropathogens and conventional biochemical test was used for identification.

For rota virus EIA technique, 10% suspension of fecal specimen was prepared by mixing 100µl (two drops) of liquid stool and 1ml of sample diluent (specific buffered saline provided with the kit). The mixture was homogenized by vortex mixer, then allowed to settle and 100µl suspension was taken from the supernatant and added to EIA test plate wells based on the plate setup on our work sheet. Once all the test and control samples are added, 100µl of conjugate (enzyme labeled antibody against rota virus) was added to each well and incubated for an hour.

After one hour incubation, the EIA plate was washed five times by ELISA washer with diluted wash buffer (1:5) to remove excess specimen and unbound conjugates. Plate was inverted and tapped on absorbent paper to avoid last traces of wash buffer remained. Then 100µl substrate was added to each test plate well and incubated for 10 minutes to allow the enzyme (if any) to break the substrate. Finally enzyme substrate reaction is stopped by adding 100µl stop solution (0.46 mol/L sulpheric acid) to each well.

The color intensity of wells were read by ELISA plate reader at 450 nm within 30 minutes from addition of sulpheric acid. The cut off value was determined by adding 0.2 on the negative control optical density (OD). Cut off varied for each run based on the negative control OD.

The result of a test sample was recoded as positive when the OD of test well was greater than the cut off value and negative if lower. Samples with equal OD with cut off value or within 0.001difference were equivocal and repeated.

### 4.9 Quality Control

Sample preparation and testing at Virology laboratory was according to the manufacture's instruction. Positive and negative controls (provided by the manufacturer) were run in each run and test results were interpreted against the controls included. Standard Operational Procedure (SOP) was followed strictly.

About 10% of all positive and negative samples were rechecked by the same kit and there was no significant variation between repeated and previous results.

Data was accessed by principal investigator (PI) only and will be kept up to a year in the PI personal computer after the study completed and presented. No third body has access to the hard copies.

National Polio and Measles Laboratory is an accredited laboratory and receives proficiency panel samples every year and sends 10% quality control samples quarterly. The lab scored $\geq$ 95 % efficiency in proficiency panel testing and 10% quality control samples in the last three years. This was promising for quality bench work to be conducted in it.

### 4.10 Data Processing and Statistical Analysis

The data was entered by using Epi-Info software version 3.5.4 and analyzed by SPSS version 20 statistical soft wares. Percentages and frequencies were used to show distribution of descriptive data using tables. Both bi-variant and multi-variant analysis were employed using logistic regression model. Results were interpreted based on 95% confidence level, statistically significant when p-value <0.05. Odds ratio was used to see the strength of association between dependent and independent variables.

### 4.11 Ethical Consideration

The study was ethically cleared and approved by Department Research and Ethical Review Committee (DRERC) of Addis Ababa University and Scientific and Ethical Review Office (SERO) of EPHI. Support letter was obtained from the Department of Medical Laboratory Science to Addis Ababa City Administration Health Bureau and the study was reviewed and approved by Institution review board of Addis Ababa City Administration Health Bureau once

again. Then support letter was obtained for each site from the health bureau. Informed consent was obtained from caregivers. Results were reported to the clinicians. Collection of stool sample does not have any harm to the child health.

### 4.12 Dissemination of Findings

The finding of this thesis has been submitted to the School of Graduate Studies, Addis Ababa University. Oral presentation of the thesis will be made according to the school schedule. Copy of this thesis has been submitted for EPHI and Addis Ababa Administration Health Bureau. In addition the findings of this study will be published in peer review journals and abstract will be presented in local and international scientific conferences.

## 5. Results

### 5.1 Socio-Demographic Characteristics and Clinical features of Children

In the study period from January to March 2014, a total of 246 diarrheic stool samples were collected from under five children and investigated for rota virus, bacterial and parasitic enteropathogens. Of all, one hundred nineteen (48.4%) were females. The mean age of study children was 28.5 months (ranges 2-59 months with standard deviation, SD, of ±15.5). Age wise distribution of diarrheic children showed maximum number of patients 125 (51%) in the age group 25-59 months and majority of them (81%) were not vaccinated for rota virus according to their guardian reports (Tab 5.1).

Of these 246 children with GE, 74 (30.1%) attend day care and 10 (4.1%) were exclusive breast feeding children. The mean episode of diarrhea per day was 3.5 (range 1-10, ±1.34 SD) and stayed with GE for an average 3 days (range 1-10, ±1.8 SD). Regarding the sign and symptoms, 133 (54.1%) had vomiting, 163 (66.3%) had abdominal pain, 47 (19.1%) had tenesmus, and 116 (47.2%) had fever along with diarrhea. The mean episodes of vomiting was 2.2 per day (SD±1.4, range 1-10). One hundred twenty (48.8%) of the collected stools were mucoid. The children had an average body temperature of $37^0$c, ranges 35-39 $^0$c (±$0.55^0$c SD) (Tab 5.1).

**Table 5.1. Socio-Demographic and Clinical features of children investigated for Rota virus in Selam, Semen, Addis Ketema and Kolfe Health Centers of Addis Ababa, Ethiopia, 2014 (N=246)**

| Variable | Category | Frequency | Percent |
|---|---|---|---|
| Sex | Female | 119 | 48.4 |
| | Male | 127 | 51.6 |
| Age Group (Months) | 0-6 | 20 | 8.1 |
| | 7-12 | 22 | 8.9 |
| | 13-24 | 79 | 32.1 |
| | 25-59 | 125 | 50.8 |
| Feeding Practice | Exclusive breast | 10 | 4.1 |
| | Breast plus solid food | 80 | 32.5 |
| | Solid food only | 139 | 56.5 |
| | Milk Formula | 9 | 3.7 |
| | Cow Milk | 8 | 3.3 |
| Vaccination Status | Vaccinated | 7 | 2.8 |
| | Unvaccinated | 199 | 80.9 |
| | Not Known | 40 | 16.3 |
| Vomiting | Yes | 133 | 54.1 |
| | No | 113 | 45.9 |
| Day care Attendance | Yes | 74 | 30.1 |
| | No | 172 | 69.9 |
| **Stool Appearance** | Mucoid | 120 | 48.8 |
| | Watery | 114 | 46.3 |
| | Bloody | 12 | 4.9 |

## 5.2 Socio-Demographic Characteristics of Care Givers

Majority (90.2%) of care givers were females with a mean age of 29.4 years (±6.33 SD) ranging from 16 to 57 years and 93 (37.8%) were in the age group of 28-33 years. Majority of guardians (87.4%) were married, live in private rent home (49.2%), and had no extra under five child in the household (72.4%). The mean year of schooling for care givers was 7.7 years (±4.3 SD), ranges from 0 to 18 years and at least 44.3% had completed primary school education. Regarding occupation, 94 (38.2%) of the care givers were employed in private and the mean monthly income of all care givers was 1,765.5 Ethiopian birr (ETB), ranges 0 to 60,000 ETB (±3991.6 SD). Majority (61.2%) of guardians gained in the range 421-1228 ETB (22-62 USD) per month for their family (Tab 5. 2).

**Table 5.2. Socio-demographic characteristics of care givers of children investigated for Rota virus in Selam, Semen, Addis Ketema and Kolfe Health Centers of Addis Ababa, Ethiopia, 2014 (N=246)**

| Variable | Category | Frequency | Percent |
|---|---|---|---|
| Age Group (Years) | 16-21 | 21 | 8.5 |
| | 22-27 | 76 | 30.9 |
| | 28-33 | 93 | 37.8 |
| | 34-57 | 56 | 22.8 |
| Marital Status | Single | 6 | 2.4 |
| | Married | 215 | 87.4 |
| | Divorced | 15 | 6.1 |
| | Widowed | 5 | 2.0 |
| | Separated | 5 | 2.0 |
| Type of Housing | Condominium | 19 | 7.7 |
| | Private villa | 104 | 42.3 |
| | Private rent | 121 | 49.2 |
| | Others | 2 | 0.8 |
| Educational level | Illiterate | 27 | 11 |
| | Primary School | 109 | 44.3 |
| | Secondary School | 80 | 32.5 |
| | Higher Education | 30 | 12.2 |
| Extra <5 Child in home | 0 | 178 | 72.4 |
| | 1 | 60 | 24.4 |
| | 2 | 7 | 2.8 |
| | 3 | 1 | 0.4 |
| Occupation | Government | 25 | 10.2 |
| | Private employed | 94 | 38.2 |
| | Merchant | 24 | 9.8 |
| | House wife | 89 | 36.2 |
| | Jobless | 14 | 5.7 |
| Monthly Income (ETB) | <421 | 18 | 7.3 |
| | 421-1228 | 134 | 54.5 |
| | > 1228 | 94 | 38.2 |
| Source of Water | Pipe water | 241 | 98 |
| | Non-piped | 5 | 2.0 |
| Latrine | Available | 243 | 98.8 |
| | Not available | 3 | 1.2 |

19.8ETB=1USD

Majority 241 (98%) care givers used piped water source and 242 (98.4%) had a good habit of washing their hand before and after feeding their child. Two hundred forty three (98.8%) care givers had latrine and 236 (96.7%) dispose their waste through waste collectors, 6 (2.5%) incinerate and 2 (0.8%) dampened their waste (data not shown).

### 5.3 Microbial Etiology of Gastroenteritis and Association with Risk factors

Of 246 stool specimens collected from under five children with GE, rota virus was detected in 85 (34.55%) samples by EIA rota virus stool antigen detection kit. At least one parasite was identified in the stool of 65 (26.4%) patients, trophozoite of *Entamoeba histolytica/dispar* was the most prevalent parasite 29 (11.8%) and at least one bacterial pathogens were isolated from 77 (31.3%) diarrheic children, *E. coli* was the most frequent isolate, 66 (26.8%) (Tab 5.3.1).

Among all rota virus positive children, twenty six (10.6%) had bacterial pathogens, 16 (6.5%) had parasitic pathogens and 3 (1.2%) children had triple infection of rota virus, bacterial and parasitic pathogens (data not shown).

**Table 5.3.1 Distribution of Microbial Etiologic agents among children investigated for Rota virus in Selam, Semen, Addis Ketema and Kolfe Health Centers of Addis Ababa, Ethiopia, 2014 (N=246)**

| Etiology | Category | Frequency | Percent |
|---|---|---|---|
| Rota Virus | Positive | 85 | 34.55 |
| | Negative | 161 | 65.45 |
| Parasitic | *Entamoeba histolytica/dispar* (T*) | 29 | 11.8 |
| | *Entamoeba histolytica/dispar* (C*) | 6 | 2.4 |
| | *Giardia Lamblia* (T*) | 17 | 6.9 |
| | *Giardia Lamblia* (C*) | 3 | 1.2 |
| | *Hymenolopsis Nana* | 7 | 2.8 |
| | *Ascaris Lumbricoides* | 3 | 1.2 |
| | No ova/parasite seen | 181 | 73.6 |
| Bacterial | *Salmonella* | 2 | 0.8 |
| | *Shigella* | 9 | 3.66 |
| | *E. coli* | 66 | 26.8 |
| | No growth | 169 | 68.7 |

T*=Trophozoite, C*=Cyst

Rota virus positivity rate was higher among females than males (37% vs 32.2%) but sex has no significant association with rota virus infection ($p>0.05$) in this study.

Rota virus was detected at higher proportion (39.5%) among children 1-2 years but there is no statistically significant association between age and rota virus induced diarrhea in the current study ($p>0.05$). Higher (36.6%) rota virus infection was confirmed among day care attendants, however there is no significant association between day care attendance and rota virus diarrhea ($p>0.05$). Half (50%) of children who fed cow milk were positive for rota virus infection but there is no statistically significant association between feeding practice and rota virus diarrhea in the bi-variant regression analysis (Tab 5.3.2).

Rota virus detection rate was highest (43%) among children who were vaccinated for rota virus infection before a month from sample collection date relative to the non-vaccinated (37.7%) and the unknown vaccination status (17.5%) children, though rota vaccination status has no significant effect on rota virus positivity in this report ($p>0.05$) (Tab 5.3.2).

In this study children with vomiting had higher rota virus diarrhea than without vomiting (39.1% vs 29.2%) and among all rota virus positives (85, 100%), 61.2% had vomiting. However vomiting and rota virus induced diarrhea have no significant association ($p>0.05$).

Although 66% of children had abdominal pain among all rota positive cases, rota was detected similarly from children with and without abdominal pain (34.9% vs 34.4%) and no significant association is found between abdominal pain and rota virus infection ($p>0.05$).

In the present study, rota virus detection rate was slightly higher among children with tenesmus (36.2%) than without (34.2%) and among all rota positive cases only 20% had tenesmus ($p>0.05$).

Among all rota positive cases, 43.5% had fever and those children without fever had higher rate of rota diarrhea than children with fever (37% vs 32%). In this study, fever and rota diarrhea have no statistically significant association ($p>0.05$).

Children with diarrhea episodes greater than 5 per 24 hours had higher rota prevalence (44.4%) and rota detection was higher (50%) among children with diarrhea duration of greater than 7 days before coming to health facility. But both diarrhea episode and duration have no statistically significant association with rota virus infection ($p>0.05$).

Rota detection rate was highest, 36.7% (44/120) from children with mucoid appearance relative to bloody 33.3% and watery 32.5% stools. Appearance of stool has no significant association with rotavirus diarrhea.

Generally in this study, no child related risk factor was significantly associated with rota virus diarrhoea, but there was a difference in positivity rate among different categories of children (Tab 5.3.2).

**Table 5.3.2. Rota virus and its association with risk factors and clinical features of children investigated for rota virus in Selam, Semen, Addis Ketema and Kolfe Health Centers of Addis Ababa, Ethiopia, 2014 (N=246)**

| Variable | Category | Rota EIA Result | | COR (95% C.I) | P value |
|---|---|---|---|---|---|
| | | Positive (%) | Negative (%) | | |
| **Sex** | Male | 41 (32.3) | 86 (67.7) | 1.23(.73-2.1) | .440 |
| | Female | 44 (37) | 75 (63) | 1 | |
| **Age** | 0-6 | 7 (35) | 13 (65) | 1 | |
| | 7-12 | 7 (31.8) | 15 (68.2) | .906 (.336-2.44) | .846 |
| | 13-24 | 30 (38) | 49 (62) | 1.05 (.396-2.76) | .928 |
| | 25-59 | 41 (32.8) | 84 (67.2) | .797 (.44-1.4) | .450 |
| **Daycare Attendance** | Yes | 28 (37.8) | 46 (62.2) | .814 (.46-1.44) | .478 |
| | No | 57 (33.1) | 115 (66.9) | 1 | |
| **Feeding Practice** | Breast Only | 3 (30) | 7 (70) | 1 | |
| | Breast and Solid | 27 (33.75) | 53 (66.25) | 2.333 (.34-16.2) | .391 |
| | Solid Food Only | 50 (36) | 89 (64) | 1.96 (.455-8.5)) | .366 |
| | Milk formula | 1 (11.1) | 8 (88.9) | 1.8 (.43-7.43) | .429 |
| | Cow Milk | 4 (50) | 4 (50) | 8.0 (.66-97) | .103 |
| **Vaccination status** | Yes | 3 (42.86) | 4 (57.14) | 1 | |
| | No | 75 (37.7) | 124 (62.3) | 1.24 (.27-5.7) | .782 |
| | Not Known | 7 (17.5) | 33 (82.5) | 3.5 (.46-19) | .146 |
| **Vomiting** | Yes | 52 (39.1) | 81 (60.9) | .64 (.38-1.1) | .105 |
| | No | 33 (29.2) | 80 (70.8) | 1 | |
| **Abdominal Pain** | Yes | 56 (34.4) | 107 (65.6) | 1.0 (.59-1.8) | .927 |
| | No | 29 (34.9) | 54 (65.1) | 1 | |
| **Tenesmus** | Yes | 17 (36.2) | 30 (63.8) | .92 (.47-1.8) | .795 |
| | No | 68 (34.2) | 131 (65.8) | 1 | |
| **Fever** | Yes | 37 (31.9) | 79 (68.1) | 1.3 (.74-2.1) | .408 |
| | No | 48 (36.9) | 82 (63.1) | 1 | |
| **Diarrhea Episode** | 1-5 | 77 (33.8) | 151 (66.2) | 1 | |
| | 6-10 | 8 (44.4) | 10 (55.6) | 1.6 (.6-4.14) | .363 |
| **Diarrheal Days** | 1-7 | 83 (34.3) | 159 (65.7) | 1 | |
| | 8-10 | 2 (50) | 2 (50) | 1.9 (.265-14) | .519 |
| **Stool Appearance** | Watery | 37 (32.5) | 77 (67.5) | 1.0 (.3-3.7) | .951 |
| | Mucoid | 44 (36.7) | 76 (63.3) | .86 (.25-3) | .819 |
| | Bloody | 4 (33.3) | 8 (66.7) | 1 | |

The prevalence of rota virus and it association with child's guardian related risk factors are presented in table 5.3.3. In the current study, rota virus was detected in higher frequency among children whose close guardians are males (41%) than females (34.6%) and among age group of above 35 years (37.5%). However both sex and age of guardian have no significant association with rota virus infection ($p>0.05$).

Rota was detected frequently (44.4%) among children whose guardians were illiterate, but rotavirus diarrhea and guardian educational level have no significant association in this study. Children from families with extra under five children had lower rota infection rate but highest from private villa resident families even though both presence of extra under five children and type of home have no significant association with rota virus induced diarrhea (Tab 5.3.3).

The prevalence of rota virus infection was highest (50%) among children from jobless care givers and least from children of government workers (20%). But there is no statistically significant association between occupation and rota virus induced diarrhea.

The prevalence of rota virus induced diarrhea was highest (36.8%) among children from middle income families (421-1228 ETB) (21-62USD). But there is no statistically significant association between rota virus diarrhea and care giver monthly income level in this study.

Although there is no significant association between rota virus and hand washing practice in this study, rota virus detection rate was higher (75%) among children whose care givers did not wash their hand before and after feeding them. The prevalence of rota virus was higher (37%) among children from guardians that did not use soap than soap users (33%) for hand washing but still hand washing and soap usage have no statistically significant association with rota virus infection (Tab 5.3.3).

Detection rate of rota virus associated diarrhea was higher (40%) among children of non-piped water users than piped water users (34.4%). However there is no significant association between rota virus diarrhea and type of water source.

The prevalence of rota virus diarrhea was higher (66.7%) among children having latrine than without (34%) and slightly higher (34.7%) among public latrine users than private latrine users (33.8%). But both availability and type of latrine have no significant association with rota virus detection rate (Tab 5.3.3).

**Table 5.3.3. Rota virus and risk factor association of children investigated for Rota virus in Selam, Semen, Addis Ketema and Kolfe Health Centers of Addis Ababa, Ethiopia, 2014 (N=246)**

| Variable | Category | Rota EIA Result | | COR (95% C.I) | P value |
|---|---|---|---|---|---|
| | | Positive, N(%) | Negative, N(%) | | |
| **Sex of Care giver** | Male | 9 (40.9) | 15 (59.1) | .87 (.36-2.1) | .749 |
| | Female | 76 (34.2) | 146 (65.8) | 1 | |
| **Care giver Age** | 16-24 | 17 (37) | 29 (63) | 1. (.4-2.6) | .961 |
| | 25-35 | 56 (33.3) | 112 (66.7) | 1.2 (.55-2.6) | .649 |
| | 36-57 | 12 (37.5) | 20 (62.5) | 1 | |
| **Educational Level** | Illiterate | 12 (44.4) | 15 (55.6) | .54 (.18-1.6) | .261 |
| | Primary | 34 (31.2) | 75 (68.8) | .95 (.39-2.3) | .900 |
| | Secondary | 30 (37.5) | 50 (62.5) | .7 (.29-1.76) | .465 |
| | Higher | 9 (30) | 21 (70) | 1 | |
| **Extra <5 Child** | 0 | 62 (34.8) | 116 (65.2) | 1 | |
| | 1-3 | 23 (33.8) | 45 (66.2) | .96 (.53-1.7) | .88 |
| **Occupation** | Government | 5 (20) | 20 (80) | 1 | |
| | Private | 33 (35.1) | 61 (64.9) | 4.0 (.95-16.8) | .058 |
| | Merchant | 10 (41.7) | 14 (58.3) | 1.85 (.6-5.7) | .287 |
| | House wife | 30 (33.7) | 59 (66.3) | 1.4 (.37-5.3) | .619 |
| | Jobless | 7 (50) | 7 (50) | 2 (.63-6.1) | .243 |
| **Income Level** | <421 | 6 (33.3) | 12 (66.7) | .94 (.3-2.7) | .906 |
| | 421-1228 | 49 (36.6) | 85 (63.4) | .813 (.465-1.4) | .468 |
| | >1228 | 30 (31.9) | 64 (68.1) | 1 | |
| **Housing** | Condominium | 4 (21) | 15 (79) | 1.9 (.61-6.2) | .263 |
| | Private Villa | 39 (37.5) | 65 (62.5) | .86 (.5-1.5) | .599 |
| | Private Rent | 42 (34.1) | 81 (65.9) | 1 | |
| **Hand Washing** | Yes | 82 (33.9) | 160 (66.1) | 1 | |
| | No | 3 (75) | 1 (25) | 5.8 (.6-57.2) | .129 |
| **Soap Usage** | Yes | 62 (33) | 126 (67) | 1 | |
| | No | 20 (37) | 34 (63) | 1.3 (.73-2.45) | .351 |
| **Water Source** | Non-Piped | 2 (40) | 3 (60) | 1.3 (.21-7.7) | .796 |
| | Piped | 83 (34.4) | 158 (65.6) | 1 | |
| **Latrine** | Not available | 2 (66.7) | 1 (33.3) | 3.8 (.345-43) | .273 |
| | Available | 83 (34.2) | 160 (65.8) | 1 | |
| **Type of Latrine** | Public | 33 (34.7) | 62 (65.3) | 1.1 (.64-1.86) | .755 |
| | Private | 50 (33.8) | 98 (66.2) | 1 | |
| **Waste Disposal** | Dampened | 1 (50) | 1 (50) | .26 (.023-2.9) | .274 |
| | Incineration | 2 (33.3) | 4 (66.7) | 1.0 (.186-5.8) | .966 |
| | Collectors | 82 (34.5) | 156 (65.5) | 1 | |

COR=Crude odds ratio, C.I=Confidence Interval

## 6. Discussion

The millennium development goal 4 (MDG4) target on child health is to reduce child mortality by two-third from 1990-2015. Under five child deaths in Ethiopia have reduced by 67% over two decades (204/1000 in 1990 to 68 death per 1000 live birth in 2012) reaching the target two years early. Diarrhea is still the major cause of under-five child mortality and morbidity in Ethiopia, with 230,000 deaths annually. Rota virus is responsible for an estimated 28% of all under-five diarrhea hospitalizations in Ethiopia (12, 16, 19, 49).

The present study revealed that rota virus is an important etiologic agent of acute gastroenteritis accounting for 34.6% of all diarrhea (Tab 5.3.1). This prevalence of rota virus infection was higher than the findings of other studies conducted in different parts of Ethiopia like Addis Ababa 20% (47), Jimma 26.6% (45) and Hawassa 22% (46). This might be due to the difference of method, variation in geographic location, season and period of sample collection and target population.

The prevalence of this study was consistent with studies conducted outside Ethiopia like studies in Sudan (36%) (44), Italy (34.9%) (24), China (33.7%) (50) and Nepal (36.6%) (33), and this shows rota virus infection is a challenge of both developed and developing world.

The prevalence of rota virus found in this study was lower than the findings of other studies conducted in African and Asian countries like 45.5% of Uganda (43), 58% of Ghana (51), 44.6% of Egypt (40), 42.8% of Cameroon (35), 65.5% of Saudi Arabiya (29) and 67% of Pakistan (52), but higher than other studies previously conducted in developed and developing countries like 21% of France (25), 30.1% of Ivory Coast (53), 30% of Burkina Faso (37), 27.3% of Tunisia (38), 20.7% of Tanzania (42), 13.8% of Nigeria (36), 13.6% of Kenya (54), 9.2% of Botswana (13) and 11.7% of Sudanese study among displaced communities (1). These difference might be due to the methods used, sample size, season of data collection, time frame, case definition (onset, stool appearance), difference in countries control strategy, geographic variation, life styles, health education and prevention programs and patient department the study done (27, 47, 55).

Although there was a difference in rota virus prevalence among females and males, there is no significant association between sex and rota virus infection in the present study. This finding was consistent with the result of other studies conducted in Iraq, India, and Israel (26, 28, 56)

but in disagreement with the finding of Indian study (58) (males more affected). But still there is no any scientific background in biological perspective and no life style difference at this age of children that supports sex preference of rota virus infection.

In the findings of this study, rota virus was detected throughout all age groups (2-59 months) almost equally which was in agreement with the findings of studies done in Jimma Ethiopia (45) and India (26) but in disagreement with the findings of studies done in Addis Ababa Ethiopia (47) where children 6-12 months were highly affected, Ghana(51) children 0-5 months were mostly affected, Ivory Coast (53) children 6-11 months were highly affected, Jordan (32) children under 24 months were most affected, Saudi Arabia (29) children under one year were highly affected and a study of China (50) where children 12-24 months of age were the mostly affected. All of the aforementioned six studies had showed a significant association of rota virus diarrhea and age of children. This might be due to a difference in study design and population, and method.

In the current study, rota virus was confirmed at higher rate in stools of day care attendant children, but there is no significant association between day care attendance and rota virus diarrhoea ($p>0.05$). This result agrees with the result of studies done in Israel (28) and Nigeria (58) in which day care attendance has no significant association with rota virus infection but disagrees with findings of a study done in USA where attending day care was a significant risk for rota virus diarrhea of children (21). This might be due to the difference in target population (age of day care registration), day care school entrance criteria applied in each country and study type (cohort).

Rota virus was detected in a higher proportion of vaccinated children than unvaccinated, and this might be due to shedding of virus after a month of vaccination or new infection by a different strain of rota virus (other than G1P8, vaccine strain). However this result is not conclusive due to small number of samples in this category.

Although rota detection rate was highest among patients who fed cow milk and least from milk formula fed children, there is no significant association between feeding practice and rota diarrhea in this study($p>0.05$), matching with findings of a study done in Sudan (59) but disagrees with the findings of studies conducted in Uganda (43) and Nigeria (58) that stated breast fed children were most exposed for rota infection and studies done in Egypt (40), USA (21) and India (26) showed lower detection of rota virus among breast fed children and breast

feeding was protective against rota virus hospitalization for infants in theses studies. These controversial results might be due to difference in intensity and frequency of breast feeding, hygienic condition of breast feeding mother and method used (study type, study population and sample size).

In consistent with the findings of a study conducted in Ghana (51), the episodes of diarrhea per day has no significant association with rota virus infection ($p>0.05$) in this study, though the prevalence of rota virus was slightly higher among children with greater than five episodes of diarrhea daily.

In this study rota virus was detected at higher rate among children who stayed with diarrhea for more than seven days before coming to health facility for diagnosis and treatment, but there is no significant association detected between diarrhea duration and rota virus infection which agrees with the findings of studies done in Ethiopian (45), Ghana (51) and Israel (28).

In agreement with findings of studies conducted in Ivory Coast (53), Ghana (51) and Israel (28) in this study fever has no significant association with rota virus infection and the finding of a Jordan study (32), both vomiting and fever have no significant association with rota virus. In disagreement with the findings of studies conducted in Ethiopia (45, 47), Egypt (40) and Indonesia (57), vomiting had significant association with rota virus infection and studies in Tunisia (38) and Cameroon (35) where both vomiting and fever has significant association with rota virus, in this study both vomiting and fever are not significantly associated with rota virus infection. This might be due to inter personal difference in recording of signs and symptoms, collector bias or non-specific symptoms.

In contrast with studies conducted in Jimma, Ethiopia (45) and Israel (28), in this study there is no significant association between appearance of stool and rota virus infection. This might be a sample collector bias in recording appearance or double infection with protozoan parasites and other bacterial pathogens.

Rota virus was detected in higher frequency among children whose close guardians were males but there is no significant association between care giver sex and rota virus infection like findings of a study conducted in Nigeria (58). This finding is not conclusive due to small number of males as care givers in this study.

The prevalence of rota virus diarrhea has no significant association with guardian age group, but rota virus proportion was higher among children of guardian age greater than 35 years in this study. But a study in USA (21) showed that children from guardian age below 25 years were at greater risk for rota virus. This difference might be due to better educated young generation and better experience of aged guardians in child care in our country.

Although there is no significant association between educational level of guardian and rota virus diarrhea of children in this study, rota virus detection rate was highest among children coming from illiterate guardian and least from children whose guardian complete higher education and this agrees with the findings previous study conducted in Jimma Ethiopia (45), but disagrees with the findings of studies in Sudan (59) where children from illiterate family were at greater risk and USA (21) in which rota virus was detected among children whose care giver did not complete secondary education. This might be due to better access of health education in health facilities and home-based by health extension workers in the study sites.

In the present study, the prevalence of rota virus was almost similar in children from care givers with or without additional under five children in the house hold and significant association is not found between rota virus infection and availability of extra children. This disagree with the finding of a study conducted in USA (21) in which presence of additional under two children was risks for higher rota infection. This might be due to difference in the age of children included in the study.

In accordance with the findings of study conducted in Jimma Ethiopia (45), in this study there is no significant association between occupation of care giver and rota virus diarrhea of children, even though the prevalence of rota virus diarrhoea was highest among children from jobless care givers and lowest among children from government working family. This might be due to similar living standards and high human interaction.

In agreement with the findings of a study carried out in Jimma Ethiopia (45), the prevalence of rota virus has no statistically significant association with marital status but the prevalence was relatively highest among children whose guardians were separated by place. In contrast with the findings of a study conducted in Ethiopia (60) where income of family had significant association with total diarrhea, there is no significant association detected among income and rota virus diarrhea in this study. This might be due to the increased social interaction, similar

way of life and diarrhea agents other than rota virus and the fact rota virus cannot be controlled through improved water supply and sanitation alone.

The similar incidence of rotavirus infections in both industrialized and developing countries suggests that it cannot be controlled through improved water supply and sanitation alone (5). Although rota virus detection rate was higher among children whose care givers did not wash hands before and after feeding, did not use soap, did not get piped water and those did not have latrine and used public latrine, none of them have statistically significant association with rota virus infection. This disagrees with the findings of a study in West Gojam, Ethiopia (60) where total diarrhea was significantly associated with water supply quality and latrine availability. This might be due to the difference in etiologic agents of diarrhea and small number of samples in these categories with higher rota virus infection.

Over all in the current study, no socio-economic status of care givers has statically significant association with rota virus of children. This result was valid due the fact that many people of our country live with poverty and with great social interaction and this result is comparable with the findings of previous studies done in Jimma Ethiopia (45) and in Zaria Nigeria (58).

## 7. Strength and Limitation of the Study

### 7.1 Strength of the Study

- This study tried to identify the prevalence and possible risk factors of rota virus diarrhea.
- The study identify other enteropathogens like bacteria and parasites
- Ten percent quality control samples (positive and negative) were reanalyzed and there was no significant variation detected in the result.
- All laboratory operations were done in accredited laboratory (WHO disease specific accreditation).

### 7.2 Limitation of the Study

- This study was health facility based and hence the results were unlikely to be a true reflection of the disease burden in the community.
- The study was cross-sectional and control samples were not included to assess risk factors.
- Sites were selected conveniently, site selection bias.
- Children were not followed for final outcome to decide the case fatality rate of disease.
- As disease history and clinical features were reported by care givers not by patients, respondent bias was not avoidable.
- The study was unable to identify other viral enteropathogens that cause diarrhea and unable to characterize (genotype) the rotavirus positive samples.

# 8. Conclusion and Recommendation

## 8.1 Conclusion

The results of this study confirmed that rotavirus was the most important etiology of diarrhea in under five children relative to bacteria and parasite, high burden of rota virus infection in the study sites. Rotavirus is a significant cause of gastroenteritis in children, suggesting as a major cause of childhood morbidity in the area. In this study, rota virus infection affect all age groups of under five children regardless of their care giver socio-economic background, there was no any risk factor significantly associated with rota virus diarrhea.

## 8.2 Recommendation

Based on our finding the following recommendations have been given.

- Long term community based surveys and epidemiological studies at regional and national levels are important to provide broader picture of rota virus burden in children.
- Continuous monitoring of viral enteric pathogens is important for successful treatment of patients and control of diarrheal disease.
- Genotyping rota virus positive specimens is important to see the emergence of new strains.
- Immunization is possibly an important preventive strategy towards control of rota virus infection.
- Vaccine efficacy study in children of Ethiopia is vital.
- Good hygiene is another basic important rota virus infection prevention method.

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## Annexes

## Annex I Participant Information sheet and Questionnaire in English

### A. Participant Information Sheet

Addis Ababa University, School of Allied Health Sciences, Department of Medical Laboratory Science, Addis Ababa, Ethiopia.

Title**: Prevalence of Rotavirus and Associated Risk Factors among children under five Years with gastroenteritis in selected health facilities of Addis Ababa, Ethiopia**.

First, we would like to thank you in advance for your cooperation and consent in participation in this study. Please read/listen when readout for you about general information of the study. If you have any question about the study, do not hastate to contact us by PI address.

**Summary**

**Background**: Diarrhoeal diseases affect millions of people around the world and have the greatest impact on children, especially those in developing countries. Rotavirus disease is responsible for 36% of hospitalizations due diarrhea among children under five years of age.

**Aim of the Study:** This study will be conducted to discover the Prevalence of Rota virus among <5 children with gastroenteritis and the risk factors that determine rota virus prevalence in Addis Ababa. The study will provide baseline data for health planners and care providers to design control strategy in our country.

**Benefit from the Study:** There is no financial incentive for study participants but the result of the lab will be communicated with their physician.

**Confidentiality:** Name will not be recorded and the sample will be coded, confidential. No third party has access to your information.

**Assurance of Principal Investigator:** I, the under signed to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all concerned of the research project.

Mekonen Getahun (PI) Signature....., Date ...../...../.....

PI address: Mekonen Getahun, National Polio and Measles Laboratory, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

E-mail: mekonengetahun@gmail.com Telephone: **+251919 961 961/ 13031796**

**B. Questionnaire for Socio-Demographic Characteristics of Children and Care Giver**

Addis Ababa University, School of Allied Health Sciences, Department of Clinical Laboratory Science questionnaire to assess the microbial etiology, demographic characteristics and risk factor of rota virus among children in Addis Ababa, Ethiopia.

Name of Site: .....Medical Record No .....

Address.....City, District.....

| S. No | Questions | Choice or Space to be filled | If |
|---|---|---|---|
| **1. Socio-Demographic characteristics of the child** | | | |
| 1.1 | Age | ..... Months or ……Years | |
| 1.2 | Sex | 1. Male 2. Female | |
| **2. Clinical Information** | | | |
| 2.1 | Number of days child has diarrhea | ……. | |
| 2.2 | Episodes of diarrhea in 24 hours | …… | |
| 2.3 | Vomiting | 1. Yes 2. No | 1 |
| 2.3.1 | Episodes of vomiting in 24 hours | …… | |
| 2.4 | Abdominal pain | 1. Yes 2. No | |
| 2.5 | Tenesmus | 1. Yes 2. No | |
| 2.6 | Fever | 1. Yes 2. No | |
| 2.7 | Dehydration status | 1. Severe 2. Some | |
| 2.8 | Did your child vaccinated for Rota virus | 1. Yes 2. No | 1 |
| 2.8.1 | Type of vaccine taken | 1. Rotateck 2. Rotarix | |
| 2.8.2 | How many dose the child took | 1. One 2. Two 3. Three 4. Not Known | |
| 2.9 | Temperature | .....$^{O}$C | |
| **3. Laboratory Information** | | | |
| 3.1 | Date of stool collected | ...../...../..... | |
| 3.2 | Stool identification number | ..... | |

| | | | |
|---|---|---|---|
| 3.3 | Appearance of stool | 1. Watery 2. Mucoid 3. Bloody | |
| 3.4 | Result of direct microscopy | …… | |
| **4. Family / Guardian Information** | | | |
| 4.1 | Age | .....Years | |
| 4.2 | Sex | 1. Male 2. Female | |
| 4.3 | Marital Status | 1. Single 2. Married 3. Divorced 4. Widowed 5. Separated 6. Other…… | |
| 4.4 | Years of schooling | ……. | |
| 4.5 | Did your child attend day care centre? | 1. Yes 2. No | |
| 4.6 | Number of <% children in home | …….. | |
| 4.7 | Occupation of Parents | 1. Government employed 2.Private employed 3. Merchant 4. Student 5. Others (Specify)..... | |
| 4.8 | Monthly income in birr | ……. | |
| 4.9 | Type of housing | 1. Condominium 2. Private villa 3. Rent from private | |
| 4.10 | How do you feed your child? | 1. Exclusive breast feeding 2. Breast plus solid food 3. Solid food only 4. Milk formula 5. Cow milk 6. Others (Specify)..... | |
| 4.11 | Do wash your hands before and after feeding the child? | 1. Yes 2. No | 1 |
| 4.11.1 | Do you use soap when washing hands? | 1. Yes 2. No | |
| 4.12 | Source of water for your family | 1. Piped 2. Non piped 3.Packed water | |
| 4.13 | Do you have latrine? | 1 Yes 2. No | 1 |
| 4.13.1 | Type of latrine | 1. Public 2. Private | |
| 4.14 | Waste disposal practices at home | 1. Dampened 2. Incineration3. Collectors 4. Others..... | |

Thank You for Your Genuine Information!

**Annex II የመረጃ ፤ የስምምነት ና የቃለ-መጠይቅ ቅፆች በአማርኛ**

## i. <u>የመረጃ ቅፅ</u>

**አዲስ አባባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ፤ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል**

**አርዕስት፡** ከ 5 ዓመት በታች በሚገኙ በተቅማጥ ከተያዙት ህፃናቶች መካከል የሮታቫይረስበሽታንስርጭት ማወቅ እና መንስኤዎቹን ማሳየት፡፡ በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን መንኛውም ነገር በሙሉ በነፃነት ይጠይቁ፡፡

**መግቢያ፡** የተቅማጥ በሽታ በተለያዩ ህዋሳቶች የሚመጣ የጤና ችግር ሲሆን በዓለም ላይ የሚያደርሰው የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም በታዳጊ ሀግሮች ውስጥ ለሚገኙ ህፃናቶች ሞት እና ጉዳት ዋነኛ ምክንያት ነው፡፡ ለዚህም ዋነኛ መንስኤዎቹ ባክቴሪያ፤ ቫይረስ እና ጥገኛ ትላትሎችሲሆኑ በሚያመጡት የተቅማጥ በሽታ ምክንያት የሚሞቱት ህፃናት ከፍተኛውን ቁጥር ይይዛሉ፡፡ ሥለሆነም እነዚህ በሽታዎች ከፍተኛ ጉዳት ከማስከተላቸው በፊት ለመከላከል የህፃናቶችን ሞትም ለመቀነስ ያላቸውን ስርጭት ማዎቅ በጣም አስፈላጊ ነው፡፡

**የጥናቱ ዓላማ፡** ይህ ጥናት ዋና አላማው በአዲስ አበባ ከተማ በሚገኙ የተመረጡ የጤና ተቁዋሞች አገልግሎት ለማግኘት የሚመጡትን ተቅማጥ ያለባቸውን ህፃናቶች የሮታ ቫይረስስርጭት እና መንስኤዎቹን ማሳየት ይሆናል፡፡

**ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም፡** በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም:: ነገር ግን በጥናቱ መሰረት በሽታው ለተገኘባቸው ተሳታፊዎች ከሚመለከታቸው የጤና ድርጅቱ ባለሙያዎች ጋር በመነጋገር አስፈሊጊ የሆነ ህክምና እንዲደረግላቸው ይደረጋል፡፡

**የመረጃ ሚስጥራዊ አጠባበቅ፡** የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በሏላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ በሃኪምዎ በኩል ሊያገኙት የሚችሉ ይሆናል፡፡

**ያስታውሱ:** ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ መጠይቁን ከሚሞላው ባለሙያ ሊያገኙ ይችላሉ፡፡ ተጨማሪ መረጃ ከፈለጉ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

**የዋና ተመራማሪው አድራሻ፡** መኮንን ጌታሁን

የቫይሮሎጅ ጥናት ቡድን፣

ኢትዮጵያ ጤናና ሥነ-ምግብ ምርምር ኢንስቲቱት

አዲስ አበባ፣ ኢትዮጵያ

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**0919 961 961**

**ii. <u>የተቅማጥ ና ትውከት ምልክቶች መጠይቅ፡</u>**

የአዲስ አባባ ዩኒቨርሲቲ፡ ጤና ሳይንስ ኮሌጅ፤ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል፡ ይህ መጠይቅ የተዘጋጀዉ በአዲስ አባባ ከተማ የሚኖሩ ሕፃናትን ለአጣዳፊ ተቅማጥ እና ተዉከት የሚያጋላጡ ምክንያቶችን አና መንስኤዎችን ለማወቅ ሲሆን ጥናቱ በተመረጡ የጤና ተቋዋሞች ዉስጥ ብቻ ቢካሔድም ለዕቅድ አዉጭዎች እና ለወደፊት ተመራማሪዎች ግን ከፍተኛ ግብአት እንዳለዉ እናረጋግጣለን፡፡ ሥለሆነም ሁሉም የመጠየቁ ተሳታፊዎች ጥያቄወቹን በታማኝነት እንዲሞሉ በትህትና እንጠይቅዎታለን፡፡

አስታዉሱ ፤ ሥም መፃፍ አያስፈልግም ፡ ማብራሪያ ቢያስፈልግዎ አስተባባሪው(ዋ)ን ያነጋግሩ፡፡

የጤና ድርጅቱ ሥም----------የህክምና ካርድ መለያ ቁጥር----------

አደራሻ----------ከተማ (ዞን)----------

| ተ.ቁ | ጥያቄዎች | ምላሽና መለያ | |
|---|---|---|---|
| **1. የታካሚዉን መረጃ በተመልከተ፡** | | | |
| 1.1 | ዕድሜ | ..... ወር | |
| 1.2 | ፆታ | 1. ወንድ 2. ሴት | |
| **2. ክሊኒካል መረጃ፡** | | | |
| 2.1 | ስንት ቀን አስቀመጠው (አመመው/ማት) | …… | |
| 2.2 | በቀን ስንት ጊዜ ያስቀምጠዋል/ጣታል | …… | |
| 2.3 | ትውከት(የማስመለስምልክት) | 1. አለው 2. የለውም | 1 |
| 2.3.1 | በቀን ስንትጊዜ ያስመልሰዋል | …….. | |
| 2.4 | የሆድ ህመም | 1. አለው 2. የለውም | |
| 2.5 | የሆድ ድርቀት (ሲፀዳዱ ማስቸገር) | 1. አለው 2. የለውም | |
| 2.6 | የማተኮስ ምልክት | 1. አለው 2. የለውም | |
| 2.7 | የሰውነት ድርቀት መጠን | 1. ከፍተኛ 2. መጠነኛ | |
| 2.8 | ልጅዎ የሮታ ቫይረስ ክትባት ወስዱዋል/ዳለች | 1.አዎ ወስዱዋል/ዳለች 2. አልወሰደም(ችም)3. አይታወቅም | 1 |
| 2.8.1 | የትኛውን የሮታ ክትባት ወሰደ/ች | 1. ሮታ ቴክ 2. ሮታሪክስ 3. አይታዎቅም | |
| 2.8.2 | ክትባቱን ስንት ጊዜ ወሰደ/ች | 1.አንድ 2. ሁለት 3. ሦስት 4. አይታዎቅም | |
| 2.9 | የሰውነት ሙቀት መጠን | .....$^{O}$C | |
| **3.የላቦራቶሪ ናሙና መረጃ** | | | |
| 3.1 | ሠገራ የተሰበሰበበት ቀን | ...../...../..... | |
| 3.2 | የሠገራው መለያ ቁጥር | ..... | |
| 3.3 | የሠገራው መልክ /አይነት / ገጽታ | 1. ፈሳሽ 2. ነጭ የተቀለቀለበት 3. ደምየተቀላቀለበት | |
| 3.4 | የቀጥታ ማይክሮስኮፒ እይታ ዉጤት | …… …… | |

| | | | |
|---|---|---|---|
| **4. የቤተሰብ /አሳዳጊ/ መረጃ በተመለከተ** | | | |
| 4.1 | ዕድሜ | ..... አመት | |
| 4.2 | ፆታ | 1. ወንድ 2. ሴት | |
| 4.3 | የህፃን ወላጅ ጋብቻ ሁኔታ | 1. ያላገባ 2. ያገባ 3. የተፋታ 4. በሞት የተለየ<br>5. በቦታ ተለያይቶ የሚኖር | |
| 4.4 | የትምህርት አመታት | …….. | |
| 4.5 | ህጻንዎ መዋእለ ህጻናት | 1. ይከታተላል 2. አይከታተልም | |
| 4.6 | በቤቱ ውስጥ ያሉ ከአምስት አመት በታች ህጻናት ብዛት | …… | |
| 4.7 | የወላጅ መተዳደሪያ ሥራ | 1. የመንግስት ሠራተኛ 2. የግል ሠራተኛ 3. ነጋዴ<br>4. የቤት እመቤት 5. ተማሪ6. ሌሎች(ግለጥ)..... | |
| 4.8 | ወርሃዊ ገቢ (በብር) | …… | |
| 4.9 | ያላችሁበት የመኖሪያ ቤት አይነት | 1. የጋራ መኖሪያ ቤት (ኮንዶሚንየም) 2. የግል (የኪራይ ቤት) ግቢ<br>3. የግለሰብ ተከራይ | |
| 4.10 | ህጻኑ/ኗን ምን ትመግባላችሁ? | 1. ጡት ብቻ 2. የጡትወተትናሌሎችምግቦችን<br>3. ሌሎችምግቦችንብቻ 4. የወተትተዋፅኦ (Milk formula)<br>5. የላም ወተት 6. ሌላካለቢጠቅሱ ..... | |
| 4.11 | ህጻኑን ከመመገብዎ በፊት ና በሁዋላ እጅዎን ይታጠባሉ? | 1. አዎ እታጠባለሁ 2. አልታጠብም | 1 |
| 4.11.1 | ሲታጠቡ ሳሙና ይጠቀማሉ | 1. አዎ እጠቀማለሁ 2. አልጠቀምም | |
| 4.12 | የሚጠቀሙት የውሃ ምንጭ (ለቤተሰብ) | 1. የቧንቧ 2. የቧንቧ ያልሆነ 3. ሌላቢጠቅሱ..... | |
| 4.13 | ሽንት ቤት አላችሁ? | 1. አዎ 2. የለንም | 1 |
| 4.13.1 | የሽንት ቤቱ አይነት | 1. የግል2. የህዝብ | |
| 4.14 | የቆሻሻ አወጋገድ ስርኣታችሁ | 1. መቅበር 2. ማቃጠል 3. ማጠራቀም እና መስጠት<br>4. ሌሎች..... | |

**ለትብብርዎ ከልብ እናመሰግናለን!**

## Annex III Stool collection and Rota virus antigen Detection SOP

### a) Background

Rota virus is the leading cause of under five children diarrhea killer in Ethiopia. The result of a laboratory test depends on accurate case investigation, proper sample collection, storage and transport.

### b) Principle

3 ml stool specimen collected using clean screw capped container and transported immediately or stored at an appropriate temperature (-20) until detection or transportation. Rota virus infection is confirmed by stool antigen detection by EIA.

The wells of EIA plate are impregnated with antibodies that can attach with rota virus antigens in stool. 100 microliter of diluted (10%) stool specimen is added to test well and 100 microliter of conjugate is added and stored for an hour. During this incubation period, antigens in the stool will be attached and the conjugate (enzyme labeled antibody against rota virus proteins) will attach the antigen, making the viral antigen sandwiched.

The plate will be washed to avoid unattached conjugates and stool debris by ELISA washer five times. Antigens attached to the plate and conjugate attached with antigen will remain attached. Then substrate is added and incubated for 10 minutes to allow the labeled enzyme break the substrate. Reaction will be stopped by weak acid (low concentration) and color development will be read by ELISA reader at 450 nanometer wave length.

### c) Materials Required

Clean Screw cup stool container
Plastic sheet
Spatula
Marker
Gloves
Cold box
Icepack
Micro-titration Plate
Enzyme Conjugate
Positive control
Negative Control
Sample Diluent
Wash Buffer
Color Substrate
Stop solution
Precision micropipettes
Disposable tips 50, 100 and 1000 µl

While transporting the Sample, please make sure the case investigation form is complete, dried Ice packs in ice box, the stool cups are tightly closed, stool samples between the ice packs and Close the ice box firmly.

**d) Safety**

All stool specimen should be treated as potentially infectious

Wash hands before and after collection

Materials used should be disinfected before being discarded

Clean protective gloves and Lab coat should be worn at all times

Spills should be contained using 5% hypo-chlorate or 70 % alcohol

**e) Specimen Preparation**

Add 1ml of Sample Diluent to a suitable labeled container and use to prepare a 10% suspension or dilution of fecal specimen by addition of approximately 100 µl of liquid faeces using transfer pipettes. Mix thoroughly and leave transfer pipette in container for later use.

**f) Rota stool antigen Detection Procedure**

1. Open the foil pouch, remove the required number of Microplate strips and place into a microplate strip holder. Use one well for the negative control and one for positive control.
2. Add two drops (100 µl) of each diluted specimen and negative and positive control samples to the respective wells
3. After addition of all specimens and controls, add two drops of conjugate to each microwell and Cover the plate and incubate the microwells at 20-30 $^0$C for 60 $\pm$ 5 minutes
4. Wash by ELISA washer with diluted Wash Buffer (5 times) and invert plate and tap on absorbent paper to remove the last traces of wash buffer.
5. Add two drops of substrate to each microwell and Cover the plate and incubate the plate at 20-30 $^0$C for 10 minutes
6. Stop the substrate reaction by adding two drops of stop solution to each microwell.
7. Read color change within 30 minutes by ELISA reader at 450ηm wavelength.

**g) Quality Control and Result Interpretation**

At least one positive and one negative control will be run each assay. Negative control value should be less than 0.150 absorbance unites. The positive control value must be greater than 0.500 absorbance unit.

The cut off value will be plus 0.200 absorbance unit to the negative control value.

Positive: clinical sample absorbance value > the cut off value, Negative: clinical sample absorbance value < the cut off value and Equivocal: clinical sample absorbance value within 0.010 absorbance units of the cut off value. Equivocal samples will be repeated.

**Declaration**

I the under signed declare that this MSc thesis work is my original work, has not been presented for a degree in this or any other university and that all source of materials used for this thesis have been duly acknowledged.

Name: - Mekonen Getahun

Signature …….……

Place: - Addis Ababa University

Date of submission ……/……../…..…….

This thesis has been submitted with our approval as university advisor

Name:- Mr. Kassu Desta

Signature ……..

Place : - Addis Ababa University

Date of submission ……./……./…..…….