ISSN No:-2456-2165

Rotavirus Infection in Children Less than 5 Years of Age in Sana'a City- Yemen, Post-Vaccine Era

Najla Al-Sonboli1 , Saeed Alghalibi2, Rawan Al-Sanabani2.
1Faculty of Medicine and Health Sciences, Pediatrics Department, Sana'a University, Sana'a, Yemen.
2 Faculty of Sciences, Department of Biology, Microbiology Division, Sana'a University, Sana'a, Yemen.

Abstract

- ***Objectives:***

To describe the seroprevalence of rotavirus infection among children who were admitted with acute diarrhea to Al-Sabeen Hospital for Women and Children after the introduction of Rotavirus vaccine as part of the National Yemeni Immunization Program in Sana'a city, Yemen and to determine some risk factors for getting rotavirus infection.

- ***Methods:***

It was a cross-sectional prospective study that conducted from January 2016 to November 2016 on children aged ≤ 5 years with acute watery diarrhea (≤14 days duration) and admitted for the treatment of gastroenteritis as a primary illness. The demographic and clinical data of the patients were collected according to a structured questionnaire. Four to eight grams of stool samples were collected from each participant and Rotavirus was detected by ELISA according to the manufacture instruction enclosed (ProSpecT™ RV Microplate Assay, Oxoid Ltd, UK).

- ***Results:***

A total of 400 children were enrolled. Rotavirus infection was detected in 118 (29.5%) children. Among those children who infected with RV there were 60 (50.8%) vaccinated and 58 (49.2%) were unvaccinated.

- ***Conclusions:***

Rotavirus infection is widespread among Yemeni children with diarrhea despite the introduction of the Rotavirus vaccine in the Yemeni National Program for Immunization in Sana'a city. The efficiency of the Rotavirus vaccine is questionable and further studies are needed to reveal the reasons of this low efficacy rate of the vaccine.

Keywords:- *Rotavirus, Vaccine, Yemen.*

I. INTRODUCTION

Acute gastroenteritis (AGE) is one of the infectious diseases which causes major morbidity and mortality in the world. Diarrheal diseases are the second most important infectious cause of mortality among children under five years of age after pneumonia, [1,2].

Diarrheal diseases account for approximately 17% of the 10.4 million deaths among children aged below 5 years globally [3] and in 2011, diarrheal diseases accounted for 9.9% of the 6.9 million deaths in children under 5 years of age [2]. It is estimated that acute gastroenteritis causes 580 000 – 750 000 deaths in children under 5 years of age in the world every year [4].

Acute infective gastroenteritis is a major global health problem, which defined as ≥ 3 watery or loose stool per day that may last ≤ 14 days, with or without fever and vomiting. Children under five years of age are above all vulnerable, and global estimates indicated a mean of between three and a half to seven episodes of severe diarrhea during the first two years of life, and the greatest weight is in the developing countries due of the poor sanitation, lack of safe drinking water, and bad sanitary habits [5].

Diarrhea can be due to a diversity of bacterial, viral and parasitic organisms. *Rotavirus* (RV) was documented as the single most important reason of severe infantile gastroenteritis universally [6,7]. RV infections account for more than forty percent of hospital admissions of AGE infections in children less than five years [8]. Almost every child contracts an episode of diarrhea in the first five years of his/her life [9]. Severe diarrhea due to RV infection still represents a major public health problem in developed and developing countries [10]. It is estimated that RV caused gastroenteritis leading to 2.4-3.3 million deaths annually in the developing countries [11].

The disease burden in morbidity and mortality due to AGE is massive, but in the countries where the RV vaccines have been introduced in their national immunization programs (NIPs), the scale of pediatric infectious diseases in inpatients has changed. These vaccines have also had an impact on childhood deaths[12,13].

In Yemen, the WHO estimates infant and under-five years mortality rates are 40.4 and 51.3 per 1000 live births, respectively [14]. In Yemen, Rotarix™ vaccine (GlaxoSmithKline Biologicals, Belgium) was introduced in the NIP in October 2012 with support from the Global Alliance for Vaccines Initiative (GAVI) and is given free of charge to all children. Rotarix is a monovalent live attenuated human RV vaccine based on the G1P[8] strain. The national vaccination coverage rates for the second dose of Rotarix was twenty-three percent in 2012 and ninety percent in 2013 [15]. In Yemen, there were few studies after the introduction of the RV vaccine in the NIP.

However, the rate of diarrheal diseases still high among children especially under five years old, so this study aimed to reveal the burden of RV infection during post- vaccine era

II. MATERIALS AND MEHODS

➢ *Study Area:*

Sana'a has a population of 3 million. It is situated at an altitude of 2300 meters and has a dry, mild climate with 200 mm of rainfall annually and minimum–maximum average monthly temperatures of 6–30 °C [16]. The study was done in Sana'a city in Al-Sabeen Hospital for Women and Children (SHW&C). It is a tertiary hospital with a capacity of 112 beds for children. It is the only hospital in Sana'a city that contains center for diarrhea treatment. It serves the population of Sana'a city and suburb, which is now nearly three million as a result of the internal displacement due to the conflict and the war in Yemen. In addition, it receives patients from the neighbor governorates.

➢ *Study population and design:*

A cross-sectional prospective study was conducted by recruiting case-series of children who have acute diarrhea in SHW&C in Sana'a city from the period of January 2016 to November 2016.

➢ *Case definition and patient enrollment:*

According to the standard operating procedures (SOPs) that was used by the RV gastroenteritis surveillance network in the EMRO Region are described in the WHO generic protocol for RV surveillance, children with an age of < 5 years with acute watery diarrhea (≤14 days) and admitted for treatment of AGE as a primary illness [17]**.** Any child who attended the participating hospital with the inclusion criteria was enrolled in the study after a signed informed consent by the parents/legal guardian was obtained.

➢ *Specimens collection:*

Stool collected from referred children with diagnosis of AGE and who fulfill the inclusion criteria. Stool collected from inpatients (Emergency Room) and outpatients (Oral Rehydration Room) in diarrhea treatment center (DTC) in the hospital. Each stool specimen (4-8 gm) was collected in a sterile plastic container, in each case the mother was instructed to collect the specimen in the specified container. All specimens were labeled with a specific study numbered serially and questionnaire was given the same number of the specimen. Information in the questionnaire including demographic and clinical were filled in after the specimen collection by the mothers or the child's guardian.

➢ *Serological kits:*

All collected stool specimens were analyzed by ELISA according to the manufacture instruction enclosed ProSpecT™ RV Microplate Assay, (Oxoid Ltd, UK).

➢ *Statistical methods:*

Laboratory data and data from the interviews were checked for errors and imported from Microsoft Office Excel 2007 into SPSS software, version 21 (IBM, Armonk, NY, USA). We used descriptive statistics to calculate the annual prevalence of severe RV gastroenteritis in children. The frequencies and percentage also were determined using univariate table significant difference between the proportions and the groups or variables were determined using chi-square > 3.9 were significant, *P- value* of 0.05 were considered significant. Relative risk (RR) and 95% confidence interval (CI) also were calculated. The exponential E (adjusted relative risk) was also determined using multilogestic regression to determine the independent risk factors.

➢ *Ethical consideration*

Mothers or child's guardian received a simple explanation for the aim of the study. If agreed to participate verbally, a stool sample was collected and an interview was conducted. Mothers and child's guardian were reassured that their child will be treated as usual if they refuse to participate and that the participation is entirely voluntary. Confidentiality of the collected data was achieved by keeping data record in a locked room with limited access to the research team only. Ethical approval was obtained from the ethical committee of the Faculty of Science and the Faculty of Medicine and Health Sciences, Sana'a University and SHW&C.

III. RESULTS

A. Prevalence of RV antigen by ELISA:

Totally, 400 children with diarrhea (patients) were enrolled. Out of them, 118 (29.5%) were positive for RV infection, while 282 (70.5%) were negative for RV infection **Monthly distribution of RV infection:**

Out of 118 patients who are positive for RV, three (2.5%) children were enrolled during January, 0 (0%) in February, 6 (5%) in March, 2 (1.7%) in April, 1 (0.84%) in May, 3 (2.5%) in June, 3 (2.5%) in July,4(3.4%) in August,14(11.86%) in September, 53 (44.9%) in October and 29 (24.57%) children in November **(Table 1 & Figure 1)**.

B. Characteristics of children:

➢ *Socio-demographic features of study population:*

A total of 400 children were enrolled. Three hundred and three (75.8%) patients were in an age group of ≤12 months, 72 (18%) were in the age group of >12-24 months and 25 (6.2%) in the age group of >24-59 month**s.** Two hundred and thirty (57.5%) were males and 170 (42.5%) were females. One hundred and thirty-nine (34.8%) children were living in rural areas, while 261 (65.2%) children lived in urban areas. Two hundred and thirty-two (58%) children were enrolled from the outpatient clinic, while 168 (42%) children were enrolled from inpatients wards **(Table 2)**.

➢ *Vaccination status of study population:*

One hundred and ninety-eight (49.5%) patients were vaccinated and 202 (50.5%) were unvaccinated. Among vaccinated patients, there were 36 (18.2 %) patients took one dose and 162 (81.8 %) took two doses **(Table 3)**.

C. Univariate analysis of the potential risk factors associated with diarrhea:

➢ *Socio-demographic by positivity of RV:*

The mean (SD) in ≤12 Month group age of children with RV diarrhea was 6.5 (3.2) months while the mean (SD) in >12 months group age of children with RV diarrhea was 21.9 (8.6). Eighty-eight (74.6%) were positive for RV in age group of ≤12 months while 30 (24.4%) were in age group of >12 months **(Table 4)**. There was no statistical significant differences between age groups and positive results of RV (*P value* > 0.05). Males 79 (67%) were more prone to be infected with RV than females 39 (33%) **(Table 4).** There was statistical significant differences between sex and positive results of RV *P value* ≤ 0.05). RV infection was reported more significantly among children who live in urban areas 87 (73.7%) (*P value* ≤ 0.05). **(Table 4).** RV infection was reported more significantly among children who were enrolled from the outpatient clinics 84 (71.2%) than who were enrolled from inpatient ward 34 (28.8%) (*P value* ≤ 0.05) **(Table 4).**

➢ *The vaccination status by RV positivity:*

Among the children who infected with RV there were 60 (50.8%) vaccinated and 58 (49.2%) were unvaccinated (*P value* > 0.05). The seropositivity of RV infection in vaccinated children that given one dose was 12 (20%) and 48 (80%) in those that given two doses **(Table 5)**. There was no significant association between doses of vaccine and seroprevalence of RV (*P value* > 0.05)**.**

D. Clinical presentation of the study population by positivity:

➢ *Diarrhea:*

- *Type of diarrhea:*

Rotavirus infection was reported more significantly among children who had watery diarrhea 85 (72%) than those who had watery diarrhea with mucus 33 (28%) (*P value* ≤ 0.05) **(Table 6).**

- *Frequency and duration of diarrhea:*

The mean (SD) in ≤5 frequency of diarrhea group in children with RV was 4.2 (0.87) times while the mean (SD) in > 5 frequency of diarrhea group in children with RV was 10.9 (5.4) times. Among children with RV diarrhea, those who had a frequency of diarrhea >5 times /day were 89 (75.4) and those who had a frequency of diarrhea ≤5 times /day were 29 (24.6%). There was statistical significant association between frequency of diarrhea and seroprevalence of RV (*P value* ≤ 0.05).

The mean (SD) in ≤5 days duration of diarrhea group in children with RV was 3.2 (1.2) days while the mean (SD) in the group of 6-14 days duration of diarrhea in children with RV was 8.9 (1.2) days. The positivity of RV infection in patients who had diarrhea ≤ 5 days were 98 (83%) and those who had diarrhea from 6 to 14 days were 20 (17%). There was statistical significant association between duration of diarrhea and seroprevalence of RV (*P value* ≤ 0.05) **(Table 6).**

➢ *Fever:*

Rotavirus infection was reported more among children who had fever (95, 80.5%) than those who hadn't fever (23. 19.5%) **(Table 6).** There was no significant association between fever and seroprevalence of RV (*P value* > 0.05).

➢ *Vomiting:*

Rotavirus infection was reported more significantly among children who had vomiting (106, 89.8%) than who hadn't vomiting (12, 10.2%) (*P value* ≤ 0.05) **(Table 6).**

- *The frequency and duration of vomiting:*

The mean (SD) in ≤5 frequency of vomiting group in children with RV was 3.3 (1.2) times while the mean (SD) in > 5 frequency of vomiting group in children with RV was 11.2 (5.5) times. The patients with RV diarrhea and had vomiting > 5 times /day were 57 (53.8%) while those who had vomiting ≤5 times /day were 49 (46.2%) **(Table 6).** There was no significant association between frequency of vomiting and seroprevalence of RV (*P value* > 0.05).

The mean (SD) in the group of ≤5 days duration the of vomiting in children with RV was 2.9 (1.2) days while the mean (SD) in the group of 6-14 days duration of vomiting in children with RV was 8.2 (2.4) days. More children with RV diarrhea (92, 86.8%) had vomiting ≤5 days than those who had vomiting from 6 to 14 days (14, 13.2%) **(Table 3.5.3).** There was significant association between duration of vomiting and seroprevalence of RV (*P value* ≤ 0.05).

➢ *Dehydration:*

Ninety-five (80.5%) children with RV diarrhea had dehydration while, 239 (84.8%) children with negative RV diarrhea had dehydration **(Table 3.5.3).** There was no significant association between dehydration and seroprevalence of RV (*P value* > 0.05).

- *Type of dehydration:*

Among children with RV diarrhea, 56 (59%) children had severe and moderate dehydration and 39 (41%) had mild dehydration **(Table 3.5.3).** There was no significant association between type of dehydration and seroprevalence of RV (*P value* > 0.05).

E. Risk factors for positivity of RV:

➢ *Univariate Analysis:*

The following variables **(Table 7)** were found to be statically significant for RV positivity using univariate analysis. They were selected to be subjected for multivariate analysis to find out the independent risk factor for getting infected by RV.

➢ *Multivariate Analysis:*

The variables that were found statically significant for RV positivity by uni-varite analysis (p value <0.05) were analyzed using multivarite conditional logistic regression model to identify risk factors that were independently associated with diarrhea and to control confounders. In multivariate analysis, sex, type of diarrhea, duration of diarrhea and vomiting were found to be independent risk factors for getting RV positive. **(Table 8).**

IV. DISCUSSION

To our knowledge this was the second study that performed to determine the prevalence of RV among infants with diarrhea in Sana'a city after the introduction of RV vaccine as part of the NIP in 2012. The overall seroprevalence of RV infection among children in this study was 29.5%. Comparing to other studies carried out in different cities in Yemen, the present study's result agreed with a study done in Sana'a city before the introduction of RV vaccine as part of NIP, which stated that RV infection was 27 % [16] . A higher rate of RV infection was reported before the introduction of RV vaccine as part of NIP by Al-Badani et al in Taiz city (45.2%) [18] and by Al-Hobishi in Ibb city (46%) [19]. In Sana'a city, the incidence was increased instead of decrease after the vaccine introduction and this may be due to the emergence of new genotypes in the city that were not covered by the vaccine[20] However, the percentages of RV infection that were reported in Taiz city before (43.79%) and after (10.54%) [21] showed strong effect for the vaccine. Similarly, a study that conducted in Aden and Taiz cities by Banajeh and Abu-Asba, that compared the percentage of RV infection before introduction of RV vaccine showed a significant decline from 42.9% pre-vaccine era to 18.5% post-vaccine era [15]. In comparison to other studies carried out in different countries in the world, the present study' results were in agreement with studies conducted in Iran (Tehran) (28.8%) [22], Egypt (Cairo) (28.3%) [23], while higher rate of RV infections was recorded in Iraq (93.88%) [24]. On the other hand, lower rates of RV infection were reported in many countries, in Iraq (18.75%) [25], Sudan (16%) [26], and in Northwestern Nigeria (Katsina State) (5.3%) [27]. These differences in the studies could be attributed to the differences in the geographic locations, climates, type of investigations & technique, environmental conditions, economic, personal hygiene, educational levels of infants' families and immunity states of the infants.

During the period of the present study RV infection occurred throughout the year, the proportion of children with RV infection followed a well-defined monthly pattern that peaked in September (11.86%), October (44.9%), November (24.57%) and was zero in February and lowest in May (0.84%), April (1.7%), January (2.5%), June (2.5%), July (2.5%), and August (3.4%). This result agreed with studies done in Taiz and Aden cities [15], RV infection was more frequent in October, November, and December and also was agreed with different studies in Izmiri (Turkey) [28], Latin America [29], Vietnam [30], Mashhad (Iran) [31]. On the other hand, this result was disagreed with a study carried in Yemen (Taiz city) where RV was identified in May and June months [18], South West Iran [32], and in Egypt (Cairo) where RV was identified in April and May months [23]. These differences may be due to the change of weather and seasons from a country to another.

In the present study, the occurrence of RV infection was higher in the first 12 months of life (88, 74.6%) than in the other age groups and these differences were without statistically significant (*P value* > 0.05). Similar results were observed in previous studies in developing countries [33] and local previous studies in Yemen (Taiz, and Aden cities) [15,18, 19] and similar to other studies in different countries in the Middle East, Africa and Europe [23,24,26,27, 32, 34, 35]. This finding may be explained by the decline of maternal antibodies with immature immune systems which protect the newborns from pathogens during the first months of life [36]. On the another hand, the present study was in contrast with other studies that found the occurrence of RV infection was higher in children who were more than one year old which were done in Iran (Tehran) [22], Uganda [376] **and** Vientiane [38].

In the present study, the prevalence of RV was found higher among the male patients than among female patients with a rate of 67 % in the males and 33% in the females, these differences were statistically significant (*P value* ≤ 0.05). This result was similar to studies that were conducted in Yemen [15, 18], and with other studies in different countries in the region and Asia, [21,23,32,35], and disagreed with studies that were conducted in Tanzania (Moshi) [39] and Baghdad (Iraq) [40]. The low differences among sex may be due to the higher number of males infants RV in the present study was found higher among infants who lived in the urban (87, 73.7%) than the rural infants 31 (26.3%), and this difference was statistical significant (*P value* ≤ 0.05) which was similar to results of a study done in Ibb city, Yemen [19]. This result was agreed with a study in Tanzania (Moshi) [39] and disagreed with a study from Iraq [24]. That means the diarrhea related to RV was more circular in urban than in rural which may be due to the higher crowded in urban and then faster and higher transmission through the direct and non-direct oro- fecal route.

In the present study, the high prevalence of RV infection was among outpatients 84 (71.2%), which was statistically significant (*P value* ≤ 0.05) in comparison with the prevalence of RV infection in inpatients 34 (28.8%). This explain the role of RV in hospitalization of infants and children less than five years of age and the severity of diarrhea due to RV in practical medicine. Many other

studies showed the burden of hospitalization due to RV infections, which was consistent with previous study done in Taiz city, Yemen [18].

In the present study, the prevalence of RV infection was found slightly higher among vaccinated patients than unvaccinated patients with a rate of 60 (50.8%) in vaccinated and 58 (49.2%) in unvaccinated, however, this difference was not statistically significant (*P value* > 0.05), this raise the question about the efficacy of the vaccine Rotarix (GlaxoSmithKline Biologicals) in Sana'a city. In vaccinated patients, the prevalence of RV was 12 (20%) in those who took one dose and 48 (80%) in those who took two doses. The present study was agreed with two studies conducted in Tanzania (Moshi) [40], Tanzania (Dar es Salaam) [41], and Nepal (Kathmandu) [42] that reported the vaccine Rotarix (GlaxoSmithKline Biologicals) is not efficient to reduce RV infections. On the other hand, this result was disagreed with study conducted in Yemen (Aden and Taiz cities) [15,21], United Kingdom [43], and Egypt (Cairo) [23], where these studies reported that the vaccine Rotarix (GlaxoSmithKline Biologicals) is efficient to reduce RV infections in their countries.

The first case that was infected with cholera epidemic in Sana'a city was in October in the same period of the present study and most of the cases that were suspected for cholera in these two months were positive for RV and negative for *Vibrio cholera* this may be due to emerging of a new strain for RV that may give similar character of stool as cholera (water-rice stool). In the present study, there was statistically significant differences between type of diarrhea and the occurrence of RV *(P value* ≤ 0.05), where the watery diarrhea was the most common (72%) followed by the watery diarrhea with mucus was (28%). This result agreed with studies that was done in Yemen [16,19], in Indonesia (Surabaya) [35] and in Nigeria [44]. The frequent association of fever, vomiting and the significant association of vomiting with RV diarrhea are not conclusive in the clinical diagnosis of RV infection, because such symptoms are constitutional and present with other causes of diarrhea. Nonetheless, the frequent vomiting with diarrhea lead to the risk of dehydration in RV infection more than other diarrheal infections and therefore increase the need for hospitalization.

The reported prevalence of RV in the present study was higher among infants who had vomiting (89.8%) with statistically significance (*P value* ≤ 0.05) which was agreed with studies in Southwest Iran [32] and Indonesia (Surabaya) [35] and was higher among infants who had fever (80.5%) with no statistically significance (*P value* > 0.05) which was disagreed with a study in Southwest Iran [32]. In the present study, RV infection rate was higher among children with severe dehydration (59%). Previous studies in Northwestern Nigeria [27] and in Indonesia by Wardana *et al*. (2015) has made a similar observation.

In the present study, the frequency of diarrhea (times/day) in patients who were infected with RV was higher in the group of >5 times/day (75.4%) and the lower prevalence was in the group with a frequency of ≤5 times/day (24.6%). These differences were statistically significant ((*P value* ≤ 0.05) which was agreed with a study in Yemen by Al-Hobishi (2012).

In the present study, the duration of diarrhea (days) in the patients who were infected with RV was higher in the group of a duration of ≤5 days (83%) and lower in the group with a duration of 6-14 days (17%). These differences were statistically significant (*P value* ≤ 0.05). The duration of illness is generally 5 to 7 days [45], in Taiz and Ibb cities (Yemen), the higher prevalence was reported in the group with a duration of 4-6 days of diarrhea and in Vientiane, the higher prevalence was in the group with a duration of 3-5 days [38] which emphasis the current results.

In the present study, the frequency of vomiting (times/day) in patients who were infected with RV was higher in the group of ≥ 6 times / day (53.8%) and lower in the group of ≤ 5 times\day (46.2%). These differences were not statistically significant (*P value* ≤ 0.05). In the present study, the duration of vomiting in patients who were infected with RV was higher in the group of ≤5 days duration and lower in the group of 10-14 days duration. These differences were statistically significant (*P value* ≤ 0.05). There were no previous studies that studied the relationship between RV positivity and frequency and duration of vomiting.

In the present study, sex, type of diarrhea, duration of diarrhea and vomiting were found to be independent risk factors for getting RV positive. There were no previous studies that studied the independent risk factors for getting RV positive.

V. ACKNOWLEDGEMENTS

The study could not have been successful without the cooperation of the Paediatric staff in Al-Sabeen Hospital, especially the doctors and the nurses in the DTC of the department. We are very grateful to Dr. Dhekra Al-Shekh and Dr. Khaled Al-Rubasi in the laboratory department for their great role in this work and many thanks to Prof. Abdulwahed Al-Sororri and Dr. Mohammed Al-Emad for their corporation with the data analysis.

REFERENCES

[1]. Liu, L.; Johnson, H.L.; Cousens, S.; Perin, J.; Scott, S.; Lawn, J.E.; Rudan, I.; Campbell, H.; Cibulskis, R.; Li, M. and Mathers, C. (2012): Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *The Lancet*. 379(9832): 2151-2161.

[2]. Lanata, C.F.; Fischer-Walker, C.L.; Olascoaga, A.C.; Torres, C.X.; Aryee, M.J. and Black, R.E. (2013): Global causes of diarrheal disease mortality in children< 5 years of age: a systematic review. *PloS One*. 8(9): 72788.

[3]. Parashar, U.D.; Gibson, C.J.; Bresee, J.S. and Glass, R.I. (2006): Rotavirus and severe childhood diarrhea. *Emerging Infectious Diseases*. 12(2): 304.
[4]. Liu, L.; Oza, S.; Hogan, D.; Perin, J.; Rudan, I.; Lawn, J.E.; Cousens, S.; Mathers, C. and Black, R.E. (2015): Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *The Lancet*. 385(9966): 430-440.
[5]. WHO (World Health Organization) (2009a): Diarrhoeal Disease. FactSheet.;330. http://www.who.int/mediacentre/factsheets/fs330/en/index.html. [accessed in 1/12/2017].
[6]. Staat, M.A.; Rice, M.A.; Donauer, S.; Payne, D.C.; Bresee, J.S.; Mast, T.C.; Curns, A.T.; Cortese, M.M.; Connelly, B.; McNeal, M. and Ward, R.L. (2010): Estimating the Rotavirus hospitalization disease burden and trends, using capture-recapture methods. *The Pediatric Infectious Disease Journal*. 29(12):1083-1087.
[7]. Glass, R.I.; Parashar, U.D.; Bresee, J.S.; Turcios, R.; Fischer, T.K.; Widdowson, M.A.; Jiang, B. and Gentsch, J.R. (2006): Rotavirus vaccines: current prospects and future challenges. *The Lancet*. 368(9532): 323-332.
[8]. Mwenda, J.M.; Ntoto, K.M.; Abebe, A.; Enweronu-Laryea, C.; Amina, I.; Mchomvu, J.; Kisakye, A.; Mpabalwani, E.M.; Pazvakavambwa, I.; Armah, G.E. and Seheri, L.M. (2010): Burden and epidemiology of Rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. *Journal of Infectious Diseases*. 202(1): S5-S11.
[9]. Basu, G.; Rossouw, J.; Sebunya, T.K.; Gashe, B.A.; De Beer, M.; Dewar, J.B. and Steele, A.D. (2003): Prevalence of Rotavirus, Adenovirus and Astrovirus infection in young children with gastroenteritis in Gaborone, Botswana. *East African Medical Journal*. 80(12): 652-655.
[10]. Temu, A.; Kamugisha, E.; Mwizamholya, D.L.; Hokororo, A.; Seni, J. and Mshana, S.E. (2011): Prevalence and factors associated with Group A Rotavirus infection among children with acute diarrhea in Mwanza, Tanzania. *The Journal of Infection in Developing Countries*. 6 (06): 508-515.
[11]. Kiulia, N.M.; Nyaga, M.M.; Seheri, M.L.; Wolfaardt, M.; Van Zyl, W.B.; Esona, M.D.; Irimu, G.; Inoti, M.; Gatinu, B.W.; Njenga, P.K. and Taylor, M.B. (2014): Rotavirus G and P types circulating in the eastern region of Kenya: predominance of G9 and emergence of G12 genotypes. *The Pediatric Infectious Disease Journal*. 33: 85-88.
[12]. WHO (World Health Organization) (2013): Rotavirus vaccines: WHO position paper—January 2013= Vaccins antirotavirus: Note de synthèse de l'OMS. *Weekly Epidemiological Record.* 88(05):49-64.
[13]. Gastañaduy, P.A.; Sánchez-Uribe, E.; Esparza-Aguilar, M.; Desai, R.; Parashar, U.D.; Patel, M. and Richardson, V. (2013): Effect of Rotavirus vaccine on diarrhea mortality in different socioeconomic regions of Mexico. *Pediatrics*. 131(4): 1115-1120.
[14]. WHO (World Health Organization) (2015): Regional Office for the Eastern Mediterranean. Eastern Mediterranean Region: framework for health information systems and core indicators for monitoring health situation and health system performance. 2014. *applications. emro. who. int/dsaf/EMROPUB_2014_EN_1792. pdf. Accessed May, 7*.
[15]. Banajeh, S.M. and Abu-Asba, B.A. (2015): The epidemiology of all-cause and Rotavirus acute gastroenteritis and the characteristics of Rotavirus circulating strains before and after Rotavirus vaccine introduction in Yemen: analysis of hospital-based surveillance data. *BMC Infectious Diseases*. 15(1): 418.
[16]. Kirby, A.; Al-Eryani, A.; Al-Sonboli, N.; Hafiz, T.; Beyer, M.; Al-Aghbari, N.; Al-Moheri, N.; Dove, W.; Cunliffe, N.A. and Cuevas, L.E. (2011): Rotavirus and Norovirus infections in children in Sana'a, Yemen. *Tropical Medicine & International Health*. 16(6): 680-684.
[17]. WHO (World Health Organization) (2002): Generic portocols for (i) hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children and (ii) a community-based survey on utilization of health care services for gastroenteritis in children: field test version.
[18]. AL-Badani, A.; Al-Areqi, L.; Majily, A.; AL-Sallami, S.; AL-Madhagi; A. and Amood, A.K. (2014): Rotavirus diarrhea among children in Taiz, Yemen: prevalence—risk factors and detection of genotypes. *International Journal of Pediatrics*. 2014: 9.
[19]. AL-Hobishi, E.A (2012): Prevalence of Rotavirus among infants with diarrhea in Ibb City. MSc. Thesis. Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, Yemen.
[20]. Al-Sonboli, N. N;. Bin Mohnna M.;. Al-Aghbari, N.A; Al-Shaekh, Z.; Abo Asba, B. Genotypes of Rotavirus among Yemeni Children with Acute Diarrhea. Ijisrt. Vol.4 (12) 2019
[21]. AL-Kamarany, M.A.; Al-Areqi, L.; Mujally, A.; Alkarshy, F.; Nasser, A. and Jumaan, A.O. (2016): Diarrheal diseases hospitalization in Yemen before and after Rotavirus Vaccination. Scientifica. 2016: 6.
[22]. Nasab, S.D.M.; Sabahi, F.; Makvandi, M.; Samiee, S.M.; Nadji, S.A. and Ravanshad, M. (2016): Epidemiology of Rotavirus-Norovirus co-infection and determination of Norovirus genogrouping among children with acute gastroenteritis in Tehran, Iran. Iranian Biomedical Journal. 20(5): 280.
[23]. Shaheen, M.; El-Daim, A.S.E.; Hosseney, E.N.; Shoeib, A.R. and Ali, M.A. (2017): Molecular Characterization of Rotavirus Strains Causing Gastroenteritis in Children under 5 Years in Cairo, Egypt. MOJ Public Health. 6(5): 00187.
[24]. Salman,A.D (2017):Prevalence of Rota Virus detection by reverse transcriptase- polymerase chain reaction in stool from children fewer than five age

with acute diarrhea in Diyala Province in Iraq. IOSR Journal of Pharmacy and Biological Sciences. 12(1): 94-99.
[25]. AL-Ezzy, A.I.A. and Abood, W.N. (2013): Immuno-Microbiological diagnosis of co-infections with Rota Virus, Giardia lamblia, Entamoeba histolytica and Salmonella enterica as a possible pathological strategy for acute diarrhea among Iraqi children. International Journal of Advanced Research. 1(9): 216-224.
[26]. Magzoub, M.A.; Bilal, N.E.; Bilal, J.A. and Osman, O.F. (2014): Rotavirus infection among Sudanese children younger than 5 years of age: a cross sectional hospital-based study. Pan African Medical Journal. 16(1): 2519.
[27]. Mukhtar, G.L.; Aminu, M.; Hayatuddeen, M.R. and Bello, I. (2016): Prevalence and risk factors associated with Rotavirus diarrhoea in children less than five years in Katsina State, northwestern Nigeria. Bayero Journal of Pure and Applied Sciences. 9(2):12-16.
[28]. Kurugol, Z.; Geylani, S.; Karaca, Y.; Umay, F.; Erensoy, S.; Vardar, F.; Bak, M.; Yaprak, I.; Ozkinay, F. and Ozkinay, C. (2003): Rotavirus gastroenteritis among children under five years of age in Izmir, Turkey. Turkish Journal of Pediatrics. 45(4): 290-294.
[29]. Castello, A.A.; Arvay, M.L.; Glass, R.I. and Gentsch, J. (2004): Rotavirus strain surveillance in Latin America: a review of the last nine years. The Pediatric Infectious Disease Journal. 23(10): 168-172.
[30]. Jin, Y.; Ye, X.H.; Fang, Z.Y.; Li, Y.N.; Yang, X.M.; Dong, Q.L. and Huang, X. (2008): Molecular epidemic features and variation of Rotavirus among children with diarrhea in Lanzhou, China, 2001–2006. World Journal of Pediatrics. 4(3): 197-201.
[31]. Sadeghian A; Hamedi A; Sadeghian M; et al. (2010): Incidence of Rotavirus diarrhea in children under 6 years referred to pediatric emergency and clinic of Ghaem hospital, Mashhad, Iran. Acta Medica Iranica. 48 (4): 263-265.
[32]. Azaran, A.; Makvandi, M.; Samarbafzadeh, A.; Neisi, N.; Hoseinzadeh, M.; Rasti, M.; Teymurirad, M.; Teimoori, A.; Varnaseri, M. and Makvandi, K. (2016): Study on Rotavirus infection and its genotyping in children below 5 years in south west iran. Iranian Journal of Pediatrics. 26(2): 2080.
[33]. El Qazoui, M.; Oumzil, H.; Baassi, L.; El Omari, N.; Sadki, K.; Amzazi, S.; Benhafid, M. and El Aouad, R. (2014): Rotavirus and Norovirus infections among acute gastroenteritis children in Morocco. BMC Infectious Diseases. 14(1): 300.
[34]. Rodrigues, F.; Lopes, A.I.; Iturriza-Gomara, M.; Nawaz, S.; Cruz, A.; Antunes, H., Loreto, H.; Amil Dias, J.; Cunha, J.; Varandas, L. and Silveira, L. (2015): Acute Rotavirus gastroenteritis in Portugal: A Multicentre Study. Acta Pediatr Port. 46:219-25.
[35]. Wardana, O.P.; Athiyyah, A.F.; Budiono, S.O. and Soegijanto, S. (2015): The role of Rotavirus genotype in the severity of acute diarrhea in children under 5 years old at Surabaya, Indonesia. J Hum Virol Retrovirol. 2:00039
[36]. Edwards, K.M. (2015): Maternal antibodies and infant immune responses to vaccines. Vaccine. 33(47): 6469-6472.
[37]. Nakawesi, J.S.; Wobudeya, E.; Ndeezi, G.; Mworozi, E.A. and Tumwine, J.K. (2010): Prevalence and factors associated with Rotavirus infection among children admitted with acute diarrhea in Uganda. BMC Pediatrics. 10(1): 69.
[38]. Aloun, D.S.; Nyambat, B.; Phetsouvanh, R.; Douangboupha, V.; Keonakhone, P.; Xoumphonhphakdy, B.; Vongsouvath, M.; Kirkwood, C.; Bogdanovic-Sakran, N. and Kilgore, P.E. (2009): Rotavirus diarrhoea among children aged less than 5 years at Mahosot Hospital, Vientiane, Lao PDR. Vaccine. 27: 85-88.
[39]. Mchaile, D.N.; Philemon, R.N.; Kabika, S.; Albogast, E.; Morijo, K.J.; Kifaro, E. and Mmbaga, B.T. (2017): Prevalence and genotypes of Rotavirus among children under 5 years presenting with diarrhoea in Moshi, Tanzania: a hospital based cross sectional study. BMC Research Notes. 10(1): 542.
[40]. Abdulridha, A.S. (2016): Study the effects of age and gender of children on Rotavirus infection in Baghdad, Iraq. Research Journal of Pharmaceutical Biological and Chemichal Sciences.7(4):700-704.
[41]. Moyo, S.J. (2014): Viral diarrhoea in children under two years of age in Dar es Salaam, Tanzania, Clinical and molecular epidemiology. PhD thesis. University of Bergen.
[42]. Ansari, S.; Sherchand, J.B.; Rijal, B.P.; Parajuli, K.; Mishra, S.K.; Dahal, R.K.; Shrestha, S.; Tandukar, S.; Chaudhary, R.; Kattel, H.P. and Basnet, A. (2013): Characterization of Rotavirus causing acute diarrhoea in children in Kathmandu, Nepal, showing the dominance of serotype G12. Journal of Medical Microbiology. 62 (1):114-120.
[43]. Marlow, R.; Muir, P.; Vipond, B.; Lyttle, M.; Trotter, C. and Finn, A. (2015): Assessing the impacts of the first year of Rotavirus vaccination in the United Kingdom. Eurosurveillance.20(48):30077.
[44]. Grace, P. and Jerald, U. (2010): The prevalence of group A Rotavirus infection and some risk factors in pediatric diarrhea in Zaria, North central Nigeria. African Journal of Microbiology Research. 4(14):1532-1536.
[45]. Katyal, R.; Rana, S.V. and Singh, K. (2000): Rotavirus infections. Acta Virologica. 44(5): 283-288.

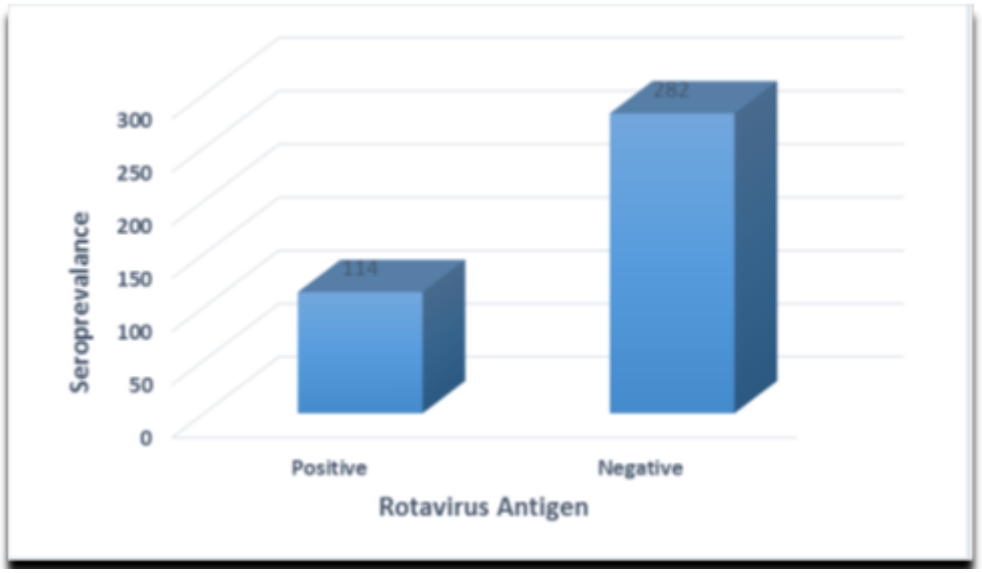

Fig 1:- Seroprevalence of RV antigen among children less than 5 years old.

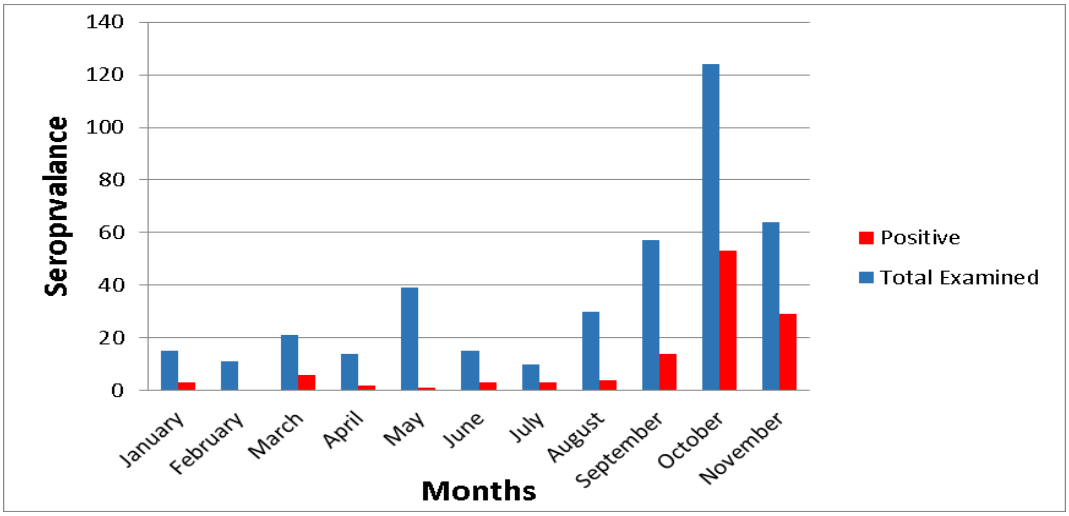

Fig 2:- Monthly distribution of diarrhea and RV infections.

Months	Participants (%) (no.=400)	Rotavirus	
		Positive (%) (no.=118)	**Negative (%)** (no.=282)
January	15 (3.75)	3 (2.5)	12(4.25)
February	11 (2.75)	0 (0)	11(3.9)
March	21 (5.25)	6 (5)	15(5.3)
April	14 (3.5)	2 (1.7)	12(4.25)
May	39 (9.75)	1 (0.84)	38(13.5)
June	15 (3.75)	3 (2.5)	12(4.25)
July	10 (2.5)	3 (2.5)	7(2.5)
August	30 (7.5)	4 (3.4)	26(9.2)
September	57 (14.25)	14 (11.86)	43(15.24)
October	124 (31)	53 (44.9)	71(25.2)
November	64 (16)	29 (24.57)	35(12.4)

Table 1:- Monthly distribution of diarrhea and RV infections.

Character	Participants (Total No.=400) No. (%)
Age groups (months):	
≤12	303 (75.8)
>12-24	72 (18)
>24-59	25 (6.2)
Sex:	
Male	230 (57.5)
Female	170 (42.5)
Area of residence:	
Rural	139 (34.8)
Urban	261 (65.2)
Place of enrolment:	
Outpatient	232 (58)
Inpatient	168 (42)

Table 2:- Socio-demographic characteristics of enrolled patients.

Character	Participants No. (%)
Vaccine: (no.=400)	
Vaccinated	198 (49.5)
Unvaccinated	202 (50.5)
Doses: (no.=198)	
One	36 (18.2)
Two	162 (81.8)

Table 3:- The vaccination status of enrolled patients.

Variable	Rotavirus		*RR (CI 95%)*	X^2	*P value*
	Positive (%) 118 (29.5)	**Negative (%)** 282 (70.5)			
Age groups (months):					
≤12	88 (74.6)	215 (76.2)	0.939 (0.665-1.327)	0.126	0.7
>12	30 (25.4)	(23.8)67			
Sex:					
Male	79 (67)	151 (53.5)	1.497 (1.078-2.079)	6.115	**0.01**
Female	39 (33)	131 (46.5)			
Area of residence:					
Urban	87 (73.7)	174(61.7)	1.495 (1.048- 2.131)	5.307	**0.02**
Rural	31 (26.3)	108 (38.3)			
Site of enrollment:					
Outpatient	84 (71.2)	148 (52.5)	1.789 (1.267-2.527)	11.947	**0.001**
Inpatient	34 (28.8)	134 (47.5)			

Table 4:- The socio-demographic features by RV positivity.
RR= Relative risk > 1 at risk, **$CI_{95\%}$ =** 95% Confidence intervals.
χ 2 = Chi-square ≥ 3.9 (significant), **p**= Probability value < 0.05 (significant).

Variable	Rotavirus		RR (CI 95%)	X^2	*P value*
	Positive (%) 118 (29.5)	**Negative (%)** 282 (70.5)			
Vaccination status:					
Vaccinated	60 (50.8)	138 (49)	0.948 (0.700-1.283)	0.122	0.7
Unvaccinated	58 (49.2)	144 (51)			
Doses:					
One (no=36)	12 (20)	24 (17.4)	0.889 (0.529 -1.494)	0.2	0.66
Two (no=162)	48 (80)	114 (82.6)			

Table 5:- The vaccination by RV positivity.
RR= Relative Risk > 1 at risk, **$CI_{95\%}$ = 95%** Confidence Intervals.
χ 2 = Chi-square ≥ 3.9 (significant), **p**= Probability Value < 0.05 (significant).

ISSN No:-2456-2165

Character	Rotavirus		*RR* (CI 95%)	X^2	*P value*
	Positive (%) 118 (29.5)	**Negative (%)** 282 (70.5)			
Type of diarrhea:					
Watery	85 (72)	151 (53.5)	1.790 (1.262-2.538)	11.755	**0.001**
Watery with mucus	33 (28)	131 (46.5)			
Frequency of diarrhea (times) / Day:					
≤ 5	29 (24.6)	113 (40)	1.689(1.172-2.435)	8.723	**0.003**
>5	89 (75.4)	169(60)			
Duration of diarrhea (days):					
≤ 5	98(83)	164(58.2)	2.582 (1.671-3.985)	22.815	**0.000**
6-14	20 (17)	118 (41.8)			
Fever:					
Yes	95 (80.5)	217 (77)	1.165 (0.789-1.719)	0.614	0.43
No	23 (19.5)	65 (23)			
Vomiting:					
Yes	106 (89.8)	195 (69.1)	2.905 (1.673-5.046)	19.105	**0.000**
No	12 (10.2)	87 (30.9)			
Frequency of vomiting (times) / Day:					
≤ 5	49 (46.2)	112(57.4)	1.338 (0.984-1.819)	3.468	0.06
5.	57 (53.8)	83 (42.6)			
Duration of vomiting (days):					
≤ 5	92 (86.8)	141(72.3)	1.918 (1.171-3.141)	8.239	**0.004**
6-14	14 (13.2)	54 (27.7)			
Dehydration:					
Yes	95 (80.5)	239 (84.8)	0.816 (0.563-1.183)	1.087	0.3
No	23 (19.5)	43(15.2)			
Type of dehydration:					
Severe + Moderate	56 (59)	156 (65.3)	0.826 (0.587-1.164)	1.173	0.27
Mild	39 (41)	83(34.7)			

RR= Relative risk > 1 at risk, **$CI_{95\%}$** = 95% Confidence intervals.
χ 2 = Chi-square ≥ 3.9 (significant), **p**= Probability value < 0.05 (significant).
Table 6:- Univariate analysis of clinical characteristics of children with RV diarrhea

Risk factors	Rotavirus		*RR* (CI 95%)	*P value*
	Positive (%) 118 (29.5)	**Negative (%)** 282 (70.5)		
Sex:				
Male	79 (67)	151 (53.5)	1.497 (1.078-2.079)	0.01
Female	39 (33)	131 (46.5)		
Area of residence:				
Urban	87 (73.7)	174(61.7)	1.495 (1.048- 2.131)	0.02
Rural	31 (26.3)	108 (38.3)		
Site of enrollment:				
Outpatient	84 (71.2)	148 (52.5)	1.789 (1.267-2.527)	0.001
Inpatient	34 (28.8)	134 (47.5)		
Type of diarrhea:				
Watery	85(72)	151 (53.5)	2.235 (1.403-3.558)	0.001
Watery with mucus	33 (28)	131 (46.5)		
Frequency of diarrhea (times) / day:				
≤ 5	29 (24.6)	113 (40)	2.052 (1.267-3.323)	0.003
>5	89 (75.4)	169 (60)		
Duration of diarrhea (days):				
≤ 5	98 (83)	164 (58.2)	3.526 (2.063-6.026)	0.000
6-14	20 (17)	118 (41.8)		
Vomiting:				
Yes	106 (89.8)	195 (69.1)	3.941 (2.061-7.536)	0.000
No	12 (10.2)	87 (30.9)		
Duration of vomiting (times) / day:				
≤ 5	92 (86.8)	141(72.3)	2.517 (1.322-4.791)	0.004
6-14	14 (13.2)	54(27.7)		

Table 7:- Risk factors for positivity of RV using univariate analysis.
RR= Relative Risk > 1 at risk, **CI95%** = 95% Confidence Intervals. **p**= Probability Value < 0.05 (significant).

Risk factors	Rotavirus		*RR* (CI 95%)	Exp (B) (CI95%)	*P* value
	Positive (%)	**Negative (%)**			
Sex:					
Male	79 (67)	151 (53.5)	1.497 (1.078-2.079)	1.1775 (1.105-2.851)	0.018
Female	39 (33)	131 (46.5)			
Type of diarrhea:					
Watery	85(72)	151 (53.5)	2.235 (1.403-3.558)	1.672 (1.008-2.774)	0.047
Watery with mucus	33 (28)	131 (46.5)			
Duration of diarrhea:					
≤ 5	98 (83)	164 (58.2)	3.526 (2.063-6.026)	2.611 (1.474-4.624)	0.001
6-14	20 (17)	118 (41.8)			
Vomiting:					
Yes	106 (89.8)	195 (69.1)	3.941 (2.061-7.536)	3.649 (1.868-7.125)	0.000
No	12 (10.2)	87 (30.9)			

Table 8:- The risk factors associated with RV diarrhea using Multivariate analysis.
RR = Relative Risk**, Exp (B) =** Exponential (B) (Adjusted relative risk).
CI95% = 95% Confidence Interval.