Revista Argentina de Clínica Psicológica
2021, Vol. XXX, N°1, 368-375
DOI: 10.24205/03276716.2020.2034

Identification and genotypic characterization of Rota - virus from children post vaccination in Aseer region of Southwestern Saudi Arabia – An ephemeral experience

Esther Paul[a], Ibrahim A Alzaydani[b], Ahmed Al-Hakami[a], Ali A Hawan[c], Ayed A Shati[d], Ihab M. Z Abdelrahim[a], Riyad Ahmad Ali Moosa[a], Sultan A Alkahtani[c], Ali H. Asiri[b], Harish C. Chandramoorthy[a]

Abstract

We intended to screen rotavirus (RV) infections post vaccination in Aseer region. Further, identification of the circulating genotypes will be a valuable information on the efficacy of the vaccination and clinical features of gastroenteritis as per the Modified Vesikari Score (MVS). 307 stool samples were collected from 450 children below five years with complaints of gastroenteritis between Nov 2017- Jan 2019. We used a questionnaire to document clinical symptoms as per Modified Vesikari Score method, history of RV vaccination and the adverse effects of immunization. The RV antigen rapid test and RT-PCR was carried out for identification and genotyping of the RV antigens in the stool. The prevalence of RV infection was 6.5% (20 samples). The most prevalent genotypes were G2P [4] (19 samples) against G1P [8] (1 sample). 90% of the positive cases had a severe MVS score (>11), while 10% of the positive cases had a mild MVS score, which was statistically significant ($p<0.001$). Continuous diarrhea, vomiting, and fever were the statistically significant adverse effects seen more among the RV positive patients. The low prevalence of RV infection with the presence of G2P [4)] strains gives us an initial picture of vaccine efficacy while envisaging any adverse effects impacting completion of vaccination doses.

Keywords: Rotavirus infection, G2P [4] genotype, Prevalence, adverse effects, Rotarix vaccine

[a]. Department of Microbiology and Clinical Parasitology, College of Medicine, King Khalid University, P.O. Box: 641, Post Code: 61421, Abha, Saudi Arabia.
[b]. Department of Pediatrics, Maternity and Children's Hospital, Ministry of Health, 62521 Emirate Al Shifa Abha, Saudi Arabia
[c]. Department of Pathology/ Microbiology and Blood Bank, Armed Forces Military hospital, Southern region, Khamis Mushayat, 62413, Saudi Arabia.
[d]. Department of Child Health, College of Medicine, King Khalid University, P.O. Box: 641, Post Code: 61421, Abha, Saudi Arabia.
Correspondence Authors:
Dr. Harish C.Chandramoorthy, Ph.D, Department of Microbiology & Clinical Parasitology, College of Medicine, King Khalid University, P.O. Box: 641, Post Code: 61421, Abha, Saudi, Arabia, Email: ccharishjabali@gmail.com
Esther Paul, MD, Department of Microbiology & Clinical Parasitology, College of Medicine, King Khalid University, P.O. Box: 641, Post Code: 61421, Abha, Saudi, Arabia, Email: dr.estherpaul@outlook.com
Running head: Rotavirus genotypes and infection in Saudi Arabia.
Highlights

- *Marginal increase in the prevalence of rotavirus infection (6.5%) in children below five years of age in the post-vaccination era*
- *A severe MVS score among RV positive patients*
- *Vomiting, fever, and continuous diarrhea were the significant adverse effects of vaccination among both the RV positive and RV negative cases.*

Introduction

Diarrheal deaths in modern times of advanced medical care and technology are unwarranted. An estimated 0.578 million deaths in children below the age group of five years are due to diarrhea, and nearly 28% of these diarrheal deaths in this age group are attributable to rotavirus (RV) infections (1,2). The RV is a three double-stranded ribonucleic acid (RNA) genome with eleven segments encoding for non-structural proteins (NSP) and six structural proteins. Based on the differences in the sequence and antigens of VP6, ten different rotavirus species have been identified. Among which species A is known to cause infections in children. The capsid proteins classify the strains of RV into G (Glycoprotein VP7) and P (protease sensitive VP4) genotypes (3). There are nearly 32 G genotypes and 47 P genotype of species A pathogenic to humans, however only six G types (G1,G2,G3, G4,

G9, G12) and three P genotypes (P[4],P[6],P[8]) dominate the world (4).The standard combinations prevalent before the introduction of the RV vaccine were G9P[8], G4P[8], G3P[8], G2P[8], and G1P[8] respectively (5).World Health Organization (WHO) estimates, before the introduction of the vaccine, RV infection contributed to 450,000 deaths every year among children below five years, with 80% of these deaths occurring in the regions of the Sub-Saharan countries and South Asia (6).Since the year 2009, WHO had advised the majority of the countries worldwide to introduce RV vaccination into their national immunization schedule. By the end of the year 2014, nearly 29% of the WHO member countries worldwide had inculcated the RV vaccine into their immunization programs. The two licensed live attenuated oral RV vaccines approved by the WHO was RV5 (RotaTeq), a pentavalent bovine-human reassortant RV vaccine (Human G1-4, P [8] surface antigens) and RV1(Rotarix) a monovalent human RV vaccine (7).

The prevalence of RV infection in the Kingdom was reported to be between 12-46% in the pre-vaccination era (8,9). Saudi Arabia included the RV1 vaccine into its national immunization schedule in the year 2013. The vaccine was administered in two doses, initially at two months and then at four months of age, along with pneumococcal conjugate vaccine and polio vaccines (10). A study from the Aseer region in the post-vaccination period reported a fall in the prevalence of RV infection to nearly 6.8% and the prevalence of G1P[8] and G2P[4] genotype as the most common genotype (11). Our research aims to explore the prevalence of RV infection, the predominant genotypes of RV existing in the post-vaccination period in the southwestern region of Saudi Arabia. Also, our study seeks to evaluate the adverse effects of the RV1 as well as the clinical presentation of gastroenteritis as per the Modified Vesikari Score (MVS) method. MVS is a useful clinical tool that can be used in different healthcare systems and settings. It uses seven variables with scores of 0-8, 9-10, and greater than 11 representing mild, moderate, and severe illness.

Materials and Methods

Study design

Our prospective study was carried out throughout fourteen months (November 2017-January 2019) in two tertiary care hospitals in Abha and Khamis Mushayat, Saudi Arabia. The Ethical committee at College of Medicine, King Khalid University granted ethical clearance for the current study vide Ref # REC#2017-03-11. Bilingual written informed consent was obtained from either set of parents of the children included in the study.

Study population and data collection

Acute gastroenteritis (AGE) included symptoms of three or more episodes of diarrhea within 24 hours, accompanied by fever, vomiting, and severe abdominal pain occurring less than 14 days before admission (12). All the children below the age group of five years with complaints of gastroenteritis were included in the study. Children above and below five years of age with symptoms other than gastroenteritis were excluded. MVS, a valid clinical tool, was used to record the clinical manifestations of children who presented in the outpatient and emergency department. MVS consists of seven variables to measure the

clinical signs of AGE (Table 1) (13). Scores of 0-8 were considered as a mild illness, 9-10 moderate illness, and ≥ 11 as severe illness (14). A questionnaire was used to collect the clinical data as well as the demographic details like age, gender, nationality, and geographical location of the patient. The RV vaccination history, the completion of the two doses, a record of any adverse effects of the vaccination following immunization was obtained from the parents and verified by the vaccination card following informed consent. Interviewees were allowed sufficient time for adequate recall of the adverse effects of vaccination, and the information was confirmed from both sets of parents and verified by the vaccination card for accuracy to control the recall bias. According to theCentre for Disease Control (CDC), the significant adverse impact of RV vaccination is intussusception, a type of intestinal blockage requiring surgical intervention. Other mild adverse effects include irritability, vomiting one or two episodes, mild diarrhea (2 episodes or less in 24 hours), continuous diarrhea (3 or more events in 24 hours), and a fever higher than 39°C developing within one week of vaccination (15).

Sample collection

Out of a total of 450 children who presented in the emergency department, outpatient clinics, and hospitalized children with complaints of AGE, stool samples were collected from 307 children after obtaining informed consent from either of the parents. One gram of the stool sample was diluted in 1:10 phosphate-buffered solutions, vortexed and centrifuged, and the clear supernatant was stored at 4-8 °C for further analysis (16).

Initial rapid antigen detection

The RV antigens were detected from the stool using the Rapid Antigen Detection Kit as per the manufacturers' instructions (Cortez Diagnostics, Inc. One Step Rotavirus Rapid Card™ InstaTest).

Reverse transcription-polymerase chain reaction (RT-PCR)

The viral genomic ribonucleic acid (RNA) was extracted by QIA amp viral RNA mini kit (QIAGEN GmbH, Germany) from all the positive stool samples. Multiplex semi-nested PCR specific for VP4 and VP7 was undertaken for the P and G genotyping for RV antigens, using the specific primers as per the WHO 2009 manual (16).

Statistical Analysis

The mean and the standard deviation and the significance of risk factors were calculated using the Fishers Exact test, Mann Whitney U test and Chi-square test from the data using the SPSS software (Version 20).

Results

Demograpic correlation of rota virus infection

A total of 307 children with symptoms of gastroenteritis enrolled in the study (Fig 1a). Among these, 174 (56.6%) were males, and 133 (43.4%) were females. The prevalence of RV infection was found to be 6.5% (20 RV positive patients). Mean age in months and standard deviation (SD) were (17.4 +/-6.6) for the RV positive and (23.5 +/- 11.9) for RV negative groups respectively (Fig 1b). Although the mean age was lower among the RV positive cases as compared to the RV negative cases, this difference was not statistically significant (*P= 0.2*). Among the RV positive cases, 12 (60%) were male, and 8 cases (40%) female (Fig 1a) . However, the male gender was not a significant risk factor for developing RV infection (*Chi-square0.0962*, a *P-value of 0.7*). 75.2% of the enrolled children belonged to the urban area, while 24.8% of the patients belonged to the rural area (Fig 2a). RV positive children were distributed more in the urban as compared to the rural area, which was statistically significant (*Chi-square 5.693, a P-value of 0.04*). RV positive children were more among the families with a higher income (>10,000 riyals) as compared to families with lower income (<10,000 riyals), which was statistically significant (*Chi-square 4.2681, a P-value of 0.03*) (Fig 2b).

Clinical symptoms associated woth rota virus postivity

The clinical manifestations of both RV positive and RV negative cases are tabulated (Table 2). 90% of the positive cases had a severe MVS score (>11) while the remaining 10% of the positive cases had a mild MVS score, which was statistically significant (*P<0.001*). 77% of the RV negative patients had a mild MVS score, 22% of them had a moderate MVS score, and the remaining 3% had a severe MVS score. The mean duration of diarrhea and vomiting among both RV positive and RV negative children are enumerated (Table 3). The Mean ± SD of the duration of diarrhea and the Mean ± SD of the duration of vomiting was higher in RV positive casesas compared to the RV negative cases (statistically significant with *P<0.001*).

Vaccination history, adverse effects of RV1 Vaccination and its relation to circulating rota virus genotype

All the children with the symptoms of AGE had been vaccinated with two doses of the RV1 and had completed the two doses. The adverse impact of RV vaccination are tabulated (Table 4). Continuous diarrhea, vomiting, and fever were the adverse effects statistically significant. These adverse effects were seen more among the RV positive patients as compared to the RV negative patients (*P<0.05*). The 20 RV positive samples were genotyped using RT-PCR. The dominant genotype by VP7Genotyping was the G2 genotype and P [4] by VP4 genotyping. The prominent genotype combination was G2P[4] (19 samples), followed by G1P[8] (1sample).

Discussion

Demographic details

Our study has reported the incidence of RV positive cases more among the 15- 30 months age group (mean age+/- S.D among the RV positive cases was 17.4 (6.6) in comparison with any other age group which is almost similar to the study from the Asir region in Saudi Arabia (13-24 months) (10).African studies have reported that male children were more prone to develop RV infection as compared to female children. The reason was that the female child was neglected and seldom hospitalized, but attention and care were provided immediately to the sick male children, thus increasing the chances of reporting of RV infection (17). However, in our study, even though the percentage of the RV infected males was twice that of the female population, the male gender did not play any statistically significant role in the development of RV infections. Worldwide studies

consider rural location and the male gender of the patients to be significant risk factors in developing RV infections (18). However, in our research contradictory to the other reviews, most of the RV positive cases belonged to the urban population, which was statistically significant. This might be due to the affluence of the urban population to water bodies like swimming pools, travel, parks and other water amusement parks than the rural conserved community. Further the food commodities which were impored were more frequently available to the urban communities than the rural areas. Accessibility to a tertiary care center and timely treatment of RV gastroenteritis in the urban areas could also explain the increase of RV positive cases in the urban areas.

Clinical symptoms & Prevalence of RV infection

Many studies from the Middle East region have demonstrated the association of RV infection with severe diarrhea and high Vesikari scores (19,20,21). The current research also shows highVesikari scores(severe=90%) among RV positive children as compared to the RV negative children (Severe= 2.7%)(statistically significant with *P<0.001*). The recent post-vaccination studies in Saudi Arabia have reported an infection rate of RVA to be in the range of 3.9%- 6.8%, respectively (11,22). These findings demonstrate the positive effects of RV vaccination. In our study, the rate of RV positive cases was 6.5%, which is almost the same as the results reported by the previous studies in the recent past. The study from the Asir region in 2016 reported G2P[4] to be the most common, followed by G9P[8] and G12P[8] strains (11). Worldwide studies from Australia, Brazil, Latin America, South America, Andaman Nicobar Islands, India, Nicaragua, and parts of Europe have reported G2P[4] genotype to be the most predominant genotype following the introduction of the RV1 vaccine into their national immunization schedule. The authors concluded that the emergence of G2P[4] genotype was not connected to the RV vaccination, but due to the fluctuation in the genotypes occurring as a normal Phenomenon (23,24). As per the global scenario and the trend in Saudi Arabia, G2P[4]strains have emerged to be the most common RV strain to persist in the post-vaccination period. In our study also, G2P[4] strains appeared to be prominent among the positive samples of RV. An Indonesian study also had observed that patients positive for the G2 genotype of the RV had more severe symptoms of gastroenteritis as compared to any other genotypes (25). These findings are similar to the present study, where the RV positive cases belonged to the G2 genotype and had more severe symptoms of gastroenteritis, which were statistically significant (*P<0.001*). However, a recent study from India and a few other studies had reported that the children with G1 serotype had more severe symptoms as compared to children with G9 and G12 strains. This observation confirms that the difference in the severity of the infection could be because of the emergence of newer strains rather than the differences in the virulence of the strains (26).

Adverse effects of the RV vaccination

Earlier vaccines like the tetravalent Rhesus vaccine caused some significant complications like intussusceptions within two weeks of the first dose. This complication, however, was not typical with RV1 vaccines and was a rare complication (27). In the present study, none of the children had any history of significant, however rare adverse effects like intussusception. The adverse effects which were statistically significant among both RV positive and negative cases were continuous diarrhea, fever, and vomiting (*P<0.05*)following either of the two doses of vaccination.

Conclusion

In conclusion, although the incidence of RV infection has decreased six folds in the post-vaccination era, the present study has exhibited a similar prevalence of RV infections, as reported in the recent past. The existence of G2P [4], although not a novelty in this part of Saudi Arabia, is one of the significant findings in our study in the post-vaccination era. The positive RV cases had severe clinical symptoms, and this is probably due to an association with G2P [4] genotypes, as observed in recent studies from the Southern region of Saudi Arabia. The significant adverse effects of RV vaccination immediately following either of the two doses of immunization were continuous diarrhea, vomiting, and fever. However, despite this finding, all the children with complaints of gastroenteritis had completed the vaccination for rotavirus, demonstrating that the adverse effects of the immunity did not stop the parents from completing the immunization schedule. The Saudi Vision 2030 is to make a tremendous change and progress in preventive medicine (28). Complete eradication of vaccine-preventable diseases can help the Kingdom to achieve its goal in preventive medicine. Further studies on the existence of RV infection caused by G2P [4] RV strains despite the completion of the

two doses of the Rotarix vaccine can prove to be beneficial at the National and the International level.

Limitations & Future Investigations

The present study was a brief investigation. However, the modest sample size in the fourteen months of duration of the study is a limitation. With the time constraint and to know the genotypes as inistial report, this study satisfies the objective. We do recommend a multicentric study with inclusion of more tertiary hospitals involving different areas of the kingdom and an increase in the duration time of the investigation spanning seasonal cycles and repeat samples or additional samples from the positive cases.

Acknowledgement

We acknowledge Denship of Scientifc Research, Grant No G.P.R 338-39, King Khalid University, Abha Saudi Arabia.

Authors' contributions

The study conception and the methodology were done by Esther Paul, Ibrahim A Alzaydani, Ahmed A Hakami, Ali A Hawan, and Ayed Abdullah Shati. Esther Paul, Ibrahim A Alzaydani, Ali H Asiri, and Sultan Alkahtani designed the questionnaire and helped in the acquisition of the clinical data. Esther Paul, Ihab Abdelrahim, Riyad Ahmad Ali Moosa and Harish C.Chandramoorthy performed the experiments. Interpretation of the data and analysis were carried out by Esther Paul, Ibrahim A Alzaydani, Ahmed A Hakami, and Harish C. Chandramoorthy. Esther Paul, Ibrahim A Alzaydani, Ahmed A Hakami, Ayed Abdullah Shati and Harish C.Chandramoorthy participated in the drafting and critical revisions of the manuscript.

Declaration of conflict of interest

None.

Availability of data and material

Data of the findings are available on request.

References

[1] Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015; 385:430-40.

[2] Walker CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, O'Brien KL, Campbell H, Black RE.Global burden of childhood pneumonia and diarrhea. Lancet. 2013; 381:1405-16.

[3] Greenberg HB, Estes, MK. Rotaviruses: from pathogenesis to vaccination. Gastroenterology. 2009; 136:1939-51.

[4] Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, Franco MA, Greenberg HB, O'Ryan M, Kang G, Desselberger U, Estes MK. Rotavirus infection. Nat.Rev.Dis.Primers. 2017; 3:17083.

[5] Bányai K, László B, Duque J, Steele AD, Nelson EA, Gentsch JR, Parashar UD. A systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. Vaccine. 2012; 30: A122-30.

[6] Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than five years before the introduction of universal rotavirus vaccination programs: a systematic review and meta-analysis.Lancet Infect Dis. 2012; 12:136-41.

[7] Agócs MM, Serhan F, Yen C, Mwenda JM, de Oliveira LH, Teleb N, Wasley A, Wijesinghe PR, Fox K, Tate JE, Gentsch JR, Parashar UD, Kang G. WHO global rotavirus surveillance network: a strategic review of the first five years, 2008-2012. MMWR Morb Mortal Wkly Rep. 2014; 63:634-7.

[8] Kheyami AM, Cunliffe NA, Hart, CA. Rotavirus infection in Saudi Arabia. Ann Saudi Med.2006; 26:184.

[9] Hegazi MA, Sayed MH, Sindi HH, Bekhit OE, El-Deek BS, Alshoudri FM, Noorelahi AK. Is rotavirus still a major cause for diarrheal illness in hospitalized pediatric patients after rotavirus vaccine introduction in the Saudi national immunization program?. Medicine. 2017; 96: e6574.

[10] WHO vaccine-preventable diseases: monitoring system. 2017 global summary. World Health Organization; 2018. http://apps.who.int/immunization_monitoring/globalsummary

[11] Al-Ayed MS, Asaad AM, Qureshi MA, Hawan AA. Epidemiology of group A rotavirus infection after the introduction of monovalent vaccine in the National Immunization Program of Saudi Arabia. J Med Virol. 2017; 89:429-34.

[12] Anca IA, Furtunescu FL, Pleşca D, Streinu-Cercel A, Rugină S, Holl K. Hospital-based surveillance

to estimate the burden of rotavirus gastroenteritis in children below five years of age in Romania. Germs. 2014; 4:30.

[13] Freedman SB, Eltorky M, Gorelick M, Pediatric Emergency Research Canada Gastroenteritis Study Group. Evaluation of a gastroenteritis severity score for use in outpatient settings. Pediatrics. 2010;125: e1278-85.

[14] Schnadower D, Tarr PI, Gorelick MH, O'Connell K, Roskind CG, Powell EC Rao J, Bhatt S, Freedman SB.Validation of the modified Vesikari score in children with gastroenteritis in 5 US emergency departments. J Pediatr Gastroenterol Nutr. 2013; 57:514 -519.

[15] CDC, 2018. Vaccines and Immunizations. Possible side-effects of vaccines. https://www.cdc.gov/vaccines/vac-gen/side-effects.htm#rota

[16] WHO Manual of rotavirus detection and characterization methods. Immunization, Vaccines, and Biologicals. World Health Organization; 2009. http://apps.who.int/iris/handle/10665/70122

[17] Magzoub MA, Bilal NE, Bilal JA, Osman OF. Rotavirus infection among Sudanese children younger five years of age: a cross-sectional hospital-based study. Pan Afr Med J. 2013; 16:88.

[18] Page A, Jusot V, Mamaty AA, Adamou L, Kaplon J, Pothier P, Djibo A, Manzo ML, Toure B, Langendorf C, Collard JM, Grais RF. et al. Rotavirus Surveillance in Urban and Rural Areas of Niger, April 2010–March 2012. Emerg Infect Dis.2014; 20:573-580.

[19] Khoury H, Ogilvie I, El Khoury AC, Duan Y, Goetghebeur MM. Burden of rotavirus gastroenteritis in the Middle Eastern and North African pediatric population.BMC Infect Dis.2011;11:9.

[20] Malek MA, Teleb N, Abu-Elyazeed R, Riddle MS, Sherif ME, Steele AD, Glass RI, Bresee JS.The epidemiology of rotavirus diarrhea in countries in the Eastern Mediterranean Region.J Infect Dis. 2010; 202(Supplement_1): S12-22.

[21] Ibrahim SB, El-Bialy AA, Mohammed MS, El-Sheikh AO, Elhewala A, Bahgat S. Detection of Rotavirus in children with acute gastroenteritis in Zagazig University Hospitals in Egypt.Electronic Physician. 2015; 7:1227-33.

[22] Zaki A, Abousekkien M, Alkholy UM, Eid A. Effectiveness and impact of rotavirus vaccines in Saudi Arabia: A single hospital-based study. Arab J Gastroenterol.2017;18:140-3.

[23] Antunes H, Afonso A, Iturriza M, Martinho I, Ribeiro C, Rocha S, Magalhães C, Carvalho L, Branca F, Gray J.G2P (4) the most prevalent rotavirus genotype in 2007 winter season in a European non-vaccinated population.J Clin Virol. 2009; 45:76-8.

[24] Nakagomi T, Cuevas LE, Gurgel RG, Elrokhsi SH, Belkhir YA, Abugalia M, Dove W, Montenegro FM, Correia JB, Nakagomi O, Cunliffe NA, Hart CA.Apparent extinction of non-G2rotavirus strains from circulation in Recife, Brazil, after the introduction of rotavirus vaccine. Arch Virol. 2008; 153:591-3.

[25] Sudarmo SM, Shigemura K, Osawa K, Wardana OP, Darma A, Ranuh R, Raharjo D, Arakawa S, Fujisawa M, Shirakawa T. Genotyping and clinical factors in pediatric diarrhea caused by rotaviruses: one-year surveillance in Surabaya, Indonesia.Gut Pathog. 2015; 7:3.

[26] Saluja T, Dhingra MS, Sharma SD, Gupta M, Kundu R, Kar S, Dutta AK, Silveira MD, Singh JV, Kamath VG, Chaudhary A, Rao V, Ravi MD, Murthy K, Arumugam R, Moureau A, Prasad R, Patnaik BN. Association of rotavirus strains and severity of gastroenteritis in Indian children.Hum VaccinImmunother.2017;13:711-716.

[27] Glass RI, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, Jiang B, Gentsch JR. Rotavirus vaccines: current prospects and future challenges. Lancet. 2006; 368:323-32.

[28] Ministry of Health Portal.National e-Health strategy, VISION 2030. 2016.Available from http://www.moh.gov.sa/en/Ministry/nehs/Pages/vision2030.aspxlast accessed on 9th May 2018.

Table 1. **Modified Vesikari Score (MVS) (Clinical criteria analyzed)**

Points	0	1	2	3
Diarrhea duration (hr.)	0	1-96	97-120	≥ 121
Max no. of diarrheal stools/24 hr. period (during the disease	0	1-3	4-5	≥ 6
Vomiting duration (hr.)	0	1-24	25-48	≥ 49
Max no. of vomiting episodes/24 hr period (during the disease)	0	1		
Max recorded fever	< 37.0°C	37.1-38.4 °C	38.5-38.9°C	≥ 39.0°C
Future healthcare visit	0	-	Primary care	Emergency Dept
Treatment	None	IV Rehydration	Hospitalization	-

Table 2. **Clinical characteristics of cases among RV positive and RV negative group**

Clinical presentation	RV positive group n= 20	RV negative group n= 287
No of diarrhea episodes		
1-3	3 (15%)	261 (90.9%)
4-5	0	17 (5.92%)
≥6	17 (85%)	9 (3.13%)
Duration of diarrhea in hours		
1-3	0	2 (0.69%)
4-5	0	0
≥6	20 (100%)	285 (99.3%)
No of vomiting episodes/day		
1	0	180 (62.7%)
2-4	2 (10%)	95 (33.1%)
≥5	18 (90%)	12 (4.18%)
Duration of vomiting		
1-24 hours	0	214 (74.56%)
25-48 hours	0	67 (23.3%)
≥49 hours	20 (100%)	6 (2.09%)
Fever		
37.1˚C – 38.4˚C	2 (10%)	228 (79.44%)
38.5˚C – 38.9˚C	0	51 (17.7%)
≥ 38.9˚C	18 (90%)	8 (2.78%)

Table 3. **Mean ± SD of the duration of diarrhea and vomiting**

Variable	RV positive (n=20)	RV negative (n=287)	*P-value*
	Mean ± SD	Mean ± SD	
Duration of diarrhea in hours	130.7 (9.3)	53.2 (37.7)	< 0.001
Duration of vomiting in hours	54.5 (3.2)	22.8 (12.7)	< 0.001

Table 4. **Adverse effects of vaccination**

Adverse effects of vaccination	RV positive group n=20	RV negative group n=287	*P-value*
Irritability	20 (100.0%)	273 (95.1%)	0.65
Vomiting	18 (90.0%)	181 (63.1%)	0.02
Mild diarrhea	15 (75.0%)	217 (75.6%)	1.0
Continuous diarrhea	8 (40.0%)	53 (18.5%)	0.04
Fever	11 (55.0%)	78 (27.2%)	0.01

Figures

Figure 1

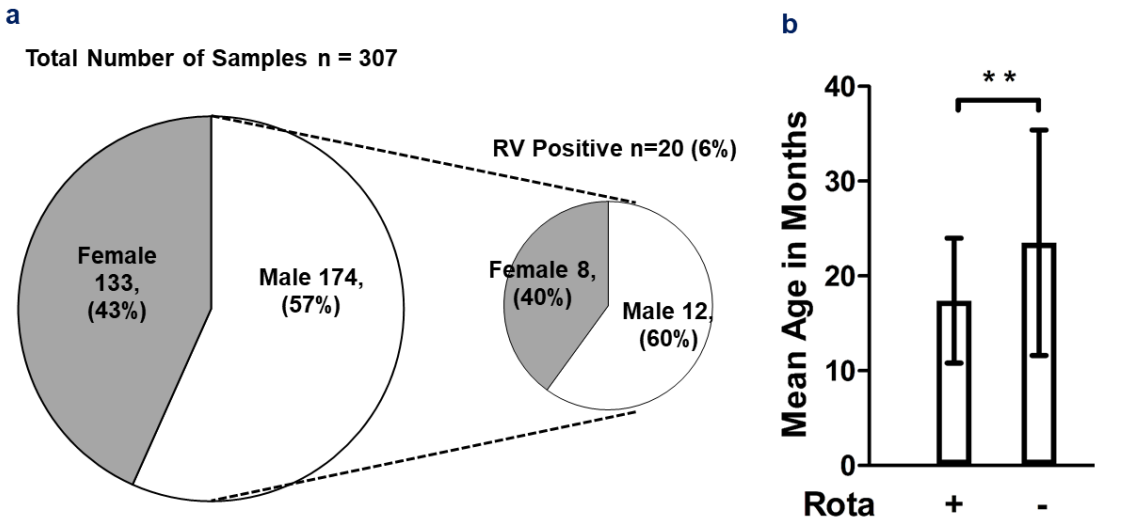

Figure 1. **a. Number of male and female samles collected during the study period. The rota virus positives among gender is depcted as separate pie chart. b. Mean age in months of rota virus positive cases.**

Figure 2

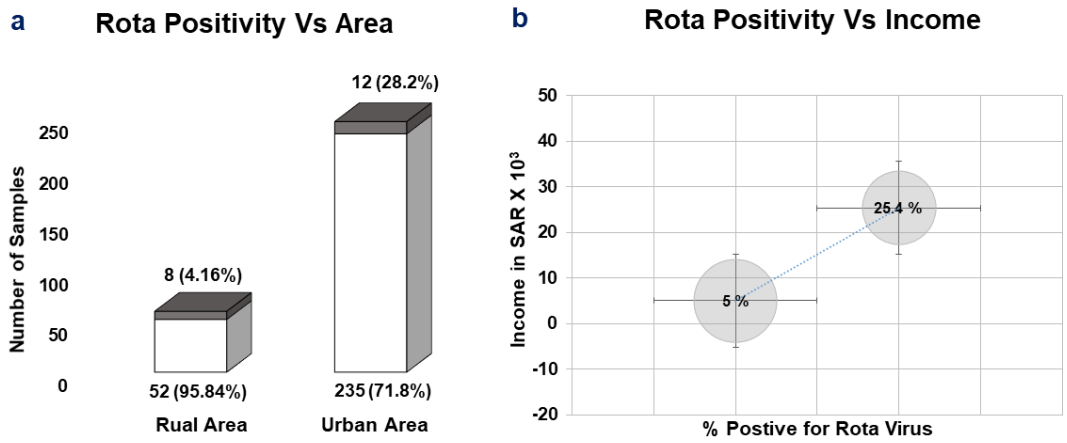

Figure 2. **a. Rota virus positive cases based on rulral and unrban area classification.The positivity in urban area were significant compared to the rural areas (*Chi-square 5.693, a P-value of 0.04*). b. Distrubtion of the rota virus positive cases among the income groups ≥10,000 SAR and ≤ 10,000 SAR. The income status and rota virus positivity were statistically significant (*Chi-square 4.2681, a P-value of 0.03*).**