American Journal of Epidemiology and Infectious Disease, 2017, Vol. 5, No. 2, 21-26
Available online at http://pubs.sciepub.com/ajeid/5/2/1

DOI:10.12691/ajeid-5-2-1

Rotavirus Infection in a Secondary Hospital, Laboratory Diagnosis and Impact of Immunization on Pediatric Hospitalization Saudi Arabia

Riyad ALkheliaf[1], Mohammed. A. Garout[2], Magda R. Abdelwadood[3,*], Amer Alkhlaif[4], Mayad R. Khelaif[5]

[1]Senior consultant Paediatric Infectious Diseases, King Saud Medical City (KSMC) / Sanad Hospital KSA
[2]Community Medicine and Public Health, Faculty of Medicine, Umm Al-Qura University, Makkah, K.S.A
[3]Microbiology and Immunology, Faculty of Medicine, ain Shams, University, Cairo, Laboratory consultant Sanad Hospital, KSA
[4]Medical Student, Almarefa Medical Collegue
[5]Medical intern, King Fahad Medical City
*Corresponding author: ramadan_magda@yahoo.com

Abstract **Rotavirus** is the most common cause of gastroenteritis (diarrhea) disease among infants and young children, Rotavirus is usually an easily managed disease of childhood, infections are most common during the winter months from November to May. However, infection with rotavirus can occur anytime of the year, There are currently 2 FDA-approved rotavirus vaccines to protect against rotavirus gastroenteritis (RotaTeq and Rotarix). These vaccines are indicated in infants aged 6-32 weeks (RotaTeq) and those aged 6-24 weeks (Rotarix). **OBJECTIVES**: Conduct viral testing on stool samples from diarrhea patients to determine ROTA viruses that as a cause of diarrhea, and effectiveness of vaccine. **STUDY DESIGN**: A retrospective cross section study was conducted using stool samples collected from children with diarrhea (n=285) between January 2015 and December 2016. Samples were tested for rotavirus, were determined by Enzyme immunoassay, Latex agglutination. **Result**: two hundred eighty five stool samples from children with acute diarrhea attend to our hospital in 2015 and 2016 were examined by commercial latex and immunoenzymatic assays for the diagnosis of rotavirus infection in laboratories. for comparison, sensitivity and specificity for the latex and ELISA assays ELISA and Latex Test specificity 98.5 % Latex sensitivity 88 % to ELISA. Around 16% of our study population with diarrhea disease were diagnosed as ROTA (45/285) AMONGE THEM 89% 40/54were previous vaccinated with RTOTARIX **CONCLUSION** Rotavirus is an infection that causes diarrhea. It's the most common cause of diarrhea in infants and children less than 5 years old worldwide, according to the Centers for CDC Rotavirus vaccines are very effective at preventing rotavirus disease, However in our study we found most of patients were vaccinated according to national vaccination program still had a disease and the vaccination only modified disease course decrease days of hospitalization for further expanded and comparative study for types of vaccination.

Keywords: *acute gastroenteritis, diarrhea oral vaccine, rotavirus, vaccine*

Cite This Article: Riyad ALkheliaf, Mohammed. A. Garout, Magda R. Abdelwadood, Amer Alkhlaif, and Mayad R. Khelaif, "Rotavirus Infection in a Secondary Hospital, Laboratory Diagnosis and Impact of Immunization on Pediatric Hospitalization Saudi Arabia." *American Journal of Epidemiology and Infectious Disease*, vol. 5, no. 2 (2017): 21-26. doi: 10.12691/ajeid-5-2-1.

1. Introduction

Rotavirus is the most common cause of diarrhea illness among infants and young children. Rotavirus infections are most common during the winter months from November to May, however infection with rotavirus can occur anytime of the year [1]. Rotavirus is usually an easily managed disease of childhood, but in 2013, rotavirus caused 37 percent of deaths of children from diarrhea and 215,000 deaths worldwide, and almost two million more become severely ill. [2] Most of these deaths occurred in developing countries. In the U.S., rotavirus infections usually peak in the fall months in the Southwest and spread to the Northeast by spring [3]. A rotavirus infection usually starts within two days of exposure to the virus initial symptoms are a fever and vomiting, followed by three to seven days of watery diarrhea. The infection can cause abdominal pain as well [4]. According to WHO 2004 estimates, 527 000 children aged <5 years die each year from vaccine-preventable rotavirus infections; most of these children live in low-income countries. WHO reiterates that rotavirus vaccines are an important measure that can be used to reduce severe rotavirus-associated diarrhea and child mortality. The use of rotavirus vaccines should be part of a comprehensive strategy to control diarrheal diseases [5]. There are currently 2 FDA-approved rotavirus vaccines to protect against rotavirus gastroenteritis (RotaTeq and Rotarix) These vaccines are indicated in infants aged 6-32 weeks (RotaTeq) and those aged 6-24 weeks (Rotarix) [6] this strategy should also

include, among other interventions, improvements in hygiene and sanitation, zinc supplementation, community-based administration of oral rehydration solution and overall improvements in case management [7]. Rotavirus infection is spread through contamination of hands, objects, food or water with infected feces. The virus is taken in by the mouth. Such routes of infection are common in: day care centers, family homes, homes for the elderly. It may also be spread by mucous membrane (the thin moist lining of many parts of the body such as the nose, mouth, throat and genitals) contact with infected airborne droplets produced by coughing and sneezing. [8] Gastroenteritis caused by rotavirus can be more severe than that caused by other pathogens. Additionally, the rates of rotavirus infection have not responded well to improvements in water supply, sanitation, or hygiene that have effectively reduced bacterial and parasitic diarrhea. For these reasons, development of rotavirus vaccines that are effective has long been a high priority [9]. The introduction of the vaccines adds momentum to ongoing efforts to understand rotavirus and to reduce the impact of rotavirus worldwide [10]. RotaTeq is indicated in the United States for the prevention of rotavirus gastroenteritis in infants and children caused by the serotypes G1, G2, G3, and G4 when administered as a three-dose series to infants between the ages of 6 to 32 weeks. RotaTeq should not be administered to infants with a demonstrated history of hypersensitivity to any component of the vaccine. Rotarix is indicated in the United States for the prevention of rotavirus gastroenteritis caused by G1 and non-G1 types (G3, G4, and G9) when administered as a two-dose series in infants and children. Rotarix should not be administered to infants with a history of uncorrected congenital malfunction of the gastrointestinal tract that would predispose to intussusceptions [11].

1.1. Objectives

Conduct viral testing on stool samples from diarrhea patients to determine ROTA viruses that as a cause of diarrhea, and effectiveness of vaccine.

1.2. Study Design

A retrospective cross section study was conducted using stool samples collected from children with diarrhea (n=285) between 2015 and 2016. Samples were tested for rotavirus, were determined by Enzyme immunoassay and Latex agglutination.

1.3. Ethics

The procedures involving the use of the stool of clinical samples from the laboratory for the detection of RV in human stool samples had the approval of the Committee on the Ethics.

2. Materials and Methods

2.1. Study Population

Samples collected randomly from two hundred and eighty five admitted cases who attended Sanad Hospital (a tertiary care hospital) during January 2015 and December 2016. Children 5 years or under 5years old who presented to the hospital with acute watery diarrhea and required hospitalization were enrolled. Verbal consent was obtained from the child's parent before inclusion in the study. The child's parent was interviewed concerning date of onset of diarrhea, associated symptoms like vomiting and fever, type of feeding, source of drinking water and past history of admission to hospital. Information from medical records was collected on the duration and frequency of diarrhea, duration and frequency of vomiting, degree of fever, gender, age, geographical location and treatment.

2.2. Specimen Collection

Two hundred and eighty five stool specimens (1 - 2 ml or 1 - 2 g) were collected at the onset of the symptoms from hospitalized children aged between 6 months - 5 years at sanad hospital in Riyadh Saudi Arabia and data collection with clinical history was recorded for each child in a special form made for this reason in this study. The stool samples were collected in clean, dry, waterproof recipients containing no detergents, preservatives or transport media, and transferred to the laboratory within 6 hours following collection and stored at 2˚C - 8˚C. The test was performed within 24 hours following collection. When the specimen was not tested within 72 hours, it was frozen at −25˚C or −6˚C and to be tested later

2.3. Specimen Preparation

The stool was brought to room temperature (15°C - 30°C) before performing the test, the cap on the vial was unscrewed and the applicator stick required to collect the specimen removed. Approximately 50 mg of stools was collected (equivalent to 1/4 of a pea) using the applicator stick in the vial. The sample inserted into the vial containing the dilution buffer. In case of liquid stools, the specimen collected by using the specimen dropper and 2 drops (approximately 50 micro liters) dispensed into the vial containing the diluted buffer, then the cap was screwed onto the vial and shook vigorously to homogenize the stool suspension

2.4. Materials

VIKIA® ROTA (Biomerieux France)12) is a qualitative test based on the immunochromatographytechnique (ICT); it is a rapid test depends on the association of monoclonal antibodies specific to rotavirus . This test uses immunological reactions performed on a test strip by migration.

The test consists of:

1) A plastic device containing: a) A chromatographic membranes to which are fixed:

• In the test region, an anti-rotavirus monoclonal antibody (test region R)

• In the control region, an anti-mouse IgG polyclonal antibody (Control region C) .

b) A test strip impregnated with a conjugate consisting of monoclonal anti-rotavirus antibody polystyrene micro

spheres 2) Vial of faecal specimen dilution buffer which is ready to use, phosphate buffer (pH 7.2) + 0.9 g/l sodium azide. 3) Waterproof recipient for specimen collection. 4) Timer. Immunochromatographic test has been processed as the following: each sample was diluted in 0.5 ml of dilution buffer and let to settle down for 1 or 2 minutes. Strips have then been incubated in the faecal solution for 5 minutes and red immediately by three different observers. After drying, strips have been stick for storage.

And EIA was performed using (Immunoassay for rotavirus Rota IIkit® (Rotascreen II-Microgen-Bioproducts- Camberley, U.K.) To evaluate the detectability parameter, positive control and negative control has been sequentially diluted in respective dilution buffer. Results have been regarded as positive as far as a signal was still observed.

3. Result

Two hundred eighty five stool samples from children with acute diarrhea attend to our hospital from January 2015 until December 2016 were examined by commercial latex and immunoenzymatic assays for the diagnosis of rotavirus infection in laboratories. Sensitivity and Specificity Parameters ELISA and Latex Test Latex, specificity 98.5 % Latex sensitivity 88 % to ELISA (Table 6). Around 16% of our study population with diarrhea disease were diagnosed as ROTA (45/285) among them 89% 40/54were previous vaccinated with RTOTARIX.

Table 1. Prevalence of Rotavirus Infection

Items	No. of patients	Percentage	P value
diarrhea	285		
Rota virus	45	15.7%	>0.05

Table 2. Prevalence of Rotavirus Infection in Relation to Gender

Gender	Male	Female	Total
diarrhea	119	166	285
Rota virus	23	22	45

Table 3. Prevalence of Rotavirus Infection According to Patient's Age

Age	Diarrhea	Rota virus	Percentage
6-8 month	29	2	6.8%
8-10 moth	30	7	23.3%
10-12 month	60	10	16.6%
12-18 moth	70	10	14.2%
18-24 m	42	8	19%
2-4 years	32	6	18.7%
5 years /above	22	2	9 %
T0tal	285	45	15.7%

Table 4. Prevalence of Rotavirus Infection According to season

Year months	No of patients with Diarrhea	No of patients with Rota virus
January	29	2
February	30	6
March	30	3
April	50	5
may	20	4
June	21	3
July	12	5
August	20	1
September	20	4
October	22	3
November	21	4
December	10	5
Total	285	45

Table 5. Prevalence of Rotavirus Infection According to the Past History of Admission to Hospital

Items	No of 1 st admission	No of 2 nd admission	P value
Rotavirus	29	16	p = 0.7726

Within children diagnosed with gastroenteritis, no significant association between viral type and history of hospital admission was observed (p = 0.7726).

Table 6. Sensitivity and Specificity Parameters l ELISA and Latex Test Latex, specificity 98.5 % Latex sensitivity 88 % to ELISA

Technique	NO. of Positive	Percentage	No of Negative	Percentage
Rapid test LATEX	39	88%	235	98.5%
ELISA	45	100%	240	100%

Sensitivity = true positive/true positive +false negative
Specificity =true negative/false positive +true negative.

Table 7. Prevalence of Rotavirus Infection in rational to vaccination

Items	No. of patients	Percentage	P value
diarrhoea	285		
Rota virus	45	15.7%	>0.05
Vaccination to Rotavirus	40	88.8%	>0.001

4. Discussion

Rotavirus is the most common cause of diarrhoeal disease among infants and young children. [1] Rotavirus is usually an easily managed disease of childhood, but in 2013, rotavirus caused 37 percent of deaths of children from diarrhoea and 215,000 deaths worldwide, and almost two million more become severely ill. [2] Most of these deaths occurred in developing countries. [3]

Children five years of age or less were investigated. Our finding shows that rotavirus is most commonly detected and responsible for 15.8 % of admissions for acute gastroenteritis (Table 1). This proportion is lower than the observed in Babylon (56%) [13], Erbil (37%) [14], Baghdad (23.33%) [15], Saudi Arabia (40.7%) [16], and Shiraz in Iran (42%) [17] in our study although vaccination we found that percentage of rotavirus in childearn with gastroenteritis 88.8% , all children receive Rotrarix vaccine (Table 7) which is the same with Dilshad etal 2016 who said that A relatively high prevalence of G2P[4] strains has been observed in several countries where Rotarix is the mainly used rotavirus vaccine. A slightly lower vaccine effectiveness has been shown against G2P[4] rotaviruses when compared to Wa-like rotaviruses (G1, G3, G4 and G9) in Belgium, which may explain the relative increase in the prevalence of G2P[4] rotaviruses in Belgium [18].

Also the author reported that We show here that the vaccine introduction in Belgium is also temporarily associated with substantial changes in the genetic makeup of G2P[4] rotaviruses in terms of the large scale transmission of bovine-like NSP4 gene segments in the human rotavirus population

Also Mark Zeller 2016 reported that the sudden increase in the relative prevalence of DS-1-like rotaviruses in Belgium. And Belgian DS-1-like rotaviruses that were isolated after vaccine which contain NSP4 genes [19].

According to WHO 2004 estimates, 527 000 children aged <5 years die each year from vaccine-preventable rotavirus infections; most of these children live in low-income countries. WHO reiterates that rotavirus vaccines are an important measure that can be used to reduce severe rotavirus-associated diarrhoea and child mortality. The use of rotavirus vaccines should be part of a comprehensive strategy to control diarrhoeal diseases; this strategy should include, among other interventions, improvements in hygiene and sanitation, zinc supplementation, community-based administration of oral rehydration solution and overall improvements in case management [5].

WHO (2010) reported that, Rotavirus is highly communicable, with a small infectious dose of < 100 virus particles and is shed in high concentrations in the stools of infected children. The virus is transmitted primarily by the fecal-oral route, both through close person-to-person contact and through fomites. Rotaviruses also are likely transmitted by other modes, such as fecally contaminated food and water and possibly via respiratory droplets. infections occur from birth to old age [20].

Our study revealed that higher percentage between 8-10 months (23.3%) and 18-24 month (19%) but no significant difference between age group (Table 3) which same with WHO 2004.

Rotavirus vaccines should be part of a comprehensive strategy to control diarrhoeal diseases; this strategy should include, among other interventions, improvements in hygiene and sanitation, zinc supplementation, community-based administration of oral rehydration solution and overall improvements in case management. (WHO 2010).

Our study proved that according to medical record reporting, in the U.S., rotavirus infections usually peak in the fall months in the Southwest and spread to the Northeast by spring, so infections are most common during the winter months from November to May. However, infection with rotavirus can occur anytime of the year (CDC) [21]. Our study revealed that rotavirus were distributed all over the year (Table 4).

In a randomized, double-blind, placebo-controlled study conducted in 6 European countries, Rotarix® was observed to be highly immunogenic. Efficacy of Rotarix® against any grade of severity of rotavirus gastroenteritis through one rotavirus season was 87.1% and against severe rotavirus gastroenteritis, as defined by >=11 on the Vesikari scale, Rotarix® reduced hospitalizations for all cause gastroenteritis regardless of presumed etiology by 74.7%. The efficacy of Rotarix® against severe rotavirus gastroenteritis through two rotavirus seasons was 90.4% ,and the efficacy of Rotarix® in reducing hospitalizations through two rotavirus seasons was 96.0% ,In contrast to the results of the trial in Latin America, good efficacy against G2,P4 serotypes (85.5%, 95% CI) in preventing severe rotavirus gastroenteritis was observed in the two-year combined

follow-up results of Rotarix efficacy among children from 6 European countries35 (Vesikari T etal 2007) [22].

Also CDC report *MMWR* 2009, in developing countries with high rotavirus disease incidence, even moderate to low vaccine efficacy translates into significant numbers of severe rotavirus gastroenteritis cases prevented and into significant public health impact. More rotavirus disease burden may be prevented in developing countries despite lower vaccine efficacy than in countries with low rotavirus disease burden and higher vaccine efficacy [23].

Paulke 2013, a relatively high prevalence of G2P[4] strains has been observed in several countries where Rotarix is the mainlyused rotavirus vaccine. A slightly lower vaccine effectiveness has been shown against G2P[4] rotaviruses when compared to Wa-like rotaviruses (G1, G3, G4 and G9) in Belgium, which may explain the relative increase in the prevalence of G2P[4] rotaviruses in Belgium. We show here that the vaccine introduction in Belgium is also temporarily associated with substantial changes in the genetic makeup of G2P[4] rotaviruses in terms of the large scale transmission of bovine-like NSP4 gene segments in the human rotavirus population [27].

ALSO Dennis 2014 reported that The analysis of complete DS-1-like genomes isolated in BrazilItaly and the USA revealed the co-circulation of multiple lineages for several gene segments, some of which arelikely of animal origin. In addition, the circulation of animal-human reassortant viruses possessing an animal NSP4 gene segment has been increasingly reported [28].

WHO 2016 reported that the public health impact of rotavirus vaccination has been demonstrated in several countries. For example, in the USA, a measurable decrease was seen in the number of rotavirus gastroenteritis hospitalizations accompanied by a suggested herd effect protecting older non-vaccinated children, while in Mexico a decline of up to 50% in diarrhoeal deaths in children < 5 years of age was attributed directly to the use of the vaccine. (WHO2016) [24].

In our study 88% of ROTA cases were vaccinated.

Our study found that 88.8 % of rotavirus infected children were vaccinated with Rotrix (Table 7).

Forster etal; 2009 also reported that the detection rate of RV in stool specimens of patients with acute diarrhea was highest using ELISA versus DAT techniques. Additionally, the results revealed that ELISA technique is more sensitive and specific, and it is more suitable for large-scale screening of patients for detection of RV infection. These results are consistent with most previous studies worldwide, affirming that, although DAT are able to quickly give results, but it has lower sensitivity versus ELISA which appeared to be an optimal method for detection of RV infection [25].

Malek et al 2010 reported that Furthermore, the results of DAT and ELISA were concordant in that RV infection was higher among urban versus rural areas. Of note, similar results were obtained by other studies [14]. It seems that the high population density and better income in urban areas increase hospitalization for RV infection.

But in our study all patient from urban area as hospital in capital of KSA

5. Conclusion

Rotavirus is an infection that causes diarrhea. It's the most common cause of diarrhea in infants and children less than 5 years old worldwide, according to the Centers for CDC Rotavirus vaccines are very effective at preventing rotavirus disease, But in our study we found most of disease were vaccinated, may be only modified disease course decrease days of hospitalization for further comparative study for types of vaccination. It can be concluded that ELISA technique is the optimal method for detection of RV in stool specimens However, latex can be used as an alternative method for screening RV infection especially during outbreak season.

References

[1] Payne DC, Baggs J, Zerr DM, et al. Protective association between rotavirus vaccination and childhood seizures in the year following vaccination in US children. Clin Infect Dis 2014; 58: 173.

[2] http://www.sahealth.sa.gov.au/wps/wcm/connect/Public+Content/SA+Health+In Jun 2016.

[3] Tate, Jacqueline E.; Burton, Anthony H.; Boschi-Pinto, Cynthia; Parashar, Umesh D. (2016). "Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000-2013". *Clinical Infectious Diseases*. 62 (Suppl 2): S96–S105.

[4] Doro, R., Farkas, S. L., Martella, V. & Banyai, K. Zoonotic transmission of rotavirus: surveillance and control. *Expert Rev. Anti Infect.Ther*. 13, 1337-1350 (2015).

[5] World Health Organization (2008). "Global networks for surveillance of rotavirus gastroenteritis, 2001-2008" (PDF). *Weekly Epidemiological Record*. 83 (47): 421-428. Retrieved 3 May 2012

[6] World Health Organization, Department of Vaccines and Biologicals. "Introduction of Rotavirus Vaccines into National Immunization Programs: Management manual including operational information for health workers". Geneva 2008. introduction.

[7] http://www.mayoclinic.org/diseases-conditions/rotavirus/symptoms-causes/dxc-20186931 (2016).

[8] http://www.sahealth.sa.gov.au/wps/wcm/connect/Public+Content/SA+Health+In Jun 2016

[9] Celik, C., Gozel, M.G., Turkay, H., *et al*. (2015) Rotavirus and Adenovirus Gastroenteritis: Time Series Analysis. *Pediatrics International*, 57, 590-596.

[10] CDC August 12, 2016 Content source: National Center for Immunization and Respiratory Diseases, Division of Viral Diseases.

[11] Rosillon D, Buyse H, Friedland LR, Ng SP, Velázquez FR, Breuer T. 2015Risk of Intussusception After Rotavirus Vaccination: Meta-analysis of Postlicensure Studies. Pediatr Infect Dis J. 2015 Jul; 34(7): 763-8.

[12] www.biomerieux-diagnostics.com).

[13] Dennehy PH (2015). "Rotavirus Infection: A Disease of the Past?". *Infectious Disease Clinics of North America*. 29 (4): 617-35.

[14] Simpson E, Wittet S, Bonilla J, Gamazina K, Cooley L, Winkler JL (2007). "Use of formative research in developing a knowledge translation approach to rotavirus vaccine introduction in developing countries". *BMC Public Health*. 7: 281.

[15] Al-Marzoqi, A.H., Shemmran, A.R. and Al-Nafee', M.K. (2010). Role of Rotavirus and Adenovirus in Acute Infantile Gastroenteritis in Infants Younger than One Year of Age in Babylon Province. *Journal of Al-Qadisiyah for PureScience*, 15, 1-7.

[16] Khalil, M., Azhar, E. and Kao, M. (2015) Gastroenteritis Attributable to Rotavirus in Hospitalized Saudi ArabianChildren in the Period 2007-2008. *Clinical Epidemiology*, 7, 129-137.

[17] Motamedifar, M., Amini, E. and Shirazi, P.T. (2013) Frequency of Rotavirus and Adenovirus Gastroenteritis amongChildren in Shiraz, Iran. *Iranian Red Crescent Medical Journal*, 15, 729-733.

[18] Dilshad O. Jaff1, Tariq A. G. Aziz2, Natalie R. Smith3: 2016 The Incidence of Rotavirus andAdenovirus Infections among Children with Diarrhea in Sulaimani Province, Iraq; Journal of Biosciences and Medicines, 2016, 4, 124-131.

[19] Mark Zeller1, Valerie Nuyts1, Elisabeth Heylen1, Sarah De Coster1, Nádia Conceição-Neto1,2,, 2016: Emergence of human G2P[4] rotaviruses containing animal derived gene segments in the post-vaccine era; *Journal of virology* 84, Published: 14 November 2016.

[20] World Health Organization, Department of Vaccines and Biologicals. "Introduction of Rotavirus Vaccines into National Immunization Programs: Management manualincluding operational information for health workers". Geneva 2010.

[21] CDC http://www.who.int/immunization/topics/rotavirus/en/ 2010.

[22] Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomized, double-blind controlled study. *Lancet* 2007; 370: 1757.

[23] Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 2009; 58: 1-26.

[24] WHO Organization, W. H. Vaccine Preventable Diseases Surveillance. Global Rotavirus Surveillance and Information Bulletin. Vol. 10, Data period 2013 http://www.who.int/immunization/monitoring_surveillance/resources/WHO_Global_RV_Surv_Bulletin_Jan_2015_Final.pdf (2016).

[25] Forster, J.; Guarino, A.; Parez, N.; Moraga, F.; Roman, E.; Mory, O.; Tozzi, A.E.; de Aguileta, A.L.; wahn, U.; Graham, C.; Berner, R.; Ninan, T.; Barberousse, C.; Meyer, N. and Soriano-Gabarro, M. The rotavirus study group. Hospital-based surveillance to estimate the burden of rotavirus gastroenteritis among European children younger than 5 years of age. Pediatrics, 2009; 123 (3): 393-400.

[26] Malek, M.A.; Teleb, N.; Abu-Elyazeed,R.; Riddle, M.S.; Sherif, M.E.; Steele, A.D.; Glass, R.I. and Bresee, J.S. The epidemiology of rotavirus diarrhea in countries in the Eastern Mediterranean Region. J. infect. Dis. 2010; 202 Suppl: S12-22.

[27] Paulke-Korinek, M. *et al.* Sustained low hospitalization rates after four years of rotavirus mass vaccination in Austria. *Vaccine* 31, 2686-2691.

[28] Dennis, A. F. *et al.* Molecular epidemiology of contemporary G2P[4] human rotaviruses cocirculating in a single U.S. community: footprints of a globally transitioning genotype. *Journal of virology* 88, 3789-3801, (2014).