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Detection of Rotavirus in children with acute gastroenteritis in Zagazig University Hospitals in Egypt

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Abstract

Introduction: Rotavirus is the major cause of acute gastroenteritis (AGE) in infants and young children all over the world. The objective of the study was to compare different methods for detecting rotavirus and to assess the burden of rotavirus as a causative agent for AGE in children younger than five.

Methods: This case control study included 65 children with AGE and 35 healthy control children. They were chosen from the Pediatric Department of Zagazig University Hospitals from October 2014 to March 2015. Stool samples were obtained and assayed for rotavirus by the immunochromatography test (ICT), enzyme linked immunosorbent assay (ELISA) and quantitative real time RT-PCR (qr RT-PCR).

Results: Fifty out of the 65 patients (76.9%) were positive for qr RT-PCR. Forty-five (69.2%) and 44 (67.7%) were positive for ICT and ELISA, respectively. There was a significant association between the severity of the disease as determined by the Vesikari score and rotavirus infection.

Conclusion: This study demonstrated that ICT is a useful method for the rapid screening of group A rotavirus in fecal specimens, because it is rapid, inexpensive, easy to perform, and requires very little equipment. In addition, this study highlights the substantial health burden of rotavirus AGE among children less than five.

Keywords: rotavirus, acute gastroenteritis, Vesikari score, quantitative real time RT-PCR (qr RT-PCR)

1. Introduction

1.1. Background and the problem

Diarrheal disease is one of the most important worldwide causes of morbidity and mortality, accounting for an estimated 1.3 million deaths in children under five (1). Rotavirus is the major cause of acute gastroenteritis and severe dehydrating diarrhea in young children (2). The majority of morbidity and mortality caused by rotavirus gastroenteritis is experienced by children under five in developing countries. Rotavirus causes the deaths of approximately 453,000 children annually, and most of them occur in developing countries in Africa and South Asia (3). Approximately 40% of hospitalizations for diarrheal illness that occur among children under five in developing countries are due to rotavirus (4, 5). Clinically, rotavirus gastroenteritis is characterized by profuse diarrhea, mild fever, and vomiting, leading to mild to severe dehydration. However, the clinical manifestations of rotavirus diarrhea alone are not sufficiently distinctive to permit diagnosis (6), and laboratory testing is the only way to confirm the diagnosis (7). Most intractable diarrheas are treated with antibiotics, irrespective of the causative agent. If infection due to rotavirus can be diagnosed early, the misuse of antibiotics can be avoided (8). Etiological diagnosis may not be essential in the treatment of individual patients, but the knowledge of the relative importance and seasonal prevalence of different pathogens in different regions is essential for proper management of outbreaks and for the planning and implementation of control measures (8). Several techniques have been developed to detect rotavirus in stool samples, including electron microscopy, polyacrylamide gel electrophoresis of viral nucleic acid, various immuno assays, and PCR-based molecular methods (9).

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Several rapid methods, such as the immunochromatographic test and the latex agglutination test, have been evaluated and compared to other methods, such as ELISA and quantitative real time RT- PCR (qr RT-PCR), and they have shown a wide range of sensitivity and specificity (10). The performance of those rapid tests indicated that they could be used for screening but not for confirmation of the disease. Therefore, a highly specific and sensitive test is a pressing need for use in confirming rotavirus infections (11).

1.2. Objectives

This aim of this study was to compare different methods for detecting rotavirus and correlating the Vesikari Clinical Severity Scoring System with viral load. The specific objectives of the study were as follows:

1) To detect rotavirus in stool samples using immunochromatographic tests (ICT)
2) To detect rotavirus in stool samples using the ELISA technique
3) To detect rotavirus in stool samples using Quantitative Real time RT-PCR (qr RT-PCR)
4) To compare the three different methods for detecting rotavirus
5) To correlate the Vesikari Clinical Severity Scoring System with viral load

2. Material and Methods

2.1. Study setting

This work was conducted in the Microbiology & Immunology Department and the Pediatric Department, Faculty of Medicine, Zagazig University and Hospitals from October 2014 to March 2015.

2.2. Subjects

1) Patient group: Sixty-five children less than five years old (2.3 ± 0.9 years) with AGE, who were admitted in to the Pediatric Hospital or treated in the Emergency Department, were included in the study. We obtained the Institutional Review Board's approval for the study before it was initiated. The study included 37 males and 28 females. The exclusion was chronic diarrhea, which was defined as diarrhea that lasted for more than two weeks. Written informed consent for participation was obtained from the parents/guardians of the children.

2) Control group: The control group consisted of 35 healthy children who were free from diarrhea, vomiting, and fever. It included 21 males and 14 females whose ages were less than five (2.6 ± 0.8). The patient group and the control group were almost matched in age and gender.

2.3. Data collection and assessment

2.3.1. Sample collection

Fresh stool samples were obtained within 24 to 48 h of admission. Sterile, wide-necked plastic containers were used to collect and transport the samples.

2.3.2. Processing of the samples

Macroscopic examination of the samples was conducted as follows: color, consistency (formed/semiformed/liquid), presence of blood, presence of mucus, presence of segments and/or worms. The labeled stool samples were divided into separate aliquots for the detection of rotavirus antigen and nucleic acid and stored at -20 °C and -80 °C, respectively, until they were assayed.

2.3.3. Assessment of Severity

The clinical severity of the disease was assessed by a pediatrician who examined the patients and interviewed the patients' parents/guardians. The pediatrician used the 20-point Vesikari scale (12) to assess the severity of the disease. This scale is based on the frequency and severity of diarrhea, episodes of vomiting, associated fever, and dehydration.

2.3.4. Detection of rotavirus by immunochromatography test ICT

ICT RIDA® Quick Rotavirus kits (R-Biopharm, Germany) were used to detect rotavirus antigen in the stool samples. This test is a single-step, immunochromatographic lateral-flow test, and it was conducted according to the manufacturer's instructions (13).

2.3.5. Interpretation of the ICT results

Two bands should appear to indicate Rotavirus positive; the red band and the blue band are visible. If only the blue band is visible, it is rotavirus negative. If the blue band is missing, the test is invalid.

2.3.6. Detection of rotavirus by ELISA

RIDASCREEN® kit (R- Biopharm, Germany) was used to detect rotavirus antigen in stool samples. In this test, monoclonal antibodies against the product of the sixth viral gene (VP6) were used in a sandwich-type method. The assay was conducted according to the manufacturer's instructions (14).

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2.3.7. Detection of rotavirus by qr RT-PCR (11):
1) RNA extraction: Preparation of the sample: 0.5-1.0 ml of each stool sample was suspended in up to 5 ml of saline (i.e., up to 1:10 dilution) and mixed by brief vortexing. The solution was clarified by centrifugation for 20 min at 5000 × g. Then, 140 µl of the supernatant was used as the starting material for RNA following the Viral RNA Mini Spin Protocol (Quiagen, USA). RNA was extracted using the QIAamp® Viral RNA Mini Kit, which is commercially available. Extraction was done automatically using the QIAcube instrument according to the manufacturer's instructions. To determine the efficiency of the extraction protocol in removing the high levels of PCR inhibitors present in the stool samples, internal extraction control was introduced to the samples following stool clarification and carried through the preparation, amplification, and detection protocols of the specimen.
2) Real time RT-PCR: In this study, the one step qr RT-PCR approach was used, which combines the reverse transcription and real-time PCR reaction in a single closed tube. The PrimerDesign™ genesig Kit for Rotavirus A (Primer Design, Ltd., UK) was used for quantification of Rotavirus A genome using specific primer and probe mix for Rotavirus A that was detected through the FAM channel.

To determine the copy number and as a positive control for the PCR setup, a positive control template was used to generate a standard curve of Rotavirus A copy number/cycle threshold value (Ct value). The Ct value is inversely proportional to the viral load. To confirm the absence of contamination, a negative control reaction was included every time the kit was used. For this reaction, the RNAse/DNAse free water was used instead of template. A separate real-time PCR primer/probe mix was used to detect the internal RNA extraction control. The internal control was detected through the VIC channel. To confirm extraction of a valid biological template, a primer and probe mix was used to detect the Actin Beta (ACTB) gene for the selected sample. Detection of ACTB is through the FAM channel, so it was not possible to perform a multiplex for ACTB and the pathogen primers, and it was performed in a separate tube.

3. Results
This case control study was conducted on two groups, the patient group which included 65 patients with AGE and the control group which included 35 healthy children. Rotavirus was detected in 50 (76.9%) out of the 65 patients by qr RT-PCR followed by 45 (69.2%) by ICT and 44 (67.7%) by ELISA. In this study, the children within the age group of 6-12 months had the highest rate of rotavirus infection with 27 (54%), while those within the age group of 0-6 months had the least with 2 (4%). However, children within the age group of 12-24 months had the highest rotavirus negative rate, with 5 (33.3%), and those in the age group of 0-12 months had the least with 3 (20%). The data were studied statistically, and a statistically significant association was found. However, concerning gender, the incidence of positive tests for rotavirus was similar in males and females. Table 1 shows that there was a significant difference between rotavirus positive patients and rotavirus negative patients regarding vomiting, the duration of diarrhea, the severity of dehydration, and the requirement for IV rehydration. Table 2 shows that 28 (56.0%) of rotavirus positive cases had severe scores, while 16 (32%) had moderate scores, and 6 (12%) had mild scores. However, six (40%) of rotavirus negative cases had moderate scores and another six (40%) had mild scores, while only 3 (20%) had severe scores. When these data were studied statistically, we found a statistically significant association between the severity of the illness and rotavirus infection. In other words, cases with rotavirus infection were suffering from more severe illness than rotavirus negative cases.

Table 1. The spectrum of clinical manifestations among rotavirus positive and negative patients.

Clinical manifestations		Rotavirus negative patients (n=15)	Rotavirus positive patients (n=50)	t/X²	P
Fever		12 (80.0%)	42 (84.0%)	0.13	0.72
Vomiting		11 (73.3%)	46 (92.0%)	3.73	0.054
Duration of diarrhea (days)		4.6±0.9	5.5±1.5	2.15	0.04
Frequency of diarrhea (per day)		4.9±0.9	5.3±1.3	1.32	0.19
Degree of Dehydration	No dehydration	7	12	1.1	0.15
	Mild to Moderate	7	32	2.3	0.045
	Severe	2	6	2.5	0.04
Treatment received	Oral rehydration	12	22	0.8	0.65
	IV rehydration	3	28	3.2	0.03

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Table 2. Relation between rotavirus infection and Vesikari score

Severity (Vesikari scale)	Rotavirus negative (n=15)	Rotavirus positive (n=50)	X^2	P
Mild	6 (40.0%)	6 (12.0%)	0.04	0.02
Moderate	6 (40.0%)	16 (32.0%)		
Sever	3 (20.0%)	28 (56.0%)		

The severity of diarrhea, as determined by the Vesikari score, was significantly and negatively associated with the PCR Ct value ($P < 0.05$), indicating that the children with severe diarrhea excreted more virus than children with less severe disease. According to the results in Table 3, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using qr RT-PCR as a standard. Regarding ICT, these values were found to be 90, 100, 100, and 75%, respectively. For ELISA, these values were 88, 100, 100, and 71%, respectively. The results of the qr RT-PCR are shown in Figure 1. The intensity of the fluorescence was plotted against the PCR cycle number, and the intersection between the amplification curve and the threshold line is the Ct (threshold cycle), which is a measure of the concentration of the target rotavirus in the PCR reaction. Different colors represent different samples with different concentrations of nucleic acid. The CT value was plotted against the rotavirus copy number (quantity). Guided by the known copy number of the standards, the copy number of rotavirus in the unknown samples were determined.

Table 3. Comparison of the results obtained by qrRT-PCR, ICT and ELISA

Method		qr RT-PCR		Total
		Positive	Negative	
ICT	Positive	45	0	45
	Negative	5	15	20
ELISA	Positive	44	0	44
	Negative	6	15	21

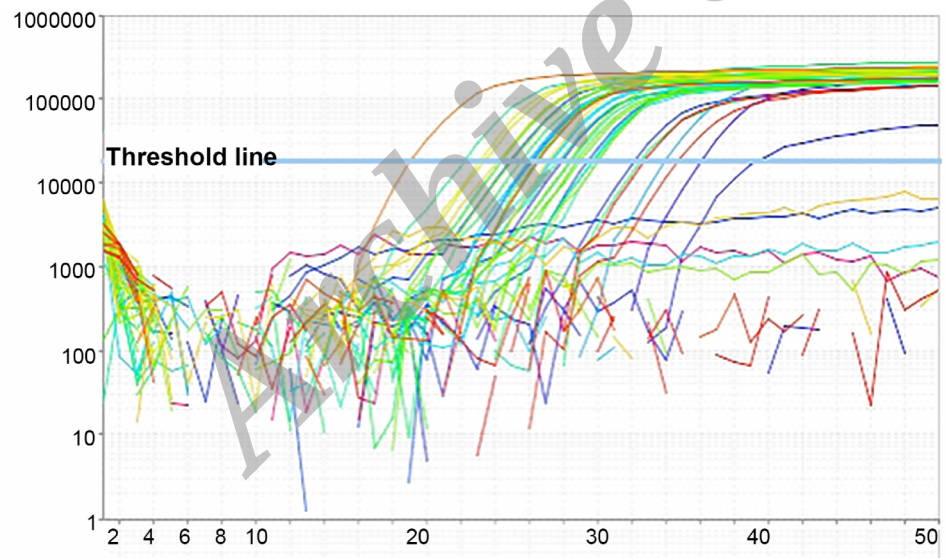

Figure 1. Amplification Plot (Intensity of flourescence vs. Cycle) for rotavirus

4. Discussion

In this study, we compared AGE patients and children in a control group regarding the detection of rotavirus by three different methods, i.e., ICT, ELISA, and qr RT-PCR. Fifty cases from a total of 65 cases (76.9%) had rotavirus in their stools by qr RT-PCR. These results were in good agreement with the results of Vainio et al. (15), who found that 72% of the children with diarrhea enrolled in their study from March 2006 to February 2008 had rotavirus in their stools by RT-PCR. Similarly, in a study conducted by Manjula (16), rotavirus accounted for 64% of the hospitalizations of children with acute diarrheal illness. However, a lower percentage was obtained by Ahmed et al. (17), who found that 40% of the cohort included in the study in Abu Homos, Beheira Governorate, Egypt, suffered

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at least one rotavirus diarrhea episode during the study period, which extended from January 2004 through April 2007. However, they reported higher frequencies (65%) in children who were less than one year old than in older children. Furthermore, rotavirus-associated diarrhea peaked from the late summer (September) to the late fall (November), similar to the trend reported in a population-based cohort study conducted by Naficy et al. (18) in Abu Homos, Egypt, during 1995-1998. In that study 90% of rotavirus diarrheal episodes occurred between July and November. However, in a much earlier study conducted in Bab El-Sha'reya University Hospital in Cairo, Egypt, by El-Mougi et al. (19), rotavirus infection peaked from September to March. The high frequency reported in our study may be explained by the fact that it was conducted from October 2014 to March 2015, and these months fall in the period of high prevalence of rotavirus. However, in a systematic review of studies conducted by Malek et al. (20) concerning the epidemiology of rotavirus diarrhea in countries in the eastern Mediterranean region, the studies demonstrated occurrences of rotavirus diarrheal episodes year-round and indicated that seasonal fluctuations were less significant in some countries, such as Egypt and Iran. This difference in seasonality was explained by Mwenda et al. (21) by the difference in the climatic conditions. There were no positive cases detected among the control group by any of the tested methods. However, the detection of rotavirus in the control group varies widely between studies (22-24). Nevertheless, because the above studies are from industrialized countries in which rotavirus vaccinations are routine and vaccine virus was detected in some of the healthy controls, these findings might not apply to developing countries in which the severity of infection, rates of asymptomatic viral shedding, and performance of the EIA may differ (24). A Ct cut-off value of 24 was proposed by Phillips et al. (25) by comparing real-time PCR results to ELISA results in cases and asymptomatic controls for interpretation of real-time PCR results and relation to clinical symptoms. Relating the Ct-value, as a quantification of viral load, to the severity of clinical symptoms to set up useful cut-off values seems to be an adequate approach to improve the interpretation of real-time PCR results. This method should solve an emerging clinical problem in the era of increased use of molecular diagnostic tools. However, the cut-off values mentioned above are not directly applicable to local real-time PCR results, because every PCR assay has different performance, and a local distribution and quantification of viruses can alter cut-off values (26). In this study, children within the age group of 6-12 months had the highest rate of rotavirus infection. Similar results were reported by Enweronu-Laryea et al. (27) and Manjula (16). However, in this study, children within the age group of 0-6 months had the lowest rate of rotavirus infection. This was in agreement with the results of Junaid et al. (28). This low incidence can be attributed to passive immunity acquired by the infants from their mothers, which wanes after six months. It also is possible that the higher rate of breast feeding in this age group protects the infants via passing of IgA anti-rotavirus antibodies to the infants. In this study, there was no statistically significant association between gender and the occurrence of rotavirus infection. These results were in good agreement with the results of Manjula (16). However, Nguyen et al. (29) findings indicated that males were affected predominantly, and Junaidet al. (28) found that males were twice as susceptible as females. In the current study, the children with rotavirus infections had infections that were more severe than the children who were rotavirus negative. These results were comparable to the results of Forster et al. (30), who found that AGE was more severe in rotavirus-positive subjects. In this study, there was a statistically significant negative association between the Vesikari score and PCR Ct value ($P < 0.05$), similar to the findings of other studies (31, 32), indicating that children with severe disease excrete more virus than children with less severe disease. Regarding the comparison between results obtained by qr RT-PCR, ICT, and ELISA, our results agreed with those of other research (33). The authors concluded that ICT kits could be used as an alternative method for the rapid screening of "group A" rotavirus in fecal specimens, especially during acute outbreaks of gastroenteritis.

5. Conclusions

This study demonstrated that ICT is a useful method for the rapid screening of group A rotavirus in fecal specimens, because it is quick, inexpensive, easy to perform, and requires very little equipment. In addition, this study highlighted the substantial health burden of rotavirus AGE among children less than five. This study provided data for assessing the potential benefits of introducing a rotavirus vaccine.

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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References

1) Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T and Liu L. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet; 2010, 375:1969–87. doi: 10.1016/S0140-6736(10)60549-1
2) Zuccotti G, Meneghin F, Dilillo D, Romanò L, Bottone R, Mantegazza C, Giacchino R, Besana R, Ricciardi G, Sterpa A, Altamura N, Andreotti M, Montrasio G, Macchi L, Pavan A, Paladini S, Zanetti A and Radaelli G. Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. BMC Infect. Dis.; 2010, 10:218-26. doi: 10.1186/1471-2334-10-218, PMid: 20649961 PMCid: PMC2918608
3) Tate JE , Burton AH, Boschi-Pinto C, Steele D, Duque J and Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. The Lancet Infectious Diseases; 2012, 12(2): 136-41. doi: 10.1016/S1473-3099(11)70253-5
4) Parashar UD, Alexander JP and Glass RI. Prevention of rotavirus gastroenteritis among infants and children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep.; 2006, 55(12): 1-13. PMid: 16902398
5) World Health Organization. Generic protocol for monitoring impact of rotavirus vaccination on gastroenteritis disease burden and viral strains. Geneva, Switzerland. 2008m Available from: http://www.who.int/vaccines-documents
6) Bass ES, Pappano DA and Humiston SG. Rotavirus. Pediatr Rev.; 2007, 28: 183-91. Doi: 10.1542/pir.28-5-183, PMid: 17473123
7) Bernstein DI, Ward RL. Rotaviruses. In: Feigin RD, Cherry JD, eds. Textbook of Pediatric Infectious Diseases. 5th ed. 2004, Vol 2. Philadelphia, PA: Saunders; 2110-33.
8) Chavan SC, Agarkhedkar S, Chavan DS, Nagdawane RP and Singhania S. Prevalence of rotavirus diarrhoea among children hospitalized in a tertiary care hospital in Western India. Int J Pharm Biomed Sci.; 2013, 4(1): 4-7.
9) Logan C, O'Leary JJ and O'Sullivan N. Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. J. Clin. Microbiol.; 2006, 44:3189-95. doi: 10.1128/JCM.00915-06, PMid: 16954246 PMCid: PMC1594742
10) Ferreira T, Becho MC, Bernardo AR, Chaves TCB, Ribeiro RS and de Lima JS. Performance of A Latex Agglutination Test in The Diagnosis of Acute Gastroenteritis by Rotavirus. Brazilian J. Microbiol.; 2006, 37:587-9. doi: 10.1590/S1517-83822006000400035
11) Yasmon A, Harahap ED, Dwipoerwantoro PG and Dewi BE. Detection of human group A and C Rotaviruses in pediatric patients with acute gastroenteritis by real time RT-PCR assay: A preliminary study. Makara Kesehatan; 2010, 14(2): 65-9.
12) Clark HF, Bernstein DI, Dennehy PH, Offit P, Pichichero M, reanor J and Heaton P. Safety, efficacy, and immunogenicity of a live, quadrivalent human-bovine reassortant rotavirus vaccine in healthy infants. J. Pediatr.; 2004, 144(2): 184-90. doi: 10.1016/j.jpeds.2003.10.054, PMid:14760258
13) Weitzel T, Reither K, Mockenhaupt FP, Stark K, Ignatius R, Saad E, Seidu-Korkor A, Bienzle U and Schreier E. Field Evaluation of a Rota- and Adenovirus Immunochromatographic Assay Using Stool Samples from Children with Acute Diarrhea in Ghana. J Clin Microbiol.; 2007, 45(8): 2695-7. doi: 10.1128/jcm.00562-07
14) Eing BR, May G, Baumeister HG and Kühn JE. Evaluation of Two Enzyme Immunoassays for Detection of Human Rotaviruses in Fecal Specimens. J Clin Microbiol.; 2001, 39 (12): 4532-4. doi: 10.1128/jcm.39.12.4532-4534.2001
15) Vainio K, Nordbø SA, Njølstad G, Størvold G, Døllner H, Midgaard C, Bosse FJ, Rognlien AG, Rojahn A, Wathne KO and Flem E. Detection and characterization of group A rotaviruses in children hospitalized with acute gastroenteritis in Norway, 2006–2008.Journal of Medical Virology; 2009, 81(10): 1839-44. doi: 10.1002/jmv.21576, PMid: 19697411
16) Manjula G. Comparison of Immunochromatography with RT-PCR for Detection of Rotavirus in Fecal Samples. International Journal Of Scientific Research; 2013, 2:479-81. doi: 10.15373/22778179/DEC2013/150
17) Ahmed SF, Mansour AM, Klena JD, Husain TS, Hassan KA, Mohamed F and Steele D. Rotavirus Genotypes Associated with Acute Diarrhea in Egyptian Infants. Pediatric Infectious Disease Journal; 2014, 33:62-8. doi: 10.1097/INF.0000000000000052, PMid: 24343617
18) Naficy AB, Abu-Elyazeed R, Holmes JL, Rao MR, Savarino SJ, Kim Y, Wierzba TF, Peruski L, Lee YJ, Gentsch JR, Glass RI and Clemens JD. Epidemiology of rotavirus diarrhea in Egyptian children and

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implications for disease control. Am J Epidemiol.; 1999, 150:770-7. doi: 10.1093/oxfordjournals.aje.a010080, PMid: 10512431
19) El-Mougi M, Amer A, El-Abhar A, Hughes J and El-ShafieA. Epidemiological and clinical features of rotavirus associated acute infantile diarrhoea in Cairo, Egypt. J Trop Pediatr.; 1989, 35:230-3. doi: 10.1093/tropej/35.5.230, PMid: 2585579
20) Malek MA, Teleb N, Abu-Elyazeed R, Riddle MS, El Sherif 1M, Steele AD, Glass RI and Bresee JS. The Epidemiology of Rotavirus Diarrhea in Countries in the Eastern Mediterranean Region. J Infect Dis.; 2010, 202(1): S12-S22. doi: 10.1086/653579, PMid: 20684691
21) Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J, Kisakye A, Mpabalwani EM, Pazvakavambwa I, Armah GE , Seheri LM, Kiulia NM, Page N, Widdowson MA and Steele AD. Burden and Epidemiology of Rotavirus Diarrhea in Selected African Countries: Preliminary Results from the African Rotavirus Surveillance Network. J Infect Dis.; 2010, 202(1): 5-11. doi: 10.1086/653557, PMid: 20684718
22) Amar CFL, East CL, Gray J, Iturriza-Gomara M, Maclure EA, Mc Lauchlin J. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control infectious intestinal disease study (1993–1996). Eur J ClinMicrobiol Infect Dis.; 2007, 26:311-23. doi: 10.1007/s10096-007-0290-8, PMid: 17447091
23) Payne DC, Staat MA, Edwards KM, Szilagyi PG, Gentsch JR, Stockman LJ, Curns AT, Griffin M, Weinberg GA, Hall CB, Fairbrother G, Alexander J and Parashar UD. Active, population-based surveillance for severe rotavirus gastroenteritis in children in the United States. Pediatrics; 2008, 122(6):1235-43. doi: 10.1542/peds.2007-3378, PMid: 19047240
24) Tate JE, Rustempasic SM, Tam KI, Lyde FC, Payne DC, Szilagyi P, Edwards K, Staat MA, Weinberg GA, Hall CB, Chappell J, McNeal M, Gentsch JR, Bowen MD and Parashar UD. Comparison of 2 Assays for Diagnosing Rotavirus and Evaluating Vaccine Effectiveness in Children with Gastroenteritis. Emerg Infect Dis; 2013, 19(8): 1245-52. doi: 10.3201/eid1908.130461, PMid: 23876518 PMCid: PMC3739503
25) Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D, Gray J. Diagnosing rotavirus A associated IID: Using ELISA to identify a cut-off for real time RT-PCR. J Clin Virol.; 2009, 44(3):242-5. doi: 10.1016/j.jcv.2008.12.001
26) Corcoran MS, van Well GT and van Loo IH. Diagnosis of viral gastroenteritis in children: interpretation of real-time PCR results and relation to clinical symptoms. Eur J Clin Microbiol Infect Dis; 2014, doi: 10.1007/s10096-014-2135-6
27) Enweronu-Laryea CC, Sagoe KW, Addy HG, Asmah RH, Mingle JA and Armah GE. Prevalence of severe acute rotavirus gastroenteritis and intussusceptions in Ghanaian children under 5 years of age. J Infect Dev Ctries.; 2012, 6(2):148-55. doi: 10.3855/jidc.1667
28) Junaid SA, Umeh C, Olabode AO and Banda JM. Incidence of rotavirus infection in children with gastroenteritis attending Jos university teaching hospital, Nigeria. Virology Journal; 2011, 8:233-40. doi: 10.1186/1743-422X-8-233, PMid: 21575246 PMCid: PMC3107812
29) Nguyen TV, Le Van P, Le Huy C and Weintraub A. Diarrhea Caused by Rotavirus in Children Less than 5 Years of Age in Hanoi, Vietnam. J ClinMicrobiol.; 2004, 42(12): 5745-50. doi: 10.1128/jcm.42.12.5745-5750.2004
30) Forster J, Guarino A, Parez N, Moraga F, Román E, Mory O, Tozzi A E., Aguileta A, Wahn U, Graham C, Berner R, Ninan T, Barberousse C, Meyer N and Gabarró MS. Hospital-Based Surveillance to Estimate the Burden of Rotavirus Gastroenteritis among European children younger than 5 years of age. Pediatrics; 2009, 123(3): e393–e400. doi: 10.1542/peds.2008-2088
31) Kang G, Iturriza-Gomara M, Wheeler JG, Crystal P, Monica B, Ramani S, Primrose B, Moses PD, Gallimore CI, Brown DW and Gray J. Quantitation of Group A Rotavirus by Real-Time Reverse-Transcription-Polymerase Chain Reaction Correlation With Clinical Severity in Children in South India. J Med Virol.; 2004, 73(1): 118–122. doi: 10.1002/jmv.20053, PMid: 15042658 PMCid: PMC2459214
32) Wolffs PF, Bruggeman CA, van Well GT and van Loo IH. Replacing Traditional Diagnostics of Fecal Viral Pathogens by a Comprehensive Panel of Real-Time PCRs. J. Clin. Microbiol.; 2011, 49 (5): 1926-31. doi: 10.1128/JCM.01925-10, PMid: 21430103 PMCid: PMC3122640
33) Wilhelmi I, Colomina J, Martín-Rodrigo D, Roman E, Sánchez-Fauquier A. New immunochromatographic method for rapid detection of rotaviruses in stool samples compared with standard enzyme immunoassay and latex agglutination techniques. Eur J Clin Microbiol Infect Dis.; 2001, 20(10):741-3. doi: 10.1007/s100960100575

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