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Prevalence of Rotavirus among Children in Baghdad, Iraq, detected by molecular methods

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Keywords: Rotavirus; children; molecular diagnosis; VP7 gene; Baghdad
Parole chiave: Rotavirus; bambini; diagnosi molecolare; gene VP7; Baghdad

Abstract

Background. *Rotavirus, a leading cause of severe gastroenteritis in young children, has a significant global impact due to its high prevalence and potential for causing severe dehydration.*
Study design. *This study was conducted to determine the prevalence of rotavirus among children under five years old in Baghdad, Iraq using molecular technique.*
Methods. *Between November 2022 and August 2023, 120 stool specimens were collected from children exhibiting symptoms of diarrhea at a pediatric hospital. Rotavirus infection was assessed using immunochromatographic test and reverse transcriptase-polymerase chain reaction to detect and characterize the VP7 gene, a key marker of rotavirus infection.*
Results. *It was observed that 97 out of 120 specimens tested positive for rotavirus, with immunochromatographic test detecting 110 cases (91.8%) and reverse transcriptase-polymerase chain reaction identifying 97 cases (80.8%) positive for the VP7 gene. The highest infection rates were observed in males (63.92%) and children aged 13-24 months (50.5%). Statistical analysis revealed an 80.8% overall prevalence of rotavirus among the study population.*
Conclusions. *These findings underscore the significant burden of rotavirus infection in Baghdad and highlight the effectiveness of reverse transcriptase-polymerase chain reaction in detecting rotavirus strains. The results align with previous studies in Iraq, emphasizing the need for continued surveillance and vaccination efforts to control rotavirus-related diarrhea and reduce its impact on young children in the region.*

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Introduction

Rotavirus is a double-stranded RNA (dsRNA) virus, non-enveloped, and classified into eight types (A-G) (1). Its genome consists of eleven dsRNA segments that encode six viral proteins (VP1–VP4, VP6, and VP7). Among these, VP4 and VP7 are responsible for viral attachment and neutralization of antigens (2). VP4 undergoes proteolytic cleavage at the tip of VP5, producing two fragments: VP8 and VP5. VP8 and VP6 form trimers that bind to VP5 in a circular arrangement, while VP7 and VP4 trimers attach to the VP8 and VP6 trimers on the outer shell (3).

The virus particle, a three-layered coated icosahedral particle, consists of two outer shell proteins i.e., VP4 (represents P genotypes) and VP7 (represents G-genotypes), one middle shell protein (VP6) and (VP2) is the innermost protein. Based on VP4 and VP7 variants, Rotavirus A is classified into 27 G- and 35 P- genotypes. The most significant Rotavirus types that commonly causes AGE are G2P-4, G4P-8, G1P-8, G3P-8, and G9P-8 (4).

Globally, the rotavirus strains G4P-8, G3P-8, G2P-4 G1P-8 and G9P-8 are recognized as the most significant sources of human infection, with G1P-8 being the most prevalent, accounting for 37.7% of cases (5-6).

Rotavirus A (RVA) is one of the most prevalent causes of diarrhea in infants and young children globally, particularly in severe cases that can lead to fatal dehydration. RVA from Group A is a leading cause of acute dehydrating diarrhea in humans. It exhibits wide genetic and antigenic diversity, with several human strains sharing similarities with RVA strains found in animals (7). Vaccinating a child with a serious infection is crucial because it provides active immunity once maternal antibodies wane, typically between four and six months of age. As of 2019, many countries have included this vaccine in their national immunization programs. Active immunity from vaccination is particularly important during the first few years of life, as the risk of severe infections, leading to hospitalization and death, is highest between 4 to 6 months. Rotavirus infection and replication occur in mature enterocytes and endocrine cells in the small intestine, specifically in non-dividing enterocytes located at the tips and middle of the villi. This suggests that certain factors expressed by these cell types are crucial for efficient viral replication and infection (8).

This study aimed to determine the prevalence of rotavirus infection among children in Baghdad City, Iraq, and to utilize molecular detection technique to identify and characterize the circulating rotavirus strains.

Materials and Methods

A cross sectional study was conducted at at a pediatric hospital and Baghdad teaching hospital. A total of 120 children presented acute diarrhea have been collected at time of study application during the period between November 2022 and August 2023. Information including age, sex, residence, type of feeding, and clinical features (diarrhea, fever, vomiting and degree of dehydration) were taken according to the WHO criteria. The objectives and methodology of this study were explained to all parents or guardians of the patients in the study to gain their verbal consent. All vaccinated children presented with acute diarrhea from the age of 6 months to the age of 5 years were included in this study,except for the following exclusions:

1. Infant younger than 6 months.
2. Children older than 5 years.
3. Hypersensitivity to (Rotarix®)vaccine.
4. Gastrointestinal Tract Congenital Malformation.
5. History of Intussusception.
6. Severe Combined Immunodeficiency Disease.

Collection of stool specimen

At a pediatric hospital in Baghdad City, 120 stool specimens were collected from young children with diarrhea between November 2022 and August 2023. All samples were collected and analyzed under aseptic conditions.

Sample preparation

In order to analyze the specimens, each of the frozen sample was totally thawed, then 1 g was weighed and diluted in 9 ml of phosphate buffer saline (1 : 10). Themixture was vortexed vigorously for 30 s followed by centrifugation at 5000 rpm for 10 min at room temperature. The supernatant was collected forthe molecular assay, samples were held in a clean Eppendorf tube andkept at −20°C until further.

Detection of rotavirus by immune chromategraphic test

Lumi Quick, Adeno-Rota Virus Antigen Comb Test Card Immuno Chromatography (IC)

This method is rapid for the qualitative detection of RV in stool samples. According to the company,

the chromatographic immunoassay was the first instrument for detecting Rotavirus in stool samples. The sample was added to a dilution buffer supplied by the kit and thoroughly mixed, then four drops were added to the sample well in the test cassette. The result was available after 5–10 min. Two bands should appear to indicate rotavirus positive; the control band and test band are visible. If only control band is visible, it is rotavirus negative. If control band is missing, the test is invalid. The sensitivity of the test was 98,9% and the specificity was 99,6% (9). (LumiQuickAdeno-RotaVirus Antigen Comb Test, Netherlands).

Detection of rotavirus by reverse transcriptase-polymerase chain reaction

Rotavirus was also detected from stool specimens using the RT-PCR). Stool supernatant samples were processed using FavroPrep™ to isolate viral nucleic acid. Viral RNA was isolated using a micro-spin column with a silica matrix following the manufacturer's instructions. For cDNA synthesis, the BioneerAccupower® RocketScript™ RT PreMix was used. This comprehensive system included all necessary reagents for efficient first-strand cDNA synthesis from RNA templates, provided in an easy-to-use, lyophilized, single-tube format. The RTase, an RNA-dependent DNA polymerase, was employed for the synthesis of cDNA.To ensure comprehensive coverage of all RNA sequences, random hexamer primers were used during the cDNA synthesis process. The resulting first-strand cDNA was used directly for polymerase chain reaction (PCR).

Specimens and reagents were maintained at room temperature. Ten microliters of RNA were added to the reaction tube, and 20 microliters of RNase-free water were added to achieve the total volume. The materials and reaction mixture were warmed to room temperature before starting the reaction. The mixture was gently mixed and centrifuged, and then the heat cycler was set up with the reaction tube.This process generated a pool of cDNA with varying lengths. For VP7 gene amplification via PCR, the components of the master mix were brought to room temperature before use, and the PCR master mix was prepared in a separate biohazard safety cabinet. To each pre-mixed, ready-to-use PCR reaction tube, which contained Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers from Bioneer (Korea), 3 µL of the forward primer (GGCTTTAAAAGAGAGAATTTCCGTCTGG) at 10 pmol/µL and 3 µL of the reverse primer (GGTCACATCATACAATTCT) at 10 pmol/µL were added. After thorough mixing of the reaction components using a micro centrifuge (10).

Ethical approval

Ethical approval for conducting this study was obtained from the College of Medicine, University of Baghdad, Iraq (**Ref.no. of Ethical approval (0211A) on the date 16-7-2023**).

Statistical analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study (11).

Results

The results indicated that 97 out of 120 specimens tested positive for rotavirus infection in children with diarrhea, as shown in Tables 1 and 2. The ICT detected rotavirus in 110 cases (91.8%), while the RT-PCR test identified positive results for the VP7 gene in 97 cases (80.8%) of children under five years. The P- values statistically highly significant, and percentage of concordance among the two methods are non significant.

All positive specimens were further analyzed using RT-PCR to detect the VP7 gene, which is 1,062 bp in length. Primers specific to the VP7 gene were used to amplify the isolated RNA after the conversion to cDNA. Figure 1 shown the amplicon size of the VP7 gene at 1,062 bp.

Table 1 - Demographic characteristics of the participants/ Distribution according to Sex and Age groups

Factors		No (%)	P-value
Sex	Male	47 (39.1)	0.0027 **
	Female	73 (60.8)	
	Total	120 (100.0)	--
Age (year)	Age groups	No (%)	P-value
	1-12	51 (42.50%)	
	13-24	64 (53.33%)	0.0001 **
	25-48	3 (2.50%)	
	49-60	2 (1.67%)	
	Total	120 (100.0)	--

** (P≤0.01).

Table 2 - Comparing the results of the immunochromatographic test (ICT) and reverse transcriptase-polymerase chain reaction (RT-PCR) to detect rotavirus.

Method	Positive cases No (%)	Negative cases No (%)	P-value	Total No (%)
ICT	110 (91.67%)	10 (8.33%)	0.0001 **	120 (100%)
RT-PCR	97 (80.83%)	23 (19.17%)	0.0001 **	120 (100%)
P-value	0.366 NS	0.024 *	--	---

* (P≤0.05), ** (P≤0.01).

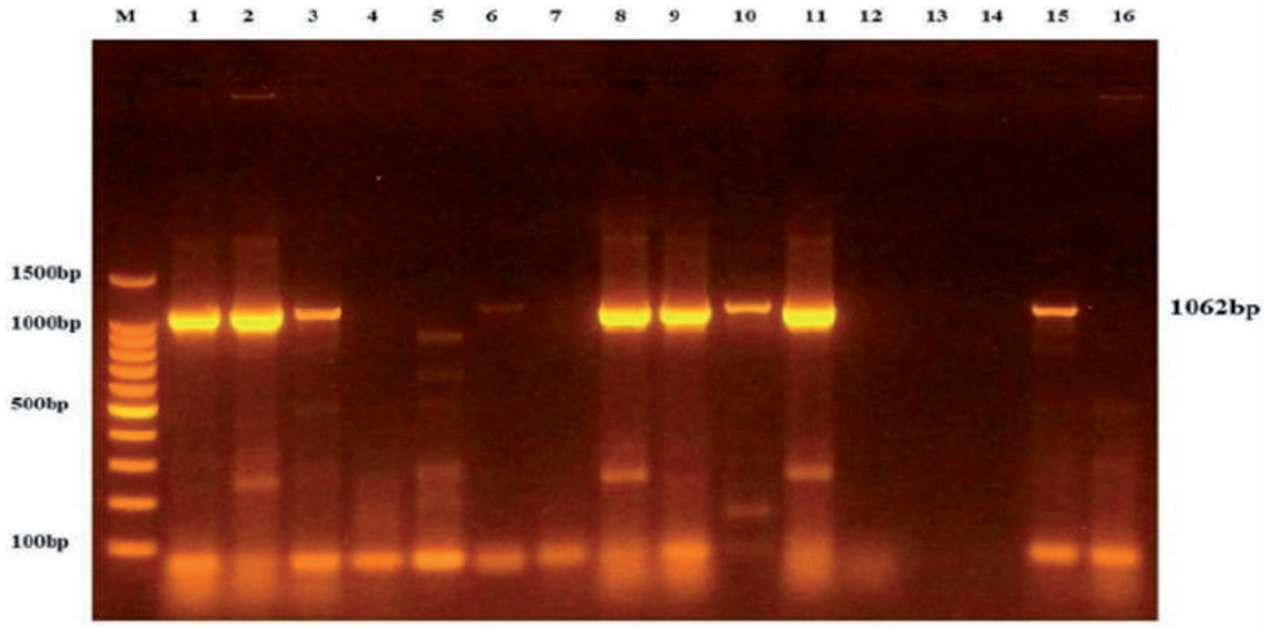

Figure 1 - Agarose gel electropherogramofreverse transcriptase-polymerase chain reaction (RT-PCR)-amplified fragment of VP7 gene of rotavirus from human.M: 100 bp DNA ladder; 1-16: Target fragment size: 1062bp.

The highest number of infected children was observed among males, with 62 cases (63.92%), compared to 35 cases (36.08%) in females. The age groups most affected were 13-24 months and 1-12 months, with 49 (50.5%) and 43 (44.3%) cases, respectively. The overall infection rate in Baghdad children under five years old was 80.8%, as shown in Tables 3 and 4. The P- values is highly significant.

Table 3 - Prevalence of human rotavirus detected by reverse transcriptase-polymerase chain reaction in Baghdad City according to

Sex	Positive cases (No (%)	Negative cases (No (%)
Female	35 (36.08%)	12 (52.17%)
Male	62 (63.92%)	11 (47.83%)
Total	97 (100%)	23 (100%)
P-value	0.0061 **	0.834 NS

** (P≤0.01).

Discussion

Rotaviruses are the leading cause of pediatric gastroenteritis globally, primarily affecting children under five years of age (2). Despite the assumption that improvements in infection control could reduce diarrheal deaths (12), there remains a significant

Table 4 - Prevalence of human rotavirus detected by reverse transcriptase-polymerase chain reaction in Baghdad City according to age group

Age stage (Month)	No. of positive cases No (%)	No. of negative cases No (%)
1-12	43 (44.3%)	8 (34.8%)
13-24	49 (50.5%)	15 (65.2%)
25-48	3 (3.1%)	0 (0%)
49-60	2 (2.1%)	0 (0%)
Total	97 (100%)	23 (100%)
P-value	0.0001 **	0.0001 **

** (P≤0.01).

risk of diarrheal disease.Rotavirus prevalence in human fecal specimens was determined using the immunochromatographictest. According to research (13), most cases of diarrhea among children under five in Iraq have been associated with rotavirus, as identified in previous studies. Based on rapid ICT results for rotavirus infection in stool specimens, 110 children in Baghdad City were positive (Table 3).These findings align with our study, where stool specimens were collected from 16 participants across five different Iraqi regions (Babil, Karbala, Missan, Qadissiya, and Wasit), showing comparable frequency and positive results using the ICT. Additionally, a study conducted on hospitalized children in Basrah, Iraq, using ICT, a simple and low-tech method with minimal equipment requirements, revealed a lower incidence of rotavirus infections (14).

Polymerase chain reaction analysis of human stool specimens was used to determine rotavirus prevalence. The PCR method is essential for identifying genes in various strains and avoids issues with primers that may not bind correctly to the template. In this study, the VP7 gene (glycoprotein) was identified due to its role in rotavirus pathogenesis and infection, serving as an outer capsid protein involved in neutralizing antibodies and facilitating entry into host cells (15). However, Isihak (2020) (16) demonstrated that RT-PCR was less effective for amplification compared to RT-qPCR when evaluating positive and negative controls. Thus, a more sensitive technique like RT-qPCR is recommended for improved PCR assay accuracy. Genotype specificity can effectively predict the origin of rotavirus strains in different host species. However, the situation is more complex, as various host species may exhibit diverse genotypes, and new or existing related host species could potentially release distinct rotavirus strains due to recognized genomic diversity across different hosts (17). Reverse transcription-PCR has been employed to identify common and uncommon VP7 (glycoprotein, G-genotype) proteins globally. The technique detected rotavirus in 80.8% of human samples (Table 3).

In contrast, a study in Iraqi Kurdistan found that only 37% of children with gastroenteritis were affected by rotavirus infection (18). This study indicated no statistically significant differences based on geographic location, gender, medical characteristics, type of therapy, or illness progression, except for the time of onset. Similarly, approximately 47.4% of acute gastroenteritis cases in diarrhea specimens showed no significant demographic differences between rotavirus and other causes of gastroenteritis, except for a higher prevalence in males. Clinical characteristics and disease progression were similar across these cases, with time of onset being the only notable exception. Among the infected children, 43 were in the 1–12-month age group, representing a 44.3% infection rate, while 49 were in the 13–24-month age group, representing a 50.5% infection rate. These findings are consistent with a study conducted in Ramadi City, Iraq (19).

Conclusion

The study's findings indicated that rotavirus infection is increasingly common among children in Baghdad, with a higher risk observed in younger children, particularly males. The RT-PCR andthe rapid ICT both showed a correlation, with RT-PCR demonstrating greater sensitivity compared to the rapid ICT.

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Conflict of interest: The authors declare that they have no competing interests.

Riassunto

Prevalenza di Rotavirus tra i bambini a Baghdad, Iraq, rilevata con metodi molecolari

Premesse. Il Rotavirus, uno dei principali agenti di gastroenterite grave nei bambini piccoli, ha un impatto globale significativo a causa della sua elevata prevalenza e della caratteristica di causare grave disidratazione.

Progetto dello studio. Questo studio è stato condotto per determinare la prevalenza di Rotavirus tra i bambini di età inferiore ai cinque anni a Baghdad, Iraq, utilizzando tecniche molecolari.

Metodi. Tra novembre 2022 e agosto 2023, sono stati raccolti 120 campioni di feci da bambini che presentavano sintomi di diarrea in un ospedale pediatrico. L'infezione da Rotavirus è stata misurata utilizzando il test immunocromatografico e la reazione a catena della polimerasi con trascrittasi inversa per rilevare e caratterizzare il gene VP7, un marcatore chiave dell'infezione da Rotavirus.

Risultati. 97 campioni su 120 sono risultati positivi al Rotavirus, con i test immunocromatografico che ha rilevato 110 casi (91,8%) e la reazione a catena della polimerasi con trascrittasi inversa che ha identificato 97 casi (80,8%) positivi per il gene VP7. I tassi di infezirone più elevati sono stati osservati nei maschi (63,92%) e nei bambini di età compresa tra 13 e 24 mesi (50,5%). L'analisi statistica ha rivelato una prevalenza complessiva di Rotavirus dell'80,8% nella

popolazione dello studio.

Conclusioni. Questi risultati sottolineano il peso significativo dell'infezione da Rotavirus a Baghdad e mettono in evidenza l'efficacia della reazione a catena della polimerasi con trascrittasi inversa nel rilevamento dei ceppi di Rotavirus. I risultati sono in linea con studi precedenti in Iraq, sottolineando la necessità di una sorveglianza continua e di sforzi di vaccinazione per controllare la diarrea correlata al Rotavirus e ridurne l'impatto sui bambini piccoli nella regione.

References

1. Matthijnssens J, Van Ranst M. Genotype constellation and evolution of group A rotaviruses infecting humans. Curr OpinVirol. 2012 Aug;**2**(4):426-33. doi: 10.1016/j.coviro.2012.04.007. Epub 2012 Jun 9. PMID: 22683209.
2. LeClair CE, McConnell KA. Rotavirus. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. PMID: 32644377.
3. Settembre EC, Chen JZ, Dormitzer PR, Grigorieff N, Harrison SC. Atomic model of an infectious rotavirus particle. EMBO J. 2011 Jan 19;**30**(2):408-16. doi: 10.1038/emboj.2010.322. Epub 2010 Dec 14. PMID: 21157433.
4. Matthijnssens J, Ciarlet M, Rahman M, Attoui H, Bányai K, Estes MK, et al. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol. 2008;**153**(8):1621-9. doi: 10.1007/s00705-008-0155-1. Epub 2008 Jul 5. PMID: 18604469; PMCID: PMC2556306.
5. Bányai K, László B, Duque J, Steele AD, Nelson EA, Gentsch JR, et al. 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 Apr 27;**30** Suppl 1:A122-30. doi: 10.1016/j.vaccine.2011.09.111. PMID: 22520121.
6. Dóró R, Farkas SL, Martella V, Bányai K. Zoonotic transmission of rotavirus: surveillance and control. Expert Rev Anti Infect Ther. 2015;**13**(11):1337-50. doi: 10.1586/14787210.2015.1089171. Epub 2015 Oct 1. PMID: 26428261.
7. Lundgren O, Svensson L. Pathogenesis of rotavirus diarrhea. Microbes Infect. 2001 Nov 7;**3**(13):1145-1156. doi: 10.1016/S1286-4579(01)01475-7. PMID: 11709295.
8. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol. 1990 Feb;**28**(2):276-82. doi: 10.1128/jcm.28.2.276-282.1990. PMID: 2155916.
9. Mijatovic-Rustempasic S, Tam KI, Kerin TK, Lewis JM, Gautam R, Quaye O, et al. Sensitive and specific quantitative detection of rotavirus A by one-step real-time reverse transcription-PCR assay without antecedent double-stranded-RNA denaturation. J Clin Microbiol. 2013 Sep;**51**(9):3047-54. doi: 10.1128/JCM.01192-13. Epub 2013 Jul 12. PMID: 23850952; PMCID: PMC3754639.
10. Chouikha A, Fodha I, Ben Hadj Fredj M, Ardhaoui M, Teleb N, Brini I, et al. Relationship between electropherotypes and VP7/VP4 genotypes of groupA rotaviruses detected between 2000 and 2007 in Tunisian children. Pathol Biol (Paris). 2011 Jun;**59**(3):e43-8. doi: 10.1016/j.patbio.2009.04.008. Epub 2009 May 29. PMID: 19481882.
11. Darren G, Mallery P. Statistical Packages of Social Sciences-SPSS/ IBM Statistics 26 step by step. 16th ed. New York: Taylor & Francis; 2019. https://doi.org/10.4324/9780429056765.
12. Mutlak QM, Abdulridha MK, Al-Huseini LMA. Updates in the prevalence of rotavirus gastroenteritis in Babylon City. Al Mustansiriyah J Pharm Sci. 2018;**18**(2):1-12. https://doi.org/10.32947/ajps.v18i2.479.
13. Fenjan MN, Jarullah BA. Molecular identification and phylogenetic analysis of rotavirus in children suffering from diarrhea under five years old in Thi-Qar Province. Iraqi J Edu Pure Sci 2019;**9**(1):82-91. http://doi.org/10.32792/utq.jceps.09.01.09.
14. Aldawmy FK, Thwiny HT, AboAlmaali HM. Epidemiological and molecular study of Rotavirus infection among humans and animals in Karbala and Basrah provinces. Iraqi J Vet Sci. 2021Apr; **35**(2):403-410. https://doi.org/10.33899/ijvs.2020.126997.1428.
15. Trask SD, McDonald SM, Patton JT. Structural insights into the coupling of virion assembly and rotavirus replication. Nat Rev Microbiol. 2012 Jan 23;**10**(3):165-77. doi: 10.1038/nrmicro2673. PMID: 22266782; PMCID: PMC3771686.
16. Isihak FA. Diagnosis of reovirus infection in broiler breeder's flocks by using PCR technique in Erbil province. Iraqi J Vet Sci. 2020;**34**(1):77-81.Available from: https://www.vetmedmosul.com/article_164199_a1c33a81fcb81b3ee6efaf9f2b976123.pdf
17. Afrad MH, Matthijnssens J, Moni S, Kabir F, Ashrafi A, Rahman MZ, et al. Genetic characterization of a rare bovine-like human VP4 mono-reassortantG6P-8 rotavirus strain detected from an infant in Bangladesh. Infect Genet Evol. 2013 Oct;**19**:120-6. doi: 10.1016/j.meegid.2013.06.030. Epub 2013 Jul 10. PMID: 23851022.
18. Mehdi SG, Niazi AD, Alhamdani FG. Burden of rotavirus gastroenteritis among under five years children at Al-AlwaiyaPaediatric Hospital. Iraqi Postgrad Med J. 2008;**7**(4):318-321. Available from: Microsoft Word - 10 (iasj.net).
19. Alani QA, Ai-Rawi SA, Salih AK, Al-Mawla SOG. Common Rota virus gastroenteritis in children under 5 years in Maternity and Children Teaching Hospital, Western Iraq. Anb Med J. 2012;**10**(1):1-7. Available from: https://amj.uoanbar.edu.iq/article_54060_5a5fe8c8c1a206086fed1e8f2a74cfa7.pdf.

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