

## Study the Distribution of Rotavirus Genotypes in Vaccinated and non Vaccinated Children in Babylon Province

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### Abstract

Rotaviruses, a major cause of gastroenteritis in children worldwide accounts for around half a million deaths annually. Vaccine against the virus has been recommended by the WHO to be involved in the national immunization program. Evaluating the genetic characterization of rotavirus among children with acute gastroenteritis in Babylon province is warranted. Children complained of diarrhea with rotavirus infection detected in their stool were involved in the study. The age of children was 6 months to less than five years whom received rotavirus vaccine (Rotarix®) from population registry of three primary health centers. Rotavirus genotypes were detected by reverse transcription-polymerase chain reaction (RT-PCR). A sample of (40) from total of (349) study children with rotavirus gastroenteritis were used to examine the rotavirus genotype. The distribution of genotypes was found to be G1, G9, and G2. Vaccinated children exhibited significantly higher percentage of G2 while G1 genotypes, meanwhile G9 genotype were presented more considerably in non vaccinated children. No significant difference in the distribution of the length of diarrhea among different genotypes. The vaccination changes the genotype distribution and this situation may create challenges to the effectiveness of rotavirus vaccines and planning for future policies.

**Keywords:** Rotavirus, (Rotarix®) Vaccine, Rotavirus Genotype.

### دراسة توزيع الطرز الوراثة لفيروس الروتا في الأطفال الملقحين وغير الملقحين في محافظة بابل قاسم مهدي مطلق\* ، منال خالد عبدالرضا\*\*<sup>1</sup> ، وليث محمد عباس الحسيني\*\*\*

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### الخلاصة

فيروسات الروتا ، وهي سبب رئيسي لالتهاب المعدة والأمعاء لدى الأطفال في جميع أنحاء العالم ، تتسبب في حوالي نصف مليون حالة وفاة سنويًا. أوصت منظمة الصحة العالمية بإشراك لقاح ضد الفيروس في برنامج التحصين الوطني. تقييم التوصيف الجيني لفيروس الروتا بين الأطفال المصابين بالتهاب المعدة والأمعاء الحاد في محافظة بابل له ما يبرره. الأطفال المشتكين من الإسهال مع عدوى فيروس الروتا المكتشفة في برازهم شاركوا في الدراسة. كان عمر الأطفال من 6 أشهر إلى أقل من خمس سنوات الذين تلقوا لقاح الفيروس العجلى (Rotarix®) من سجل السكان في ثلاثة مراكز صحية أولية. تم الكشف عن الأنماط الجينية لفيروس الروتا عن طريق النسخ العكسي - تفاعل البوليميراز المتسلسل (RT-PCR). تم استخدام عينة من (40) من مجموع (349) طفل مصاب بالتهاب المعدة والأمعاء بفيروس الروتا لفحص النمط الجيني للفيروس العجلى. تم العثور على توزيع الطرز الجينية ليكون G1 و G9 و G2. أظهر الأطفال الذين تم تلقيحهم نسبة أعلى بكثير من G2 بينما أظهر النمط الجيني G1 بشكل ملحوظ في الأطفال غير الملقحين. لا توجد فروق ذات دلالة إحصائية في توزيع طول الإسهال بين الطرز الجينية المختلفة. يغير التطعيم توزيع النمط الجيني وقد يخلق هذا الوضع تحديات لفعالية لقاحات الفيروس العجلى والتخطيط لسياسات مستقبلية. الكلمات المفتاحية: فيروس الروتا ، لقاح (Rotarix®) ، النمط الجيني لفيروس الروتا.

### Introduction

Rotavirus infection is the most significant cause of acute gastroenteritis in children worldwide (1). Annually, about 150 million episodes of diarrhea in children which need hospital care, and nearly 500,000 deaths globally for children beneath 5 years

were attributed to rotavirus infection (2). In Iraq, rotavirus is a major cause of nosocomial infectious diarrhea of nearly (18.5%), occurring primarily among children younger than 5 years of age (3,4).

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Rotavirus can infect children numerous times during their lives with first infection, after three months of age, is most probably cause severe diarrhea and dehydration<sup>5</sup>. Young children with primary rotavirus infection do not have immunity against re-infection, nevertheless such an infection protects against the progress of clinically severe disease process during rotavirus new infection<sup>6</sup>.

The primary rotavirus infection induces an antibody response which is predominantly a serotype-specific neutralizing type, while subsequent infections, yet with the same serotype of rotavirus, commonly elicit a cross-reactive broader serotype response<sup>(7)</sup>. VP4 and VP7, the two outer capsid proteins, contribute to the development of immunity by stimulating the production of neutralizing antibody<sup>(8)</sup>.

Nevertheless, after rotavirus infection, the antibody response is more generalized and involves antibodies against different other rotavirus proteins like VP2, VP6, NSP2, and NSP4<sup>9</sup>. The diversity in the strains of rotavirus, beside the genetic drift and genetic reassortment mechanism by which wild-type rotavirus strains can evolve, represent a huge challenge to modern health care<sup>3</sup>. Globally, a great diversity in circulating wild-type strains have been established with main strains causing severe disease alter from year to year and from region to region<sup>10</sup>. The findings of phase III clinical studies in several countries revealed that Rotarix® offers sustained good protection against severe cases of rotavirus gastroenteritis during the first two years of child life, with broad protection produced against each of the five main rotavirus strains that circulate globally (G1, G2, G3, G4, and G9)<sup>(7)</sup>.

Improving living standards and personal hygiene is sufficient to reduce risk of having diarrhea in children particularly in developing countries. Consequently, progress of an efficient and secure vaccine turns into a priority reduce poor clinical outcome represented by recurrent primary-care attendance, hospitalization or probably death<sup>(11,12)</sup>.

In previous reports, existing licensed rotavirus vaccines have been shown to be effective and well-tolerated<sup>(13,14)</sup>. Studies performed in infants in high income settings like Europe and North America demonstrated vaccine efficacy exceeding 90%<sup>(15)</sup>. In middle income settings of Latin America, South Africa and Far East Asia, vaccine efficacy ranged from 72% to 83%<sup>(16)</sup>, while in low income settings in Asia and Africa, vaccine efficacy ranged from 39% to 49%<sup>(17,18)</sup>. Evaluation of the outcomes of vaccination in early introduced countries is a big global difficulty for policy makers, and it is necessary to review if benefits balance the costs, and maintain wider spread of these vaccines. In the developing countries, rotavirus vaccines continued to be frequently assessed due to easy approach to reach target populations with much

more strain diversity and immunogenicity of vaccines that could decrease immunization program performance<sup>(19)</sup>.

The present study is designed to evaluate the genetic characterization of rotavirus genotyping among children less than five years old diagnosed with acute gastroenteritis in Babylon province who have received two doses of Rotarix selected from the population registry.

## Materials and Methods

### Patients

This study was done as a cross sectional one enroll a total of (349) sample population composed of children attended three primary health centers and Babylon Hospital for Maternal and Pediatrics in Babylon province were screened for positive rotavirus infection. Samples were collected during the period from October 2016 to August 2017. The study inclusions include; children older than 6 months and less than five years of age, selected from primary health centers presented with gastroenteritis induced diarrhea (the duration of diarrhea defined by the duration since onset of diarrhea until admission or examination of the children).

The children are defined as vaccinated against rotavirus according to the following categories:

• **Vaccinated:** 44 children with positive rotavirus who have received two doses of Rotarix® at age 2 months and 4 months (minimum 4 weeks in between), of which the last dose was preceding 14 days before onset of symptoms.

• **Non vaccinated:** 125 children with positive rotavirus with absence of written records for Rotarix® vaccination in the vaccination registry or medical record<sup>20</sup>.

Only 40 children sample with positive rotavirus gastroenteritis were used to examine the rotavirus genotyping distribution.

Meanwhile the study exclusion include the followings:

1. Infant < 6 months of age and children more than 5 years of age.
3. History of children hypersensitivity to the vaccine.
4. History of Gastrointestinal Tract Congenital Malformation.
5. Children with history of Intussusception.
6. History of severe combined immunodeficiency disease.

Sample size was calculated using Raosoft website sample size calculator. Assuming the margin of error of 5% and the confidence level is 95%, the total number of children registered at the above center were 3500 children then the sample size will be of minimally 347 children which is the minimum sample size for stratified analysis<sup>(21)</sup>.

### **Genotyping and Molecular Identification for Rotavirus by RT-PCR:**

All rotavirus-positive samples were confirmed by RT-PCR. The RNA extraction was carried out according to World Health Organization(2009) protocol and Faiza, L.et.al (2008) <sup>(10,11)</sup>.

#### **1. RNA extraction**

##### **1. A) Stool Sample Preparation**

- 1- Five hundred mg of stool was suspended in 5 ml of phosphate buffer saline (pH 7)
- 2- The suspension was homogenized by vortexing for 30 sec
- 3- The homogenized suspension then centrifuged at 3000 rpm for 4 min.
- 4- The clear supernatant was transferred for sterilized nucleases free tube.

##### **1. B) RNA Extraction**

- 1- From the supernatant of the clarified 10% stool suspension 250  $\mu$ l was pipetted into sterile 1.5-ml eppendorf tube and mixed with 750  $\mu$ l of TRIzol by vortexing for 30 sec and incubated for 5 min at room temperature.
- 2- Two hundreds  $\mu$ l of chloroform was added to each tube and mixed by vortexing for 30 sec, then the tubes were incubated at room temperature for 3 min
- 3- Tubes then centrifuged at 12,000 rpm for 5 min at 4°C.
- 4- The clear, upper aqueous phase was carefully transferred into a new tube.
- 5- Two volumes of cold isopropanol were added and mixed gently by inverting the tube several times.
- 6- The tubes then incubated for 20 min at -20°C.
- 7- Genomic dsRNA precipitated by centrifugation at 12,000 rpm at 4°C for 15 min.
- 8- The supernatant was pipetted carefully and the tubes were left for drying at room temperature.
- 9- The pellet then suspended in 20  $\mu$ l of nucleases free water.
- 10- Immediately the extracted dsRNA was used for downstream processes.

##### **1. C) Rotavirus Genomic dsRNA Electrophoresis**

Non-denaturing polyacrylamide gel electrophoresis was carried briefly as the following:

- 1- The electrophoresis device glasses were washed and dried by alcohol, then assembled in the device according to the manufacture directions.
- 2- Resolving gel (10%) preparation: five ml of dH<sub>2</sub>O, 3.1 ml of 30% acryl stock and 1.2 ml Resolving buffer, pH 8.8 were mixed and degased by vacuum for 5 min. then 5  $\mu$ l TEMED and 140  $\mu$ l of 10% APS were added and briefly mixed by swirling.

- 3- Immediately the mixture was poured in to the device by disposable Pasteur pipette until 1 cm beneath the comb teeth ends, covered by 3ml of water, and incubated in 37°C for 45 min to polymerize.
- 4- Spacer gel 4% was prepared by mixing 2.5 ml of dH<sub>2</sub>O, 0.6 ml of 30% acryl stock and 0.5 ml of spacer buffer, pH 6.8, the mixture was degased under vacuum for 5 min. then 2  $\mu$ l TEMED and 60  $\mu$ l of 10% APS were added and mixed briefly by swirling.
- 5- Immediately the mixture was poured in to the device by disposable Pasteur pipette and the wells forming comb was inserted gently to its location.
- 6- The gel was left for 45 min to polymerize at room temperature.
- 7- When the gel is fully polymerized, the gel was submerged in 0.5X running buffer, and the comb was removed, then the wells were carefully washed by the buffer.
- 8- Fifteen  $\mu$ l of the sample were mixed with 5  $\mu$ l of loading buffer and loaded by mechanical pipet.
- 9- The electrophoresis run was carried out under a constant voltage (150 V) for about 2h.
- 10- The gel then was dissembled and stained by silver stain as described below.

##### **1. D) Silver Staining**

- 1- The gel was fixed by 50 ml of fixing solution 1 on an orbital shaker and stay rotated at room temperature for 30 min.
- 2- The fixing solution 1 was replaced with 50 ml of fixing solution 2 and the gel was rotated for 30 min at room temperature on the orbital shaker.
- 3- The AgNO<sub>3</sub> solution (50ml) was prepared just before use and added to the gel after the aspiration of fixing solution 2. The gel was rotated for 30 min in the dark.
- 4- The silver nitrate staining solution was aspirated and the gel was washed twice with water for 2 min each time.
- 5- Preparing the developing solution by adding the NaOH to the previously prepared formaldehyde and water solution.
- 6- The developing solution was added to the gel, and agitated by hand for 30 sec to remove any black precipitate.
- 7- The developing solution was aspirated and a new developing solution was added and rotated until RNA bands were visible.
- 8- The developing solution, was aspirated and the stopping solution was added and the gel was rotated at room temperature for 5-10 min.
- 9- The gel was then rinsed in distilled water and imaged by digital camera .

**1. E) cDNA Synthesis**

- 1- Ten  $\mu\text{l}$  of extracted nucleic acid was transferred to a PCR tube and denatured at  $97^{\circ}\text{C}$  for 5 min. then chilled immediately on ice for 2 min.
- 2- Five  $\mu\text{l}$  of the denatured dsRNA and 1  $\mu\text{l}$  of each Primer VP7-F and VP7-R (20 pmoles/ $\mu\text{l}$ ) were added to RT-mastermix (Bioneer), the total volume was completed to 20  $\mu\text{l}$  by nucleases free water.
- 3- The tubes were Incubated at  $37^{\circ}\text{C}$  for 1h. and then the reaction was stopped by incubation at  $95^{\circ}\text{C}$  for 5 min.
- 4- The tubes then were chilled on ice for 2 min.
- 5- The synthesized cDNA can be used or stored at  $20^{\circ}\text{C}$  until use.

**Table 2. The PCR mixture thermocycling**

no	Stage	Temperature	Incubation time	Cycle number
	Initial denaturation	$94^{\circ}\text{C}$	2min	1
	denaturation	$94^{\circ}\text{C}$	1 min	35
	Annealing	$52^{\circ}\text{C}$	1min	
	Polymerization	$72^{\circ}\text{C}$	1min	
	Final polymerization	$72^{\circ}\text{C}$	5min	1

**1. G) Genotyping PCR**

Rotavirus genotyping was carried out according to World Health Organization (2009) protocol by nested multiplex PCR. The PCR reaction mixture components are listed in table (3).

The PCR reaction was carried out by employing thermocycling protocol listed in table (4).

**1. F) First-Round PCR**

The first round PCR reaction mixture compositions are listed in table (1);

**Table1. First round PCR reaction mixture.**

no	Component	Volume
1	cDNA	5 $\mu\text{l}$
	Master mix (promega)	10 $\mu\text{l}$
	VP7-F primer	1 $\mu\text{l}$
	VP7-R primer	1 $\mu\text{l}$
	Nucleases free water	3 $\mu\text{l}$
Total		20 $\mu\text{l}$

The PCR mixture incubated in thermocycler according to the protocol listed in table (2);

**Table 3. Reaction mixture components for rotavirus genotyping.**

no	Component	Volume
1	First round PCR product	1 $\mu\text{l}$
	Master mix (promega)	12.5
	VP7-R Primer	0.5 $\mu\text{l}$
	G1 Primer	0.5 $\mu\text{l}$
	G2 Primer	0.5 $\mu\text{l}$
	G3 Primer	0.5 $\mu\text{l}$
	G4 Primer	0.5 $\mu\text{l}$
	G8 Primer	0.5 $\mu\text{l}$
	G9 Primer	0.5 $\mu\text{l}$
	G10 Primer	0.5 $\mu\text{l}$
	G12 Primer	0.5 $\mu\text{l}$
	Nucleases free water	7 $\mu\text{l}$
Total		25 $\mu\text{l}$

**Table 4. Thermocycling conditions for rotavirus genotyping**

no	Stage	Temperature	Incubation time	Cycle number
	Initial denaturation	$94^{\circ}\text{C}$	4min	1
	denaturation	$94^{\circ}\text{C}$	1 min	30
	Annealing	$42^{\circ}\text{C}$	2min	
	Polymerization	$72^{\circ}\text{C}$	1min	
	Final polymerization	$72^{\circ}\text{C}$	5min	1

**1. H) Agarose Gel Electrophoresis**

Agarose gel electrophoresis was done according to the protocol described below in brief:

- 1- The gel (2.5%) was prepared by dissolving 1.25 g of agarose in 50 ml of 0.5X TBE buffer and heated by microwave oven for 2 min.

- 2- The homogenized agarose then cooled to 55°C by water bath.
- 3- A 50 µl of ethidium bromide stock (1 mg/ml) solution was added to the gel and mixed by swirling.
- 4- Then the gel poured in to the gel tray and let to polymerize for 30 min.
- 5- After full polymerization the gel then is transferred to the electrophoresis devise and submerged with 0.5% TBE running buffer.
- 6- PCR product (5 µl) was loaded carefully by mechanical pipet to the gel wells.
- 7- The electrophoresis was carried out by setting the device on 100 volts and 40mA for about 60 min.
- 8- The gel was then imaged and the image analyzed to determine the genotype.

**1. I) Gel Image Analyzing to Determine Rotavirus Genotypes**

According to World Health Organization (2009) protocol each genotype will produce a specific PCR product. The product for each genotype are listed in table (5).

**Table 5.** The intended PCR product for each rotavirus genotype.

No	Genotype	intended PCR product (bp)
1	first round copy of gene 9	881
	Genotype G8	754
	Genotype G3	682
	Genotype G1	618
	Genotype G2	521
	Genotype G4	452
	Genotype G10	387
	Genotype G12	266
	Genotype G9	177

**Table 6.** The PCR primer sequence .

No	Primer name	Primer sequence
1	Primer VP7-F	ATG TAT GGT ATT GAA TAT ACC AC
2	Primer VP7-R	AAC TTG CCA CCA TTT TTT CC
3	Primer G1	CAA GTA CTC AAA TCA ATG ATG G
4	Primer G2	CAA TGA TAT TAA CAC ATT TTC TGT G
5	Primer G3	ACG AAC TCA ACA CGA GAG G
6	Primer G4	CGT TTC TGG TGA GGA GTT G
7	Primer G8	GTC ACA CCA TTT GTA AAT TCG
8	Primer G9	CTT GAT GTG ACT ACA AAT AC
9	Primer G10	ATG TCA GAC TAC AGA TAC TGG
10	Primer G12	CCG ATG GACGTAACGTTGTA

**Statistical analysis**

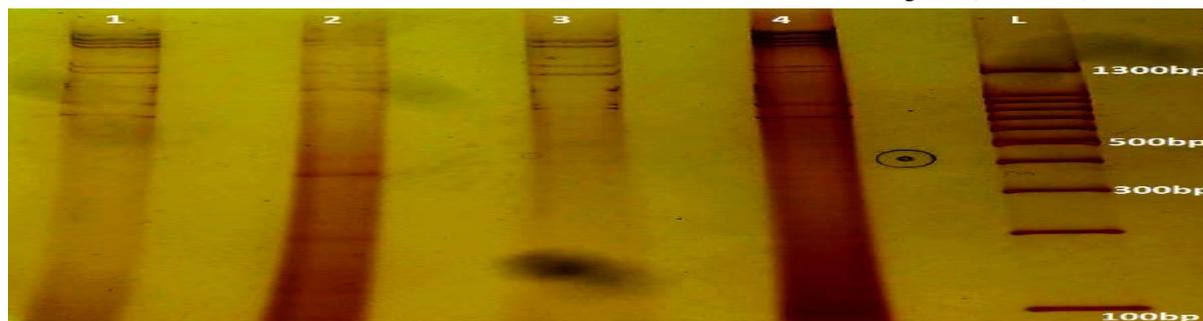
The SPSS 20.0.0, Minitab 17.1.0, GraphPad Prism 7.0 software package used to make the statistical analysis. Two samples t test used to analyze the differences in means between two groups. Discrete variables presented using their number and percentage, chi square test used to

analyze the discrete variable. *P* value considered when appropriate to be significant if less than 0.05.

**Results**

**Genotyping of rotavirus**

Results of rotavirus genomic dsRNA stain, agarose gel electrophoresis, and nested multiplex PCR were shown in Figures (1, 2 and 3).



**Figure 1.** Non denatured polyacrylamide gel electrophoresis of rotavirus genomic dsRNA stained with silver stain. (lanes 1,2,3,and 4: represent Rotavirus genomic dsRNA segments, lane L: dsDNA 100bp step ladder )

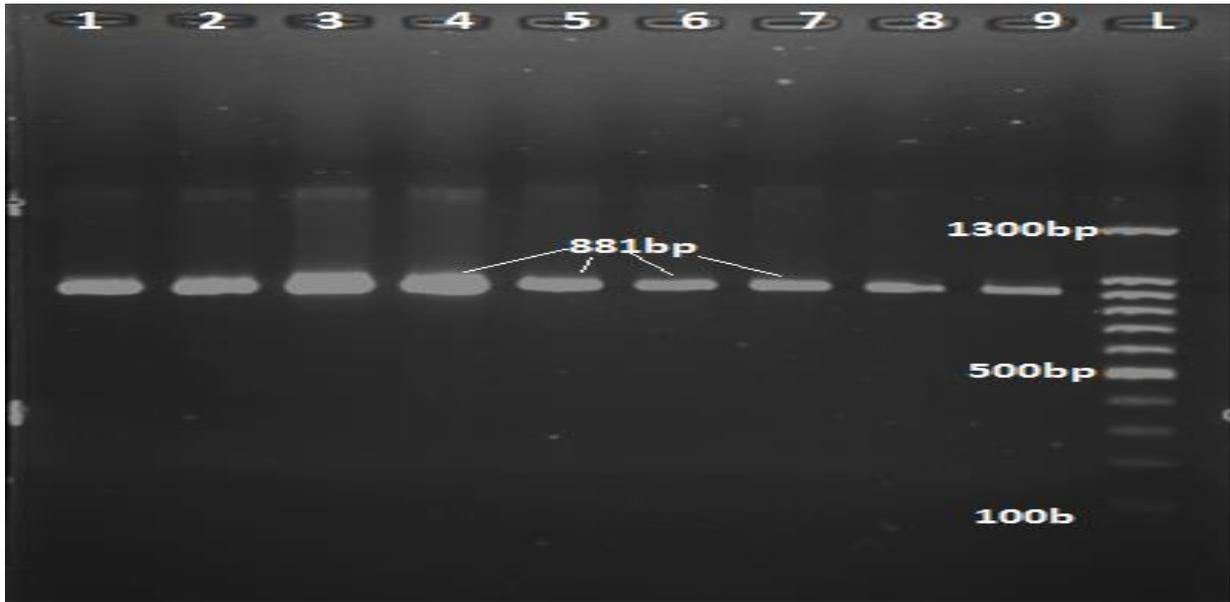


Figure 2. Agarose gel electrophoresis of first round PCR. ( Lanes 1-9 : 881 bp of VP7 amplified fragment , lane L : 100 bp step DNA ladder).

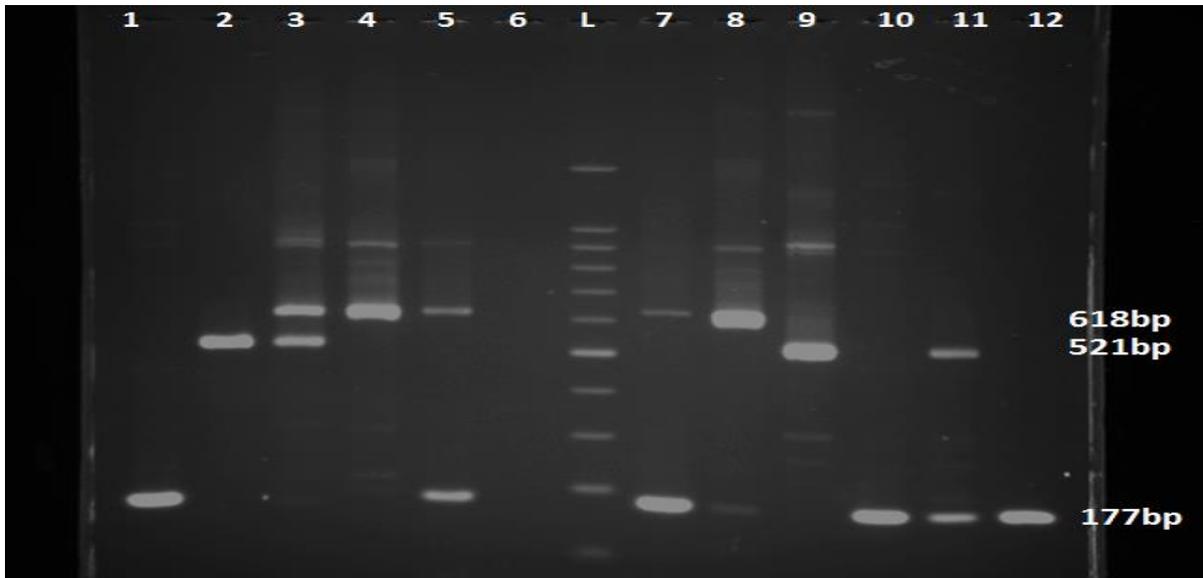


Figure 3. Rotavirus genotyping by nested multiplex PCR. ( lanes 1,7,10 and 12: G9 genotype , lanes 4 and 8: G1 genotype , lanes 2 and 9: G2 genotype , lane 3: mixed infection by G1 and G2 genotypes , lane 5: mixed infection by G1 and G9 genotypes , lane 11: mixed infection by G2 and G9 genotypes , lane 6: empty lane).

**Distribution of rotavirus genotypes**

The rotavirus genotypes were found to be G1, G2, and G9, table (7). The G1 represents the majority of positive infected children (35.0%), then G9 (32.5%), and G2 (27.5%). Mixed genotypes (G1 + G9 or G2 + G9) represent (5.0%). Significant difference was noticed among all rotavirus genotyping distribution ( $P < 0.05$ ) regardless of vaccination status of infected children.

Table 7. Distribution of rotavirus genotype (n=40).

Genotype	Number(n=40)	Percentage (%)
G1	14	35.0
G2	11	27.5
G(1 or 2) + G9	2	5.0
G9	13	32.5
P value	0.029*	

Number of patients (n) and percentage (%)

Chi-square test was used

\* P value < 0.05 ( significant)

**Duration of diarrhea and association with genotypedistribution**

In table (8a), Vaccinated children with positive rotavirus infection were significantly associated with longer duration of diarrhea

**Table 8a. Duration of diarrhea of study population**

Duration of Diarrhea(Days)		OR	95% CI of OR	P value
Not vaccinated (n=125)	Vaccinated (n=44)			
3.6 ± 1.3	4.4 ± 1.1	1.561	1.181 – 2.065	0.002**

Data presented as mean ± SD, Binary logistic regression OR: oddratio CI: confidence interval, \*\* P value < 0.01( highly significant)

**Table 8b. Association between duration of diarrhea and rotavirus genotype**

G1	G2	G9	P value
3.4 ± 1.4	3.9 ± 1.4	4.1 ± 1.4	0.470 <sup>NS</sup>

Data presented as mean ±SD of diarrhea duration (days) , NS is considered non-significant

**Association between genotype of vaccination status**

Vaccinated children exhibited significantly higher percentage of G2 (58.3%) while G1 and G9 were presented more considerably in non vaccinated children (42.3%) as in table (9).The mixed genotypes were as follows; (non vaccinated G1 + G9 or non vaccinated G 2+ G9) one samples for each mixed type were excluded from the association.

**Table 9. Association between rotavirus genotyping and vaccination status**

	Non vaccinated	Vaccinated	P value
Number	26	12	-
G1	11 (42.3%)	3 (25.0%)	0.024*
G2	4 (15.4%)	7 (58.3%)	
G9	11 (42.3%)	2 (16.7%)	

Number of patients (n) and percentage (%)

Chi-square test was used

\*P value < 0.05 ( significant)

**Discussion**

Acute diarrhea in children caused by rotavirus infection is considered as the major public health concern in the developing countries (22), including Iraq. About 70% episodes of acute infectious diarrhea caused by viruses in the pediatric age<sup>23</sup>. Especially during the first few years of life; rotavirus infection is endemic worldwide and frequently linked to morbidity and mortality in high rates particularly in developing countries due to poor nutrition and reduced health care (24,25).

The two currently licensed rotavirus vaccines, Rotarix and RotaTeq, both are very effective in reducing severe diarrhea in vaccinated and unvaccinated children although the incidence and significance of this vaccine-acquired diarrhea remain to be determined<sup>26</sup>, also both vaccines have been associated with a low risk of intussusceptions among vaccinated infants<sup>27</sup>

compared to non-vaccinated children (P < 0.01). In table (8b), no significant difference was seen between the duration of diarrhea in positive infected children according to genotype distribution.

In our previously published data (28,29), the percentage of positive rotavirus infection was (48%) among all vaccinated and non vaccinated children<sup>28</sup>, and after the assessment of the effectiveness of Rotarix® vaccine, the results revealed that non vaccinated children had a higher percentages of positive rotavirus infection (64.8%) in comparison to vaccinated children (29). large percentage of samples will need to be analyzed by RT-PCR to determine the genotypes of the strains not typeable with serotyping monoclonal antibodies (10). The results of the current showed that there were considerable differences in genotypes among both vaccinated and not vaccinated children. Vaccinated children exhibited the following genotype pattern G1 (25%), G2 (58.3%), G9 (16.7%) while unvaccinated children exhibited the following genotyping G1 (42.3%), G2 (15.4%), G9 (42.3%), with reference to the PCR primer sequence (10). In pre-vaccination era, Abood *et al.*, (2013) genotyping pattern was presented as following genotype distribution of G1 (48.57%), G2 (22.14%) and G9 (11.42%)<sup>30</sup>, which was close to the predominance pattern of the unvaccinated children<sup>22</sup>.

In an observational, prospective, multicentre, hospital-based case-control study in Belgium reported similar findings to the current study, where G2P[4] strains were more prevalent in vaccinated than in unvaccinated cases and that hospitalized rotavirus gastroenteritis caused by heterotypic G2P[4] rotavirus strains, meanwhile in the unvaccinated group, G1P[8] and G2P[4] genotypes were almost equally (31). There was a significant association (p < 0.001) between rotavirus vaccination status and genotype (29).

The immunological responses providing both homotypic and heterotypic protection normally resulted after rotavirus infection or oral vaccination, which reflect higher effectiveness of monovalent vaccine against circulating homotypic

G1P[8]rotavirus strains, compared with the circulating heterotypicG2P[4] rotavirus strains<sup>(31)</sup>.

Brazil introduced the Rotarix G1P[8] vaccine in 2006, surveillance study after using the monovalent rotavirus vaccine,the predominance of the G2P[4] genotype is temporal and that other unusual genotypes may appear with time<sup>(32,33)</sup>.

In a previous hospital-based Korean study between 2011 and 2014, no significant difference in the genotypedistribution between vaccinated children andthose unvaccinated, both of majority G2P [4], this observation could be due to small number of vaccinatedchildren (n = 19)<sup>(34)</sup>.

There was shifting in the abundance of rotavirus genotype towards G2 with greater reduction in the percentages of G1 and G9, though doesn't affect the duration of diarrhea caused by rotavirus gastroenteritis.The proposed explanation is that changes in the seasonal pattern of rotavirus disease was also observed after vaccine introduction, including delays in the start of the rotavirus season, a shorter duration of seasons and blunting of seasonal peaks<sup>(35)</sup>, thus, whether the differences in rotavirus genotypes due to natural seasonal variation or vaccine-induced selection pressure is unclear. Currently, differences in genotype-specific vaccine effectiveness and the resulting influence on distribution of co-circulating rotavirus strains, probably help to explain the increase in the proportion of G2P[4] strains.

Recent data from longitudinal study in Africa among hospitalized children aged <5 years, revealed predominance of G2 genotype [33.0%]followingRV1 introduction<sup>36</sup>.This finding based on previous studies that showed a lower point of vaccine effectiveness against rotavirus gastroenteritis caused by the heterotypic G2P[4] strains than against rotavirus gastroenteritis caused by fully homotypic G1P [8], subsequently this translates to a higher prevalence of G2P[4] strains in vaccinated cases compared with unvaccinated cases<sup>(37,38)</sup>. In line with such findings, Adlhoj *et al.* reported that G2P[4] genotypes were more frequently found in breakthrough cases vaccinated with the monovalent vaccine<sup>(39,40)</sup>.

According to all previous findings, strain diversity and genotype variation are likely driven by natural mechanisms rather than vaccine pressure, hence, monitoring genotypes, whole genomic characterization of circulating rotavirus strains before and after vaccine introduction will help assess whether vaccines are affecting the evolution of the rotavirus genome. Moreover,the changing in the model of genotype distribution of rotavirus following vaccine introduction is very crucial in deciding the future policy of immunization and assessing vaccine effectiveness. It may also explain why children are still susceptible to infection although they were fully vaccinated and why the prevalence of rotavirus gastroenteritis remains high

after five years of introducing rotavirus vaccine in the Iraqi national immunization program.

## Conclusion

Rotavirus gastroenteritis remains higher after five years of introducing rotavirus vaccine in the Iraqi national immunization program.Vaccinated children exhibited significantly higher percentage of G2rotavirus genotype.No significant difference in the distribution of the length of diarrhea among different genotypes.monitoring genotypes, whole genomic characterization of circulating rotavirus strains before and after vaccine introduction will help assess whether vaccines are affecting the evolution of the rotavirus genome.

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\* This work has been done in accordance with the suggestions of annual research plan of the Iraqi ministry of health in 2016.

\* The data used to support the findings of this study are included within the article.

\* This work was performed at Al Fadel Foundation / Babylon branch ( [www.facebook.com/Alfadhel.Babelone/](http://www.facebook.com/Alfadhel.Babelone/)).

\* The authors have no conflict of interest.

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