Scientific Research Publishing

Advances in Microbiology, 2017, 7, 863-870
http://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Genotyping of Rotavirus in Neonatal Calves with Acute Gastroenteritis in Iraq

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How to cite this paper: Abdulazeez, A.A. and Abed, M.N. (2017) Genotyping of Rotavirus in Neonatal Calves with Acute Gastroenteritis in Iraq. *Advances in Microbiology*, **7**, 863-870.
https://doi.org/10.4236/aim.2017.712066

Received: October 26, 2017
Accepted: December 23, 2017
Published: December 26, 2017

Abstract

Globally, Rotavirus is the common major etiologic agents of diarrhea in infant, young children and neonatal calves. It is very important to early diagnose the disease for effective treatment. The objective of this study was to determine the prevalence, molecular characteristics, and the effect of rotavirus strains for severe gastroenteritis in neonatal calves in five Iraqi governorates (Al-Qadissiya, Babel, Kerbala, Missan, Wassit). A total of 125-stool specimens were examined, it have been collected from calves form the period between November 2015 to March 2016. The ages were ranging from 6 to 60 weeks. The specimens were examined using Chromatographic Immunoassay, enzyme-linked immunosorbent assay (ELISA) and Polymerase-chain reaction (PCR). Our results gave us 67 (53.6%) positive by chromatographic immunoassay, 45 (36%) positive by ELISA and 32 (25.6%) positive by PCR. Genotyping were analyzed by multiplex PCR. Genotype combination G1P[8] was (30%) followed by G1P[4] (20%), G3P[4] (20%), G2P[4] (10%), G2P[8] (10%) and G9P[4] (10%). Such information will not only aid in seeking advocacy for introducing rotavirus vaccine in national immunization program in Iraq, but will also help in the evaluation of the efficacy of these vaccines in relation to the rotavirus genotyping circulation.

Keywords

Rotavirus, Genotyping, Calve, Diagnosis, PCR

1. Introduction

The wheel-like (Latin rota = wheel) particles of rotavirus were first determined as a human pathogen in 1973 by Bishop Ruth [1]. When characteristic particles were noticed in the cytoplasm of duodenal epithelial cells got from young children severing from acute diarrhea [2]. Rotavirus is a 65 - 70 nm RNA virus of the

family Reoviridae, icosahedral, with segmented double-stranded, it is classified into seven serogroups A - G; Group A subtypes 1, 2, 3, 4 constitute the main human pathogens and Group B - E infects mainly animals and birds, the rotaviruses own 3 protein shells surrounding the genome, this triple layered structure have capsomeres look like the spokes of a wheel radiating from the inner to the outer capsid [3]. Viral pathogens account for approximately 70% of episodes of acute infectious diarrhea in children, and rotavirus is the most commonly implicated virus, Group A rotaviruses are responsible for 30% - 60% of all cases of severe watery diarrhea in young children and animals [2]. Diarrhea caused by rotavirus could not be recognized clinically because the clinical symptoms (diarrhea, vomiting, fever, and dehydration) are not entirely associated with rotavirus infection [4]. The most commonly used tests in diagnosing rotavirus infections are electron microscopy, latex agglutination test, and enzyme-linked immunosorbent assay (ELISA), polyacrylamide gel electrophoresis, and immune chromatographic and polymerase chain reaction (PCR) tests [5]. Since there are many combinations of P and G genotypes in bovine group A rotavirus (BRV), research into the genotyping of BRV is very important for preventive veterinary medicine and, more specifically, for the development of a vaccine. It is also important from the point of view of ecology and public health, because interspecies transmission from cattle to humans and from humans to cattle have been reported. Particularly rotavirus P[11], G10 strains, which are commonly found in cattle had frequently been associated with asymptomatic neonatal infections in Tamil Nadu, India [6].

The aim of the present study was to identify the genotype distribution of bovine rotavirus in five governorates in Iraq and to know the vaccine isolates depending to the genotyping of rotavirus.

2. Materials and Methods

A total of 125 stool samples obtained From November 2015 to March 2016, from neonatal calves with acute gastroenteritis were randomly collected at 5 Iraqi governorates, for each governorates 25 samples have been taken (Babel, Kerbala, Missan, Qadissiya, and Wassit). 10 gm of yellow to white liquid with fatty droplet feces were examined under microscope and collected directly into sterile disposable plastic containers by rectal stimulation then stored in a cool box and transported to the laboratory, where each sample was added to specimen collection tube with extraction buffer that used in chromatographic immunoassay. The feces samples were centrifuged for 5 minute to remove particulates at 3000 rpm. Thereafter, the supernatants were stored at −20˚C until the assay day.

The chromatographic immunoassay performed to the first method that we used to detect the rotavirus in stool samples (ABON Biopharm Co., Ltd., Hangzhou, China). The qualitative Rotavirus assay ELISA was performed to the second method, which detect the rotavirus (RV) antigen (Ag), the ELISA was carried out using Cusabio kit (Cusabio Biotech Co., Ltd, China). Chromatographic immunoassay and ELISA were performed according to the manufacturers'

instructions. While the PCR and Genotyping were evaluated by using (Accu-Power PCR Premix of Bioneer Corporation) from Republic of Korea. The primers as it shown in (Table 1 and Table 2) and thermo-cycler conditions were performed according to the manufacturers' instructions that we used in RT-PCR and Genotyping for both G and P were performed according to World Health Organization manual [7].

3. Results

The present study showed the relationship among three diagnosis tests for detecting rotavirus serotype in Iraq. Chromatographic immunoassay revealed 55 (44%) The highest positive results were in Missan 60% (15 of 25 samples); however the lowest incidence was in AlQadissiya 28% (7 of 25 samples) by using chromatographic immunoassay.

Table 1. The primers and their sequences that are specific for human rotavirus genotyping. P-type-specific oligonucleotide Primers. World Health Organization [7].

No.	Primer Name	Type	Primer sequences (5'-3')	Size Product
First amplification consensus				
1	CON3 F	VP4 Forward	TGGCTTCGCTCATTTATAGACA	876 bp
2	CON 2 R	VP4 Reverse	ATTTCGGACCATTTATAACC	
Second amplification genotyping primers				
1	2T-1	P[4]	CTATTGTTAGAGGTTAGAGTC	483 bp
2	3T-1	P[6]	TGTTGATTAGTTGGATAA	267 bp
3	1T-1	P[8]	TCTACTTGGATAACGTGC	345 bp
4	4T-1	P[9]	TGAGACATGCAATTGGAC	391 bp

Table 2. The primers and their sequences that are specific for human rotavirus genotyping.G-type-specific oligonucleotide Primers.

No.	Primer Name	Type	Primer sequences (5'-3')	Size Product
First amplification consensus				
1	9CON 1F	VP7 Forward	TAGCTCCTTTTAATGTATGG	897 bp
2	VP7 R	VP7 Reverse	AACTTGCCACCATTTTTTCC	
Second amplification genotyping primers				
1	aBT1	G1	CAAGTACTCAAATCAATGATGG	158 bp
2	Act2	G2	CAATGATATTAAACATTTTCTGTG	244 bp
3	G3-AUST	G3	ACGAACTCAACACGAGARG	464 bp
4	aDT4	G4	CGTTTCTGGTGAGGAGTTG	403 bp
5	aAT8	G8	GTCACACCATTTGTAAATTCG	651 bp
6	G9 or Mg9	G9	GTTGATGTGACTAYAAATAC	110 bp

Nevertheless, ELISA test revealed 53 (42%) positive samples. The highest positive result was recorded in Missan 60% (15 of 25 samples), and the lowest in AlQadissiya 20% (5 of 25 samples). In this study, PCR technique was used for detection the two outer layer protein's VP7 and VP4. All animals samples, which had been tested using Chromatographic and ELISA, were tested by using PCR 47 (38%) were positive. The higher percent was in Missan 48% (12 of 25 samples) and the lowest was in AlQadissiya 28% (7 of 25 samples) as it shown in Table 3.

The multiplex polymerase chain reaction technique was used for genotyping of both VP7 (a glycoprotein), VP4 (a protease-sensitive protein) were revealed G1P[8] was commonly detected (30%) in animal samples followed by G1P[4] (20%), G3P[4] (20%), G2P[4] (10%), G2P[8] (10%) and G9P[4] (10%) (Figure 1).

Table 3. Comparison of the results that have been obtained from three testing method for rotavirus in 125 bovine fecal samples.

SITE	Chromatographic Immunoassay (%)	ELISA (%)	PCR (%)
AlQadissiya			
Positive	7 (28%)	5 (20%)	7 (28%)
Negative	18 (72%)	20 (80%)	18 (72%)
Total	25 (100%)	25 (100%)	25 (100%)
Babel			
Positive	11(44%)	11 (44%)	9 (36%)
Negative	14 (56%)	14(56%)	16 (64%)
Total	25 (100%)	25 (100%)	25 (100%)
Kerbala			
Positive	9 (36%)	9 (36%)	8 (32%)
Negative	16 (64%)	16 (64%)	17(68%)
Total	25 (100%)	25 (100%)	25 (100%)
Missan			
Positive	15 (60%)	15 (60%)	12 (48%)
Negative	10 (40%)	10 (40%)	13 (52%)
Total	25 (100%)	25 (100%)	25 (100%)
Wasit			
Positive	13 (52%)	13 (52%)	11 (44%)
Negative	12 (48%)	12 (48%)	14(56%)
Total	25 (100%)	25(100%)	5 (100%)
ΣPositive	**55 (44%)**	**53 (42%)**	47 (38%)
ΣNegative	**70 (56%)**	**72 (58%)**	78 (62%)

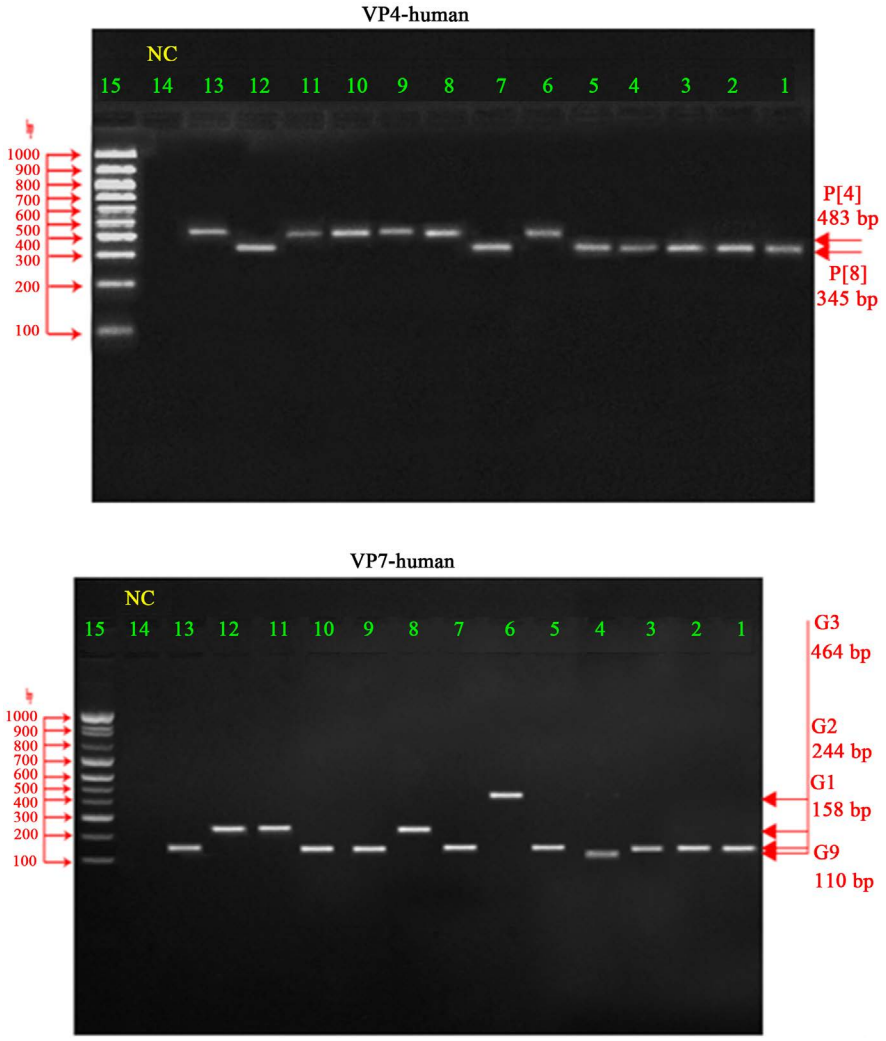

Figure 1. The presence of G and P of animal samples in the five governorates.

4. Discussion

Chromatographic Immunoassay and ELISA are the straightforward and effortless standard methods for detection of rotavirus. These methods, however, require low cost equipment and simple experience, which is available in many laboratories. Some researchers for detecting rotavirus infection have used ELISA and PCR [8]. Our study suggested that chromatographic immunoassay is a possible method for examining stool samples that have been collected from animal with suspected diarrhea that be infected by rotavirus.

This study suggested that chromatographic immunoassay was screening test which can be performed without need of trained personnel or expensive equipment also it can read with naked eye, making it easy to perform in every laboratory. The positive results that have been obtained by ELISA method were approximately similar to the results by using chromatographic method, which is probably responsible to the reported superior accuracy of the method. By chromatographic immunoassay 55 (44%) were positive samples, and by ELISA revealed 53

(42%) positives. This study agrees with a study that conducted in American United States examined 63 feces samples, which were collected from infant that infected with diarrhea, by chromatographic immunoassay and Rotazyme ELISA, the obtained results were 37 positive samples and 26 negative and it were 36 positive and 27 negative by ELISA [9]. Moreover, it agrees with a study conducted in Iran, which detected Rotavirus by chromatographic immunoassay and ELISA, which were (34.8%), (28.3%) positive samples respectively. The sensitivity, specificity of ELISA were 94.6%, 94.4% and to Chromatographic immunoassay were 82.6%, 81.6% respectively [10]. In addition, another research in Babylon, which obtained the rotavirus in 108 out of 236 samples (45.76%) by ELISA and 112 out of 236 samples (47.45%) by Chromatographic immunoassay. Results of rotavirus detection by Chromatographic immunoassay for the sensitivity and specificity were (97.2%), (94.5%) respectively, whereas by ELISA the sensitivity and specificity were 97.2%, 97.1%, respectively [11].

The sensitivity and specificity of the chromatographic immunoassay are 99.9% and 97.8% respectively. The test for ELISA is very high (sensitivity at least 98%) and (excellent specificity 98%). The kappa between PCR and Chromatographic 0.42 while the kappa between PCR and ELISA is 0.45 the kappa percent refer to a good agreement between these three tests.

In the present study, consensus primers were used to amplify the partial-length copies of the rotavirus VP4 gene which yielded the expected amplicon size of 876 bp and full length copies of VP7 gene, which yielded 897 bp product in all the nine field samples, which in turn was used as a template in multiplex PCR to identify all the possible G and P genotype present in bovine rotavirus strains as reported by [12].

The result of present study in genotyping G1P[8], G1P[4] was found to be the most frequently infected in animal samples, whereas the G2P[4], G2P[8] were the lowest strain. Our results were identify G and P genotypes were similar to the results presented [13], they showed that G1P[8] and G1P[4] were most frequent in Iran followed by P[8] (66.4%) then P[4] (9.2%) and G1 (76.3%). In another study conducted in Saudi Arabia [14], the higher rate of identified genotype were G1P[8] (44%) followed by G2P[4] (20%).

The G10P[11] genotype strain was an important genotype of group A BRV because of its zoonotic transmission from humans to cattle and also from cattle to humans, as reported by [6]. In this study, we describe the detection of bovine P[11], G10 genotype strains in Tamil Nadu which may be recognized as an important contributor to the diversity of rotaviruses found in human infections. The close interaction of the majority of the Indian population, particularly that of the Tamil Nadu population with cattle, makes possible the transmission of rotavirus from cattle to children. In conclusion multiplex PCR, which was the confirmatory serotyping for identification and typing of rotavirus genotypes, had facilitated the studies on the occurrence and distribution of individual P and G genotypes of the bovine population in 5 governorate of Iraq, in which, G1

[P8], a zoonotic important bovine genotype, was found to be present. P and G genotype associations most commonly identified in cattle were P[5], G6, P[11], G10 and P[1], G6, as reported by [15]. Hence, in this study, we attempted to discover the associations of P[5], G6 and P[11], G10 which were very common genotypes observed in bovine rotavirus, as reported by [16]. Relying on current study's results, PCR presented the greatest sensitivity and specificity while ELISA and chromatographic immunoassay kits were not as sensitive and specific as PCR test, that were the highest specific [17].

Acknowledgements

We are grateful to the many friends and colleagues who generously contributed their time and effort to help us make this paper as accurate and useful as possible. The support of our other colleagues at the ministry of health (CPHL) Dr. Faisal Al hamadani and Dr. Iman. M. Aufi who help us to use the lap for our research.

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