Benha Journal of Applied Sciences (BJAS) **print : ISSN 2356–9751**
Vol.(3) Issue(1) (2018), (107-117) **online : ISSN 2356–976x**
http:// bjas.bu.edu.eg

Investigation of Rotavirus Genotypes Infection in Egypt

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Abstract

Rotavirus have been reported as the principal causal pathogen of acute gastroenteritis and one of the leading causes of child morbidity and mortality in Egypt .This study aimed to investigate biological , serological and molecular Rotavirus genotypes infection in Egypt . The blood and stool clinical specimens were collected randomly from 100 patients .They were analyzed for the presence of antigen and the antibody using enzyme-linked immunosorbent assay (ELISA). *Rotavirus* antigen in stool and antibodies in serum were detected by ELISA among Children (78.13); Female (12.5) and male (9.38%). Rotavirus titer was determinated as $5.5x10^7$; $6.0x10^6$ and $7.5x10^7$ (PFU\ml) on vero cell of Female ; male and children respe**ctively** after 4 days Post infection. The cytopathic effects were granulation of cytoplasm , enlarged , swelling , rounded and multinucleated giant cells .The virus particles are non-enveloped with Icosahedral symmetry with about 70 nm in diameter .Rotavirus isolates showed homologous with RV isolates recorded in gene bank based on alignment and phylogenetic tree analysis. Six RV genotypes (G1P4, G1P6, G1P8 , G9P6 , G1P8, G3 P8) were detected in summer. Seven RV genotypes, (G1P4, G1P6, G1P8 , G3P4 , G3P6, G3P8 G9P8) were detected in in spring and five RV genotypes (G1P4, G1P8 , G3P4, G3P8 G9P8) were detected in winter seasons . SDS-PAGE showed seven structural and function of protein**s were** polymorphism variation among for *Rotavirus* isolates . RV genomic migration pattern of G1P4, G1P6, G1P8 , and G9P8 were varied in number , density and segmented to 12 , 9 , 10 , and 9 moved respectively **.**

Keywords: ELISA , Genotype , Rotavirus , RT-PCR , Human, Biology , Virus cultivation .

1. Introduction

Rotaviruses cause enteric infections that are subclinical in adult humans but that can cause death in new born humans . Rotaviruses are resistant to acidity, pH , heat and feces are a significant cause of spreading of infection [24]. Rotaviruses are classified in the genus Rotavirus of the subfamily Sedoreovirinae in the family Reoviridae . The genome of the virus is a double-stranded RNA with 11 segments. The virus is having icosahedral symmetry, is 70 nm in diameter and is non- enveloped. It has a three-layered capsid consisting of an outer capsid, an inner capsid and a core. VP7 (glycoprotein) and VP4 (protease-sensitive protein) in the outer capsid stimulate the production of neutralizing antibodies. Based on the differences in the genetic regions that encode these proteins, rotaviruses are classified into the G and P genotypes [15]. To date, 27 G genotypes and 37 P genotypes have been identified in humans [1]. Infections in Egypt (Cairo, Behira, Quliobia, Giza, Alexandria, Fayoum and Sharkia). are caused by strains of rotavirus in Genotypes G1, G2 and G4 represented the highest prevalence [40, 36, 3, 11, 26 ,32, 42, and 38] . Laboratory diagnosis involves the detection of Rotavirus using TEM, antigen ILFSA , ELISA and antibody ELISA techniques. Furthermore, numerous PCR methods are high sensitivity , expensive and rapid results . The RT-PCR is employed using generic or type-specific primers. At present, the VP4, VP6 and VP7 regions are most often used [1,16, 18, 19 and 5] . But there was no further extensive investigation in Egypt to determine the genetic type of Rotavirus . The aim of this study was to identify the it obtained the first data on this infection in human in the area under study , and it is the Investigation of Rotavirus genotypes infection in Egypt using biological, serological and molecular assays.

2. Materials and method

Collection and preparation of spesmans

Safety precautions

The study was done during winter, summer, out mum and spring at April 2018 to June 2019 . Seventy stool and thirty blood specimens were collected from patients responsible for acute enteric infection from hospitals and Special Clinical) from Qalubia and Cairo governorates , Egypt . The collected samples were taken from children , adult Male and Female .

2.1Preparation of clinical stool

Approximately, 0.5g of stool was resuspended in 5 ml of 10% (w/v) phosphate-buffered saline (PBS) (0.01M Tris solution (pH 7.5) , 14.5mM , NaCl and 10mM , CaCl2) . Stool solution was vortexed and clarified by centrifugation at 3000 rpm for 10 min . The clarified supernatant (1.5-2.0 ml) was collected and store at 4-8^{0}C for short term storage until use.

2.2 Preparation of clinical Serum

Blood samples were taken from the jugular veins of the patients. Blood tubes (without EDTA) were centrifuged at 3,000 × g for 10 min, and the serum samples were transferred to sterile tubes and stored in “20 °C until analyzed .

2.3Transmission Electron Microscopy(TEM)

A few drops of concentrated stool were placed on carbon-coated grids for one min, and stained with 2% PTA [2] for one min. to dry. The stained grids were examined by TEM (JEOL JEM.1400 electron microscope) in the

Regional Center of Mycology and Biotechnology El-Azhar Univ. Cairo, Egypt (RCMB).

2.4 Enzyme-LinkedImmunosorbent Assay (sandwich ELISA)

Using kits (RIDASCREEN® viral antigen and antibodies R-Biopharm AG, Germany), used for the detection RV according to the manufacturer's instructions **[9].** At the end of the test, optical densities were measured with an ELISA reader (Autobio, Zhengzhou, China) at 450 nm absorbance according to the instructions in the test procedure.

Virus cultivation

Established Vero cell line (derived from the kidney of normal African green monkey) were obtained from American Type Culture Collection (ATCC) continuous cell line established by [53].The monolayer's of Vero cells plated was inoculated in triplicates with 100 µl of serial 10-fold dilutions (10^1 to 10^7) clarify the rotavirus-positive and stool specimens. The plates were incubated for 24h at 37°C in a humidified incubator with 5% CO2. Controls consisted of Uninfected monolayer in media as negative control Vero cells continue passaging to achieve high viral titers (cell lysis is evident) .

Cytopathic effects

After 48h the cells were observed under inverted microscope before completing the assay to observe the difference in morphology between cell controls and infected one .

Titration of Virus

The monolayer cell was examined daily , starting from second day of incubation. Once the plaques have developed, usually by the fourth day post inoculation , count the number of plaques at each dilution, remove the agarose overlay and the plate was stained with 0.1% crystal violet solution and count the plaques again. The virus titer was estimated as a plaque forming units per ml (pfu /ml) as follows by counting the number of plaques at an appropriate dilution.

Viral RNA Extraction

Viral RNA was extracted from monolayer's of Vero cells plated showed cytopathic effect , cell lysis and cell death were collected at several days (up to 1 week) related to growing RV using Biozol reagent (BIOFLUX—Japan) according to the manufacturer's instructions.

CDNA synthesis

Five µl of extracted Viral RNA was shocked at 99°C for 5min. 15µl mixture containing the following constituents was added to the denatured sample ; 2 µl of 5X RT buffer (250 mMTris-HCl-pH 8.3), 375 mMKCl, 40 mM MgCl2) 0.08 µl of dNTPs and 0.25µl of primer (VP7-FATGTATGGTATTGAA TATACCAC), Reverse transcriptase from Moloney Murine Leukemia Virus, , Taq DNA polymerase and 2.5 mM dNTP) were obtained from Promega, USA and Bioline, Germany.

Amplification of VP6 **CDNA**

The c DNA was amplified by PCR technique . 1 ul cDNA was mixed with 25ul PCR mixture (RT buffer , 375 mM KCl, 40 mM MgCl2, Taq DNA polymerase and dNTP Mixture) and primer sets , VP6-F 5'-AGCACAACCTTTTCAGCACC-3'andVP6-R5'-GTGAAAACGCGTTGCAAGTT -3') directly [25]

The size of PCR product was estimated by agarose gel electrophoresis comparing with standard DNA leader. The amplified fragment of with expected size ≈ 300 bp for two samples.

Analysis of amplimer sequensing

The RT-PCR products of 4 rotavirus isolates covering all seasons were sequenced. Fifty to one hundred ul of the PCR products were purified using a high pure PCR products purification kit (Qiagen) . Sequencing was performed with an ABI prism big dye termination cycle sequencing ready reaction kit (applied biosystem) using the same primers . The DNA was sequenced with an ABI prism 310 automated DNA sequenser sequence data from both strands of the PCR products and aligned by using the clustalw and blast programs (European bioinformatics institute).

Detection of RV genotyping using multiplex RT-PCR

The second round of RT-PCR amplification of cDNA was performed with specific primer sets as in Table (1) according to [20 and 21]. P and G genotypes were determined according to amplicon size. The amplicon sizes were estimated using a DNA ladder.

The parameters for the PCR reaction were optimized for 25 µl and the final concentrations of reaction components were : 5µl of 10x PCR buffer, 4mM $MgCl_2$, 4µl of dNTPs , 0.25µl of each primers and 3 µl of ds RNA for 4RV isolates(520 µg/ml), were used as target templates. PCR cycle parameters were as follows: one cycle at 94°C for 2 min ; Amplification was carried out in 35 cycles, under the following thermal cycling conditions: 94°C for 1 min, 48°C for 1 min, 72°C for 15s and a final extension at 72°C for 10 min. The primers were used for amplification of a fragment which has a product size a 155 bp amplicon according to **(51)** All PCR reactions were performed in a programmable thermal controller (model PTC-200, MJ Research Inc., Watertown, MA, USA).

Table (1) Primer sequences pairs designed for genotyping of differentiate among isolates of *Rotavirus* (RV) specific Polymerase Chain Reaction (PCR).

Primer	Sequence 5´–3´	Target gene	Primer set	Amplicon Length	Genotype
VP7-F	ATGTATGGTATTGAATATACCAC	9 (VP7)			
VP7-R	AACTTGCCACCATTTTTTCC	9 (VP7)	VP7-F/VP7-R	881	
aBT1	CAAGTACTCAAATCAATGATGG	9 (VP7)	aBT1/VP7-R	618	G1
aCT2	CAATGATATTAACACATTTTCTGTG	9 (VP7)	aCT2/VP7-R	521	G2
G3	ACGAACTCAACACGAGAGG	9 (VP7)	G3/VP7-R	682	G3
aDT4	CGTTTCTGGTGAGGAGTTG	9 (VP7)	aDT4/VP7-R	452	G4
G8	TTRTCGCACCATTTGTGAAAT	9 (VP7)	G8V/P7-R	756	G8
G9	CTTGATGTGACTAYAAATAC	9 (VP7)	G9/ VP7-R	179	G9
G10	ATGTCAGACTACARATACTGG	9 (VP7)	G10/VP7-R	266	G10
VP4-F	TATGCTCCAGTNAATTGG	4 (VP4)			
VP4-R	ATTGCATTTCTTTCCATAATG	4 (VP4)	VP4-F/ VP4 R	663	
2T-1	CTATTGTTAGAGGTTAGAGTC	4 (VP4)	VP4-F/2T-1	483	P4
3T-1	TGTTGATTAGTTGGATTCAA	4 (VP4)	VP4-F/3T-1	267	P6
1T-1D	TCTACTGGRTTRACNTGC	4 (VP4)	VP4-F/1T1D	345	P8
4T-1	TGAGACATG CAATTGGAC	4 (VP4)	VP4-F/4T-1	391	P9
5T-1	ATCATAGTTAGTAGTCGG	4 (VP4)	VP4-F/5T-1	583	P10
P(11)	GTAAACATCCAGAATGTG	4 (VP4)	VP4-F/P(11)	312	P11

Sodium dedocyl sulfate- poly acrylamide gel electrophoresis (SDS-PAGE)

Coat protein of 4 RV isolates were analyzed banding profile qualitively and quantitatively using SDS- SDS-PAGE in 12 % acrylamide slab gels based on the method of (Laemmli, 1970), as modified by [44] .

Extraction of viral protein

Concentrated 4 partial purified of RV isolates , 450 µl into an eppendorf tube and added 50 µl of a pre-warmed solution of 1 M Na Acetate with 1% SDS followed by vortexing for 10 sec, and incubated at 37^{o}C for 15 min . Equal volume of phenol-chloroform was added to eppendorf tube then vortexed for 1 min, and incubate for an additional 15 min at 56^{o}C . The eppendorf tubes were vortexed for 1 min and then centrifuge at 12,000 rpm for 3 min. The pellets were resuspended in PBS and transferred to a fresh eppendorf tube .

Electrophoresis of viral protein

A volume of 80 µl of the protein extract was loaded on 12% gels. Control wells were loaded with standard protein marker range from 14.20 KDa to 66.00 KDa (Fermentas.Com). The voltage was increased to 200 volt until the bromophenol blue dye reached the bottom of the separating gel. The gels were stained with the staining solution(Commassie Birlliant blue R-250 ,1gm ;Methanol,455 ml ;Glacial acetic acid ,90 ml and Distilled water455 ml) . The stained gels were distained with

distaining solution (Methanol,455 ml ;Glacial acetic acid ,90 ml and Distilled water455 ml) .

Gel Analysis

Gels were photographed scanned, analyzed using Gel Doc VILBER LOURMAT system.

Extraction of viral RNA

The upper aqueous phase was transferred to a fresh tube and added 3M Sodium acetate pH 5.0 (~40 µl) and 700 µl of ice-cold absolute ethanol. The eppendorf tube were mixed by inversion and incubated at -20°C for 2 h .The eppendorf tube was centrifuged at 12000 rpm for 10 min at 4°C. The tubes were inverted onto a paper to dry for >15 min for decant ethanol . The dsRNA pellet was resuspended in 30 µl of loading buffer using the pipette .

Electrophoresis of viral RNA

the glass plates 7% acrylamide was inserted in the electrophoresis apparatus. Add The running buffer was added to the bottom reservoir, in tank. The dsRNA samples were loaded in the wells and electrophoresed at 150 V for ~2 h.

Silver staining of dsRNA

The gel was fixed in 200 ml the fixing solution contains 25% methanol, 10% glacial acetic acid and at room temperature for 30 min on shaker. The fixing solution was removed and added 200 ml of silver staining solution then shacked for 30 min at room temperature in the dark on shaker. The silver staining solution, was removed then the gel was added approximately 50 ml of developing solution (containing 1.5 ml formaldehyde and water and agitated by hand for 5 min at room temperature) and then any black precipitate RNA bands.

Gel Analysis

ıned, analyzed using Gel Doc VILBER LOURMAT system.

3. Results

Detected Rotavirus (RV)

Serological detection

RV antigen was detected in stool and serum with (35.7%) and 7 tubes of 30 with (23.33%) respectively by ELISA. RV was detected in Children with (78.13%) ; Female (12.5%) and Male (9.38%).Positive reaction was defined if the optical density (OD) of test sample exceed that of Cut off value ; ≥ 0.24 .

Biological rotavirus

Cultivated four children' stool collected in winter, spring, summer and outmum seasons onVero cell revealed different in shape, size and number plaques form . RV titer was different among seasons, female and male and children's . RV titer was 6x10^{7} , 7.5x10^{5} , 4x10 ^{6}and 6x10^{5} PFU\ml for winter, spring, summer and outmum seasons respectively on Vero cell, after 4 days Post infection in Table(2) and Fig (1).

Table (2) Titration of Rotavirus as plaque forming units (PFU\ml) on of Vero monolayer cell line**.**

Patients	Collected seasons			
	Winter	Spring	Summer	outmum
Female	$5.5x10^{5}$	$2.5x10^{4}$	$5.5x10^{4}$	$2.5x10^{3}$
Male	$6.0x10^{6}$	$5.0x10^{4}$	$2.0x10^{5}$	$4.2x10^{4}$
Children's	$6.0x10^{7}$	$7.5x10^{5}$	$4.0x10^{6}$	$6.0x10^{5}$

Cytopathic effect of RV isolates

The cytopathic effects begins with granulation of cytoplasm , the cells appear enlarged and swelling . Themacrocyte of infected cells become rounded take on are fractal appearance and undergo lytic degeneration .The second type of CPE with 5 days information of multinucleated giant cell Fig (2) **.** The staining survival cells 125 and died cells 75 out of staining cell = $2x10^{2}\mu l^{-1}$ Cell suspension**.**

Molecular detection of rotavirus

Extracted RNA from Vero Cells infected with six isolate,1 (children stool) ; isolate 2 (children plasma) , isolate 3 (female stool) , isolate 4(female plasma) isolate 5 (male stool) and isolate 6 (male plasma) collected at rour seasons . The quality of purified RNA were confirmed by UV spectrophotometer were 1.5 ,1.4 ,1.6 , 11.4 , 0.7 and 0.6 at 260/280 ratio O.D .The RNA concentration was 75 , 54 , 64 , 53 , 15 ,and 24 ug respectively .

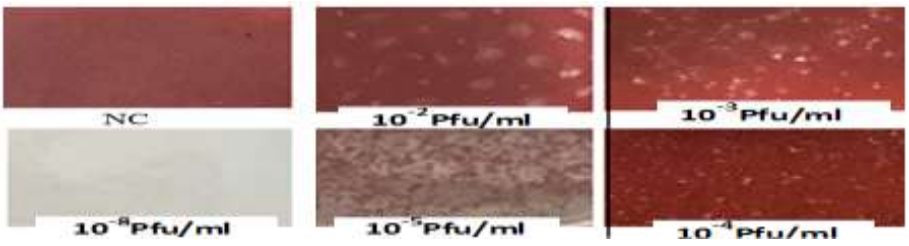

Fig (1) Vero cell line inoculated with RV showing different morphology of plaques by invert microscope.

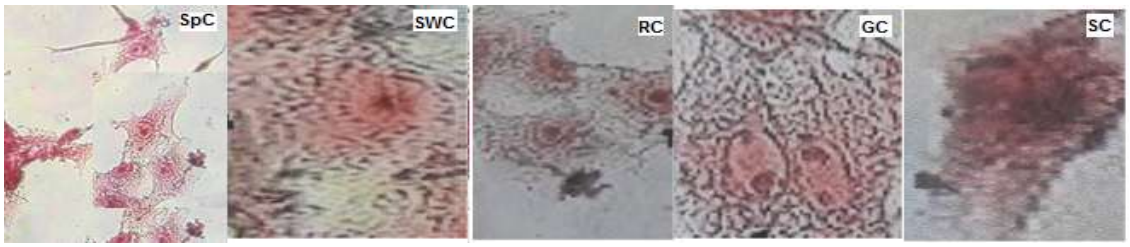

Fig (2) Cytopathic effects of Rotavirus on vero cell line showing Spindle cell (SpC) , Swelling cell(Swc) , Round cell (RC) , Spinal cell (SC) and Giant cell (Gc) survival cells.

RV morphology

The virus particles are non-enveloped with Icosahedra symmetry with about 30 nm in diameter at 80000 nm magnification Fig (3).

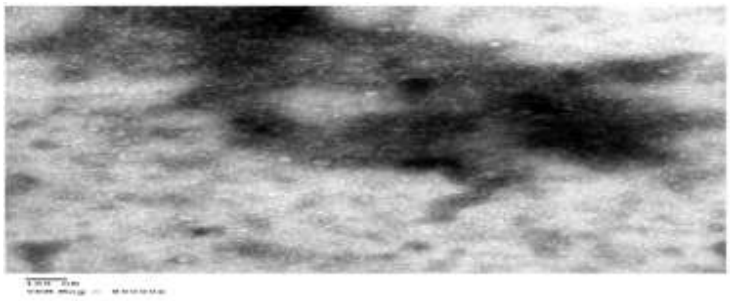

Fig (3) Electomicrographe showing the *RV* particles appear as an Icosahedra with about 30 nm in diameter using negative stain method by TEM .

cDNA of RV RNA

The obtained purified RNA from samples was transcribed to cDNA using RT and complementary primer set (reverse primer, VP6-R 5'-GTGAAAACGCGTTGCAAGTT-3').

Amplification of RV c DNA

The c DNA - RNA **Rotavirus , isolate-1** (children stool) ; isolate 2 (children plasma) , isolate 3 (female stool) , and isolate 5 (male stool) was amplified by PCR using RT-PCR reaction mixture and specific primer sets , while isolate 4 (female plasma) and isolate 6 (male plasma) non amplified by PCR ones . The efficiency of VP6 gene amplification using specific primer sets by analysis P CR product using 1.5% agarose gel electrophoresis . The amplified DNA was in the expected size calculated ≈.300bp. Fig (4) .

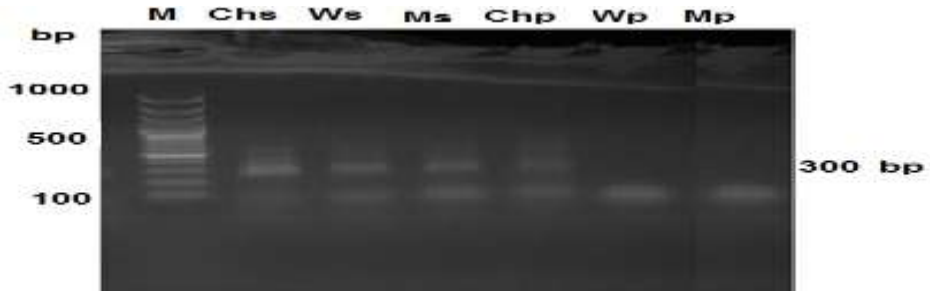

Fig (4) Electropherogram (1.5%) showing PCR amplified product from RV RNA using specific primer sets and compared DNA leader Lane (M). children stool (Chs) ; children plssma (Chp) , woman stool (Ws) , woman plasma (Wp) , Man stool (Ms) , Man plasma (Mp) and The arrow to expected size amplified fragment 300 bp.

Phylogenetic tree

Based on Multiple sequence alignment (MSA) analysis, the phylogenetic tree was performed and showed 13 clusters in which the Egypt. *Rotavirus*.showed homologous with RV isolates recorded in gene bank Fig (5).

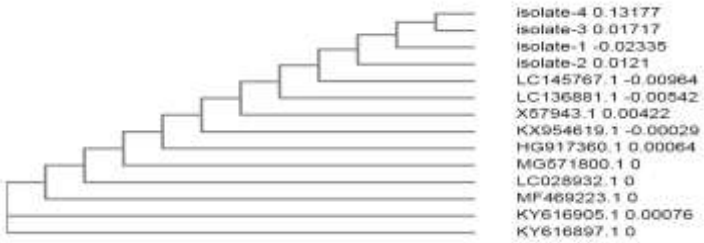

Fig (5) Phylogenetic tree representing the relationship for *Rotavirus* isolates between Egyptian isolates isolate,1(children stool ,Chs) ; isolate 2(children plasma , Ch p) , isolate 3(woman stool ,Ws) and isolate 4 (Man plasma ,Ms) based on Multiple sequence alignment of PCR amplified product from RV RNA using specific primer of vp6 genes with expected size amplified fragment 300 bp.

Genotype of rotaviruses

The Rotavirus genotypes were detected by Multiplex RT-PCR . The data shown in Table (3) and Fig (6), showed the high rate infection (G1P4, G1P6, G1P8 , G9P6 , G1P8, G31P8) genotypes in summer . Mediate rate infection (G1P4, G1P6, G1P8 , G3P4 , G3P6, G31P8 and G9P8) genotypes in spring and low rate infection (G1P4, G1P8 , G3P4, G3P8 G9P8) genotypes in outmum . On the other hand G1P8, G31P8 genotypes in winter season Table(3) .

Protein content and pattern of *Rotaviruses*

The RV protein of children stool ; children plasma , female stool and male stool isolates was red at 595 nm using the spectrophotometer and the amount of protein were calculated from the bovine serum albumin standard curve . The amount of protein were 0.69, 0.60, 0.54 and 0.62mg /ml respectively.

SDS-PAGE. showed that seven number of structure and function proteins and differed in molecular weight of *Rotaviruses* isolates with polymorphism variation whereas 7.1 % Monomorphic (common bands) , 14.4 % Polymorphic(specific bands) and 29% Unique(Genetic marker). As well as showed RV children stool has 4 Unique (Genetic marker) with molecular weight about111,30,20 and 10 KDa. , RV children plasma has 3 Unique (Genetic marker) with molecular weight about 45, 37 and 27 molecular weight .RV woman stool in Table (4) and fig (7) . it can concluded RV children stool (ChS) ; children plasma (ChP) , woman stool (WS) and Man stool (MS)are differed in their protein content qualitatively related to protein marker (M) Table (4) .

Table (4) Protein pattern and polymorphism among *Rotaviruses* isolated from stooland plasma of children , woman and man patiants .

Nucleic acid content of *Rotavirus* particles

The amount of RV **Nucleic acid** isolated from children stool (ChS) ; children plasma (ChP) , woman stool (WS) and Man stool (MS) was determined by UV Spectrophotometer at 260 nm length . The amount were 0.28 , 0.38 , 0.45 and.0.35 µg/ml respectively.

Genome pattern of *Rotavirus*

The *RV* genome were determined by 6 % polyacrylamide gel electrophoresis using selver stain . The genomic segments migration pattern of G1P4, G1P6, G1P8 , and G9P8) was observed, where segments 12 , 9 , 10 , and 9 moved in a plet. Lane (G1P4 , G1P6 , G1p8 and G9P8 respectively) showing long electropherotypes based on relative migration dye stain .. The obtained results showed that variation in number and density of genomic patters of *Rotaviruses* isolated from stool and plasma of children, woman and man patients Fig (8) .

Table (3) Seasonal distributions of Rotavirus genotypes in stool and serum of patients

Season	Samples	G-type	P-Type			Total
			P4	P6	P8	
Winter	Stool	G1	1	ND	3	4
		G3	1	ND	2	3
	Serum	G1	ND	ND	1	1
		G9	ND	ND	1	1

Table (3) Continue						
Spring	Stool	G1	1	1	3	5
		G3	1	1	2	4
	Serum	G3	ND	ND	1	1
		G9	ND	ND	1	1
Summer	Stool	G1	1	1	2	4
		G9	ND	2	3	5
	Serum	G3	ND	ND	2	2
		G9	ND	ND	1	1
	Total		5	5	22	32

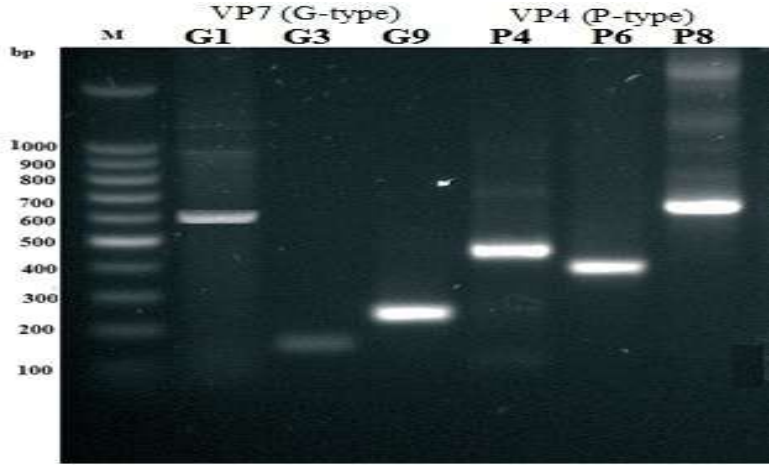

Fig (6) Gel electrophoresis positive stool sample for G9-rotavirus genotype in spring season (male less than 12 months), which have a molecular size 179 bp.

Table (4)

3	Rotaviruses isolates				polymorphism
	ChS	ChP	WS	MS	
120	+++	-	+++	++	Polymorphic
111	+++		-	-	Unique
70		++	++	+	Polymorphic
55	++++	++	++	+	Monomorphic
45	-	++	-	-	Unique
37	-	+++	-	-	Unique
35	-	++	++	++	Polymorphic
30	++++		-		Unique
27	-	+	-	-	Unique
25	++++	+	++	++	Monomorphic
20	++++	-	-	-	Unique
10	++++	-	-	-	Unique
8	-	-	+++	+++	Polymorphic
Total	7	7	7	7	

Monomorphic = common bands 7.1%
Polymorphic= specific bands 14.4 %
Unique= Genetic marker 29%
(+++) high density
(-) No detect band
(+) Low density band
(++) moderate density
(++++) Strong density

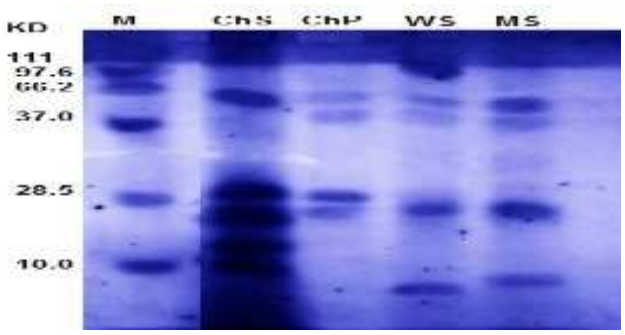

Fig (7) SDS-PAGE 12%Photogram showing protein content of purified RV isolated from children stool (ChS) ; children plasma (ChP) , woman stool (WS) and Man stool (MS) related to protein marker (M).

Fig (8) RNA-polyacrylamide gel electrophoresis of children stool (ChS) ; children plasma (ChP) , woman stool (WS) and Man stool (MS) samples for detection of Rotavirus. The genomic segments migration pattern l of G1P4, G1P6, G1P8 , and G9P8) was observed, where segments .

4.Discussion

Rotaviruses causes severe childhood diarrhea, belong to the family Reoviridae , non-enveloped and segmented ds RNA . The development of rapid and sensitive diagnostic assays is importance for diagnosing and monitoring consequently reduces hospital stays .The efficiency of diagnosis also allows for proper precautions to be taken to prevent for minimize RV spread .

In the present study, Rotavirus was serological detected in 35.72% of stool samples by antigens and in 23.33% serum samples by antibodies using ELISA collected from different hospitals belong Qalubia and Cairo governorates. It was spread between Children cases 78.13% ; female cases 12.5% and male cases with 9.38% .Clinical stool 45out of 70 collected samples were negative results Rotavirus and the infection may be related to parasites. These results are similar to the previous reports by [13 and 38]and these findings regarding the seasonal distribution are partially in agreement with the previous report which suggested no seasonal variations for adenovirus [29]. Serological investigations can only help in the diagnostic confirmation of a rotavirus enteritis, since IgM antibodies can only be detected from the fifth day after the onset of the disease. An earlier diagnosis is therefore not possible [29] . ELISA technique has been adapted by the world Health Organization as the standard method for the detection of RV antigen in samples . ELISA have the advantage of given numerical result which can be objectively interpreted but they require multiple steps in processing and usually are not cost effective for testing small number of specimens [8 and 46].

Cultivation of concentrated gave a The clinical stool positive ELISA result of RV antigen cultivated on Vero cell were revealed different in size and number plaques after 4 days Post infection . The cytopathic effects could be attributed to apoptosis induced by the virus [44]. Our result agree with the study results of [27, 37 and 10]. Rotavirus infection in vitro was associated to several sub-cellular pathological changes that ultimately culminate with cell lysis. The rotavirus and host interaction due to changes may represent a key role in the pathogenesis of diarrheic disease and death of cells has been established as being caused by lytic process [15].

The isolated RV are non-enveloped , Icosahedral symmetry with about 30 nm in diameter in concentrated stool investigated with negative stain method by TEM at 80000 nm magnification. TEM viral morphology includes virus-like-particles, i.e. electron-dense particles with ahexagonal to round shape and a diameter of RV is 30 nm [29].EM is of low sensitive as the specimens should contain approximately 10^6 viral particle /ml to be detected [30 and 35].

RNA was extracted from Vero Cell line infected with RV isolates (Chs , Chp , Fs and Ms) . The quality of purified RNA were confirmed by UV spectrophotometerat 260/280 ratio O.D and different concentration for Chs , Chp , Fs , Ms .The obtained purified RNA from samples was transcribed to cDNA using RT and complementary primer set (reverse primer, VP6-R 5'-GTGAAAACGCGTTGCAAGTT-3').

The c DNA - RNA Rotavirus was amplified by PCR technique using RT-PCR reaction mixture and specific primer sets. The efficiency of VP6 gene amplification using specific primer sets by analysis PCR product using 1.5% agarose gel electrophoresis . The amplified DNA was in the expected size calculated ≈.300bp. RT-PCR analysis (using the highly conserved the sixth viral structural proteins, VP6) of Rotaviruses of water samples and vegetable sample [11, 13 and 14]. The French report emphasized that the winter epidemics of Rotavirus infections was associated with a high level of inter human transmission, after analyzed drinking waters of children suffering from Rotaviral gastroenteritis by RT-PCR [39].

PCR assays can be developed based on genotypic differences between viruses with different host groups and can be used to better characterize sources of contamination in aquatic environment so that an appropriate and cost effective water quality remediation plan can be developed. [17]. In the case of environmental samples, amplification of viral nucleic acids by polymerase chain reaction (PCR) assays coupled to reverse transcription (RT-PCR) has been water and shellfish.[48]. Based on Multiple sequence alignment (MSA) analysis, the phylogenetic tree was performed and showed 13 clusters in which the Egypt. Rotavirus. showed homologous with RV isolates recorded in gene bank

The genotypes G and P of RV four isolates ; ChS;ChP;WS and MS were detected by Multiplex RT-PCR correspond to VP7 (glycoprotein) and VP4 (protease protein) . The obtained data showed the high rate infection in summer . Mediate rate infection in spring and low rate infection in winter and outmum seasons . To date, twenty-seven and thirty-seven of G- and P- genotypes for human rotaviruses were identified [47]. The most common human RVA genotypes circulating in the worldwide are G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], [41]. Many previous reports targeted Rotavirus genotyping among children had been conducted in different districts in Egypt (Cairo, Behira, Quliobia, Giza, Alexandria, Fayoum and Sharkia). Genotypes G1, G2 and G4 represented the highest prevalence [3, 26 and 32].

However, in a nearby governorate (Sharkia), [22] recorded higher prevalence of G1, G9 and G3 (55%, 14.5% and 22.2% Strain combinations G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] have been shown to contribute to over 90% of rotavirus infections worldwide. Of these, the G1P[8] genotype is predominant in most region [41]. Further, all the PAGE positive samples were genotyped (G and P) using the multiplex PCR assay [52].

The viral protein was read at 595 nm using the spectrophotometer and the amount of protein were calculated from the bovine serum albumin standard curve .

The structural and function proteins for *Rotaviruses* were determined using 12% SDS-polyacrylamide gel electrophoresis. The obtained results showed that seven number of protein patters were differed in molecular weight of among RV isolates with polymorphism variation and Unique (Genetic marker) .The capsid proteins are responsible for many of the serologic properties of Group A rotaviruses. Host antibodies to the VP6 protein define the rotavirus group antigen. Results from studies of animal models suggest that antibodies to VP6 and a viral nonstructural protein, NSP4, might also be involved in generation of protective immunity [6]

The obtained results showed that variation in number and density of genomic patters . Genome patterns of RV isolates were determined by 6 % PAGE using salver stain . The segmented genomic of 4 isolates were 12 , 9 , 10 and 9 segments moved in a plot. respectively. In RNA-PAGE all the RV exhibited migration pattern of its genomic segments, where segments 7, 8, 9 moved in a triplet, typical of group A rotaviruses [43]. Moreover, a genotype classification system using the sequence of all 11 genomic RNA segments has been suggested for differentiating genotypes [34 ,a and b] .

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