

## Molecular Variation between RT-PCR Detected *Rotavirus* Infection of Naturally Diarrheic Neonatal Calves and *Rotavirus* Strains of Commercial Vaccines

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### Abstract

Neonatal diarrhea is the main cause of morbidity and mortality in calves, and Rotavirus is the main viral etiology. Rotavirus vaccines are one of the main important methods for control of diarrhea in neonates' calves. In the current study, Deoxyribonucleic acid (DNA) sequencing and phylogenetic analysis of Bovine Rotavirus Group a (BRVA) were performed in our study. 1 Calf guard® vaccine genotype (G6P1) and 5 different field genotypes (2 G6P5, 1 G10P5, G10P? and 1 G10P11) were subjected to DNA sequencing. We observed that at the nucleotide level, G10P5 and G10P? Sequences were 100 % identical with each other, two G6P5 sequences were 100% identical with each other and there was no significant similarity between sequences of G10P11 with sequences of G6P5, G10P5, and G10P?. The phylogenetic analysis of G10P5 and G10P? Isolates showed a close cluster with G10 isolates of Sharkia governorate, Egypt, phylogenetic analysis of two G6P5 and one G10P11 isolate showed a close cluster with the VP4 gene of Rotavirus isolates of Dakahlia Governorate, Egypt. Molecular comparison between detected and typed Rotaviruses' genotypes with other genotypes of common vaccines indicated that there were genetically close or distance between field and vaccine Rotavirus strains. Our results can be concluded as the following, Molecular comparison between detected and typed Rotaviruses' genotypes with other genotypes of common vaccines indicated that there was genetically close or distance between field and vaccinal Rotavirus strains. Also, we suggest that Rotavac vaccine containing G6P5 Rotavirus strain and Scour guard vaccine containing can be used in Assiut governorate due to circulating of G6P5 and G10P11 strains of Rotavirus in Assiut.

**Keywords:** Rotavirus, DNA Sequencing, Vaccines

### Introduction

BRV is one of the main etiological agents of neonatal enteritis in calves worldwide. Morbidity and mortality rates due to BRV infection are high, which can lead to direct and indirect economic losses to beef and dairy production [1, 2]. Rotavirus is non-enveloped virus of genus Rotavirus, which belongs to family Reoviridae and has a genome of 11 segments of double stranded ribonucleic acid (dsRNA) that is enclosed within a triple layered capsid protein. Rotavirus encodes six viral structural proteins (VP) (VP1–VP4, VP6 and VP7) and six non-structural proteins (NSP) (NSP1–NSP6) [3]. Based on VP6, Rotaviruses are classified into ten serogroup (A-J) [4]. Rotavirus has two neutralization proteins, VP4 (protease-sensitive protein) and VP7 (glycoprotein) on its outer capsid, these two proteins define the P and G types, respectively. Rotaviruses are classified into 36 G genotypes and 51 P

genotypes based on nucleotide sequences of VP7 and VP4 genes, respectively [5]. This last classification combined with complete genome sequencing and phylogenetic analysis has been applied to indicate the evolutionary mechanisms through which the strain under study has emerged and to detect potential origins of new strains. This has led to obtain information and important insights on the complex genetic diversity of Rotavirus strains [6]. Vaccination of cows and buffalo at the end of pregnancy is one of the main health management strategies for control and prophylaxis of BRV infection. An increase in anti-Rotavirus antibody titer and adequate colostrum intake promote improvement in passive immunity that protects calves from Rotavirus infection in the first weeks of life [7]. According to Rotavirus antigen was serologically detected in fecal samples of enteric cases of examined calves coming from vaccinated dams that were vaccinated by a commercially prepared

inactivated Rotavac vaccine containing Rotavirus serotype G6P5, Coronavirus and Enterotoxigenic Escherichia coli E-coli strain F5 (K99). Immunity to Rotavirus infection is homotypic, i.e. genotype specific [8, 2]. Considering emergence of strains with variant G and P genotypes in a vaccinated herd, it is possible to confirm that there is homologous protection [7]. Still, no information is available regarding the sequence data of circulating BRV amongst livestock population of Assiut Governorate. Therefore, the current work aimed to perform a molecular comparison between detected and typed Rotaviruses' genotypes with other genotypes of the common commercial vaccines in Egyptian field.

## Material and Methods

### Sampling

A Calf guard® vaccine and a total of 5 molecularly positive fecal samples of enteric calves based on VP7 and VP4 genes of BRV by using Reverse transcriptase polymerase chain reaction (RT-PCR) were subjected to DNA sequencing.

### DNA Sequencing

Six PCR products samples (amplicons of the VP7 and VP4 genes) (five fecal samples and one Calf guard® vaccine) were used for DNA sequencing. These PCR products were purified by using a commercial kit (QIAquick® PCR Purification Kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol. The purified PCR products were used as a template for sequencing using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied biosystems, USA) as per kit protocol and sequenced on an automated sequencer (ABI PRISM® 310 Genetic Analyzer, applied biosystem, USA), (Molecular Biology Research unit, Assiut University, Egypt) using the forward primer.

### Phylogenetic Analysis

All nucleotide sequence data obtained were analyzed by using Basic Local Alignment Search Tool (BLAST) software (<http://www.ncbi.nlm.nih.gov/BLAST>). All nucleotide sequence data presented in this study had been deposited in National Center for Biotechnology Information (NCBI) GenBank database under the following accession numbers: MW656245, MW751820, MW751824, MW751825, MW751826 and MW714568. All nucleotide sequences were subjected to BLAST analysis to search for sequence similarity against GenBank database. Reference sequences were downloaded from GenBank for phylogenetic analysis. Multiple sequences were aligned by using Clustal X2 (version 2.1) software. All aligned query sequences and reference sequences were extracted using fasta format. The aligned sequences were imputed into MEGA-X (version 10.2.4) software. Multiple alignments were performed by using ClustalW program in MEGA-X. Genetic distances were calculated with Kimura-2 parameter model in MEGA-X. The confidence values of internal nodes in phylogenetic trees were calculated by performing 1000 bootstrap replicates of sequence alignment datasets, and phylograms were constructed using Neighbor-Joining method with MEGA-X.

### Data Analysis

All data were also analyzed by using Chi-square of independence in (SPSS) version 16 software program (2007). Statistically analyzed by using Chi-square of independence formula manually according to [9].

## Results

### DNA Sequencing and Phylogenetic Analysis

Currently, Calf guard® vaccine genotype (G6P1) had accession number (MW714568) and 5 different field genotypes (2 G6P5, 1 G10P5, 1G10P? and 1G10P11) that had accession numbers (MW656245, MW751820, MW751824, MW751825 and MW751826) were subjected to DNA sequencing. We observed that at nucleotide level in field genotypes, two G10P5 and G10P? Sequences were 100 % identical with each other, two G6P5 sequences were 100 % identical with each other and there was no significant similarity between sequence of G10P11 with sequences of G6P5, G10P5 and G10P? (Table 1). NCBI BLAST search revealed that G10P5 and G10P? sequences with published sequences in GenBank database showed overall identities of 94.91- 99.66% in nucleotide level, 2 G6P5 sequences with published sequences in GenBank database showed overall identities of 94.01- 98.10% in nucleotide level and G10P11 sequence with published sequences in GenBank database showed overall identities of 94.76- 98.75% in nucleotide level. The phylogenetic analysis of G10P5 and G10P? Isolates showed close cluster with G10 isolates of Sharkia governorate, Egypt (Fig. 1). The phylogenetic analysis of 2 G6P5 isolates showed close cluster with VP4 gene of Rotavirus isolate of Dakahlia governorate, Egypt (accession number MK961093) (Fig. 2). The phylogenetic analysis G10P11 isolate showed close cluster with VP4 gene of Rotavirus isolate of Dakahlia governorate, Egypt (accession number MK961092) (Fig. 3).

### Molecular Comparison between Detected and Typed Rotaviruses' Genotypes with Other Genotypes of Common Commercial Vaccines

In our result, NCBI BLAST search revealed that G10P5 and G10P? Sequences with G10P11 Scour guard vaccine strain showed overall identities of 97.13% in nucleotide level. NCBI BLAST search revealed that G10P5 and G10P? Sequences with G6P1 Scour guard vaccine strain and G6P5 Rotavac vaccine strain showed overall identities of 77.62% and 77.31% in nucleotide level, respectively. NCBI BLAST search revealed that G10P11 sequence with G10P11 Scour guard vaccine strain showed overall identities of 95.45% in nucleotide level. NCBI BLAST search revealed that two G6P5 sequences with G6P5 Rotavac vaccine strain showed overall identities of 89.06% in nucleotide level. NCBI BLAST search revealed that there was no significant similarity between different field strains sequences in the present study and sequence of Calf guard® vaccine strain (G6P1) and sequence of VP4 gene of G6P1 of Scour guard vaccine at nucleotide level. NCBI BLAST search revealed that there was no significant similarity between sequence of different field strains (G6P5 and G10P11) in the current

study with sequence of VP7 genes of G6P1 and G10P11 of Scourguard vaccine and G6P5 of Rotavac vaccine at nucleotide level. NCBI BLAST search revealed that there was no significant similarity between sequences of different field strains (G6P5, G10P5 and G10P?) in the present study with sequence of VP4 gene of G10P11 of Scourguard vaccine at nucleotide level. NCBI BLAST search revealed that there was no significant similarity between

sequences of different field strains (G10P5, G10P? and G10P11) in the current study with sequence of VP4 gene of G6P5 of Rotavac vaccine at nucleotide level (Table 2). Nucleotide sequences in the partial VP7 and VP4 genes of BRVA field strains were compared with those of BRVA vaccine strains. Dissimilarity of nucleotide sequences was observed in several positions in vaccines strains in comparison to field strains (Fig. 4, 5 and 6).

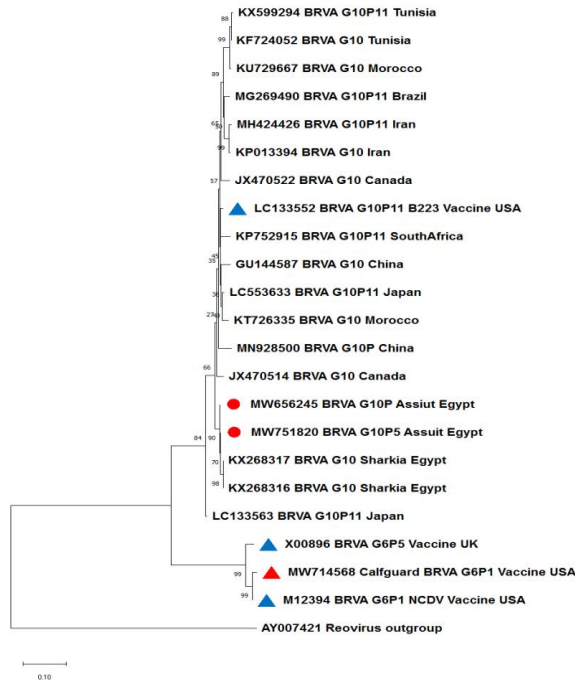
**Table 1: Comparison between Detected and Typed Rotaviruses' Genotypes by Nucleotide Identity Percentage**

Field strains	One G6P5 (VP4 gene)	One G10P5 (VP7 gene)	One G10P? (VP7 gene)
	nucleotide identity (%)		nucleotide identity (%)
One G6P5 (VP4 gene)	100%	*	*
One G10P5 (VP7 gene)	*	100%	100%
One G10P11(VP4 gene)	*	*	*

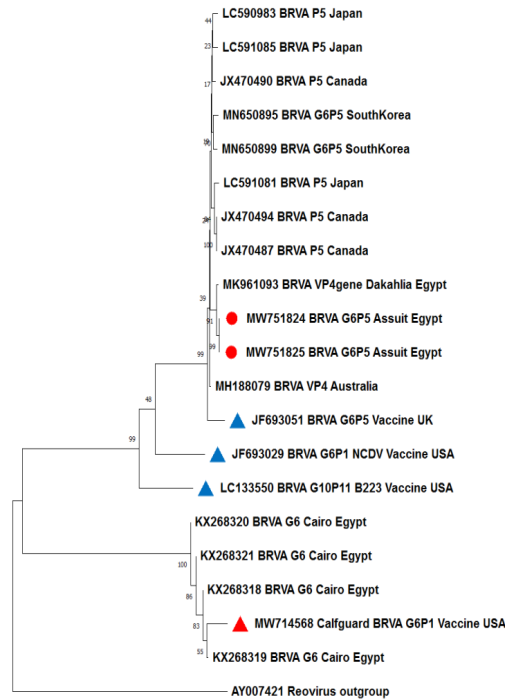
\* = No significant similarity

**Table 2: Comparison between Detected and Typed Rotaviruses' Genotypes with Other Genotypes of Common Vaccines by Nucleotide Identity Percentage**

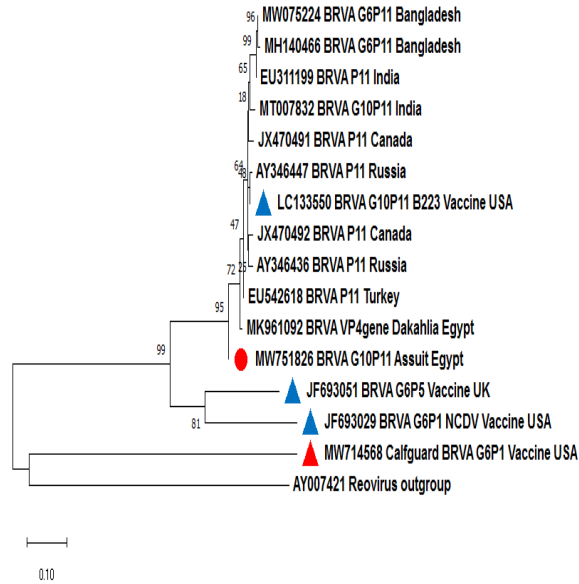
Vaccinal strains	Calfguard vaccine (G6P1) VP7 gene	Scourguard vaccine (G6P1) VP4 gene	Scourguard vaccine (G6P1) VP7 gene	Scourguard vaccine (G10P11) VP4 gene	Scourguard vaccine (G10P11) VP7 gene	Rotavac vaccine (G6P5) VP4 gene	Rotavac vaccine (G6P5) VP7 gene
	nucleotide identity (%)	nucleotide identity (%)	nucleotide identity (%)	nucleotide identity (%)	nucleotide identity (%)	nucleotide identity (%)	nucleotide identity (%)
Two G6P5 (VP4 gene)	*	*	*	*	*	89.06%	*
G10P5 (VP7 gene)	*	*	77.62%	*	97.13%	*	77.31%
G10P? (VP7 gene)	*	*	77.62%	*	97.13%	*	77.31%
G10P11(VP4 gene)	*	*	*	95.45%	*	*	*



**Figure 1:** Phylogenetic tree with 715 bp amplicon with Egyptian (Assiut) G10P5 and G10P? genotypes of BRVA strains. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The numbers adjacent to nodes represent percentage of bootstrap support (1,000 replicates) for the clusters. The Egyptian (Assiut) G10P5 and G10P? strains are marked with a filled circle and the vaccinal strains are indicated by a filled triangle. Reovirus was used as outgroup (AY007421).



**Figure 2:** Phylogenetic tree with 856 bp amplicon of VP4 gene with Egyptian (Assiut) 2 G6P5 genotypes of BRVA strains. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The numbers adjacent to nodes represent percentage of bootstrap support (1,000 replicates) for the clusters. The Egyptian (Assiut) 2 G6P5 strains are marked with a filled circle and the vaccinal strains are indicated by a filled triangle. Reovirus was used as outgroup (AY007421).



**Figure 3:** Phylogenetic tree with 335 bp amplicon with Egyptian (Assiut) G10P11 genotype of BRVA strain. The tree was constructed using neighbor-joining and Kimura two-parameter as a nucleotide substitution model. The numbers adjacent to nodes represent percentage of bootstrap support (1,000 replicates) for clusters. The Egyptian (Assiut) G10P11 strain is marked with a filled circle and the vaccinal strains are indicated by a filled triangle. Reovirus was used as outgroup (AY007421).

>MW656245_G10P?_Field	1	CTAGCGGTTAGCTCCTTTTAAATGTATGGTATTGAATATACCACATTCCTAATCTACTTGA	60
>MW751820_G10P5_Field	1	.....	60
>LC133552_G10P11_Vaccine	19	.....	78
>M12394_G6P1_Vaccine	29	.....A.T.....T.....	88
>X00896_G6P5_Vaccine	29	.....A.T.....T.....	88
>MW656245_G10P?_Field	61	TATCAATTATATTACTTAATTACATATTAAGAGTATAACTAGAAATGATGGACTATATAA	120
>MW751820_G10P5_Field	61	.....	120
>LC133552_G10P11_Vaccine	79	.....C.....	138
>M12394_G6P1_Vaccine	89	C...G...C...T.G...T...C...-TC...G.....	148
>X00896_G6P5_Vaccine	89	C...G...C...GT.G...T...C...-TC...G...A.....	148
>MW656245_G10P?_Field	121	TTTACAAATTTTGTCTTATAGTCACAATTACTTCAATTGTTGTTAATGCACAAAATTACG	180
>MW751820_G10P5_Field	121	.....	180
>LC133552_G10P11_Vaccine	139	.....C.....	198
>M12394_G6P1_Vaccine	149	.....G...C.....AGTG...--...C.CCA.AA.A...G...C..T.	208
>X00896_G6P5_Vaccine	149	.....G...C...-GTGG...-...C.CCA.GA.A...G..G..C..T.	208
>MW656245_G10P?_Field	181	GTATCAATTTACCAATAACCGGATCAATGGATACGTCATATGTGAATGCTACTAAAGATG	240
>MW751820_G10P5_Field	181	.....	240
>LC133552_G10P11_Vaccine	199	...T.....A.....	258
>M12394_G6P1_Vaccine	209	.AG.A...G...T.A.T.....TG.G...C..C...A...-	268
>X00896_G6P5_Vaccine	209	.AG.G...G...T.A.T.....TG.G...C.CA..CT...G...-	268

>MW656245_G10P?_Field	241	AGCCATTCTCAACATCGACATTATGCTTGTACTATCCAACAGAAGCTAGAACAGAAATAA	300
>MW751820_G10P5_Field	241	.....	300
>LC133552_G10P11_Vaccine	259	.....A.....T..A.....	318
>M12394_G6P1_Vaccine	269	.....T.G.....A..CC.T..T.....T.....TGTT..G...CA...-.....G	328
>X00896_G6P5_Vaccine	269	.....T.G.....A..TC.T..T.....T.....TGTT..G...CA...-.....G	328
>MW656245_G10P?_Field	301	ACGATAATGAGTGGACAAATACGTTGTCGCAGTTGTTCTTAACAAAGGGATGGCCGACTG	360
>MW751820_G10P5_Field	301	.....	360
>LC133552_G10P11_Vaccine	319	.....G.....C.....A....	378
>M12394_G6P1_Vaccine	329	CT...CC..A...A.G...C..A..A..A.....G...A.....A..A.	388
>X00896_G6P5_Vaccine	329	CT...CC..A...AGG...C..A..A..A.....G...A.....A..A.	388
>MW656245_G10P?_Field	361	GATCTGTACTTTAAAGAATATGATGATATAGTACTTTTTTCAGTGGATCCCAACTGT	420
>MW751820_G10P5_Field	361	.....	420
>LC133552_G10P11_Vaccine	379	.....T.....	438
>M12394_G6P1_Vaccine	389	...A..G...C.....C.....GG.C.....A....GT.A.	448
>X00896_G6P5_Vaccine	389	...G..G.....AC.....GG.C.....A....GT.A.	448
>MW656245_G10P?_Field	421	ATTGTGACTATAACATAGTCTGTGATGAGATACAAATTCGAGCTTAGAGCTTGATATGTCAG	480
>MW751820_G10P5_Field	421	.....	480
>LC133552_G10P11_Vaccine	439	.....T.....C..A..A.....G.	498
>M12394_G6P1_Vaccine	449	.C..C..T...TT...T.A...A..TG...T.CACA...A..A.....T.	508
>X00896_G6P5_Vaccine	449	.C...T...TT...T.A...A..TG...T..AC...A..A.....T.	508
>MW656245_G10P?_Field	481	AATTGGCAAATCTAATATGGAATGAATGGCTATGCAATCCAATGGACATTACATTGTATT	540
>MW751820_G10P5_Field	481	.....	540
>LC133552_G10P11_Vaccine	499	.....A.....	558
>M12394_G6P1_Vaccine	509	.....CG...T.....C.....G.....A..GC.A....	568
>X00896_G6P5_Vaccine	509	.....CG...T...C...C.....G.....A..GC.....	568
>MW656245_G10P?_Field	541	ATTACCAACAGACGGACGAGGCAACAAATGGATAGCAATGGGACAATCATGCACAATAA	600
>MW751820_G10P5_Field	541	.....	600
>LC133552_G10P11_Vaccine	559	.....T.....	618
>M12394_G6P1_Vaccine	569	...T.G.....T..T.A...T.....T...C...CTCT..T...GG.T.	628
>X00896_G6P5_Vaccine	569	...T.G.....T..T.A...T.....T...TTCT..T...G.C.	628
>MW656245_G10P?_Field	601	AAGTGTGCCATTGAATACCCAAACGCTAGGAATAGGATGTCAGACCACAAACTGGAA	660
>MW751820_G10P5_Field	601	.....	660
>LC133552_G10P11_Vaccine	619	.....T.....	678
>M12394_G6P1_Vaccine	629	.....A...A...A..T..T..T.....	670
>X00896_G6P5_Vaccine	629	.....A...G...A..T..T..T.....	670
>MW656245_G10P?_Field	661	CGA	663
>MW751820_G10P5_Field	661	...	663
>LC133552_G10P11_Vaccine	679	..	680
>M12394_G6P1_Vaccine			
>X00896_G6P5_Vaccine			

**Figure 4:** Comparison of the nucleotide sequences of partial VP7 gene of BRVA between Field strains (G10P5 and G10P?) and commercial vaccinal strains (G10P11, G6P1 and G6P5). Dots indicate nucleotides identity with the field strains sequences.

>MW751824_G6P5_Field	1	ATGTGGTCTTCGTGCTGTCATTGGCAGCGAATCTGAATGACGTAATGTGTTCGGCGGAG	60
>MW751825_G6P5_Field	1	.....	60
>JF693051_G6P5_Vaccine	1091	..A.....A..A.GA.....T.....GC	1150
>MW751824_G6P5_Field	61	ATTATAGCTTCGCGCTACCTGCCGGACAATGGCCTGTGATGAAAGGGGAGCGGTGACGT	120
>MW751825_G6P5_Field	61	.....	120
>JF693051_G6P5_Vaccine	1151	.....T.....TT.....G.....G.....A..G.....A....	1210
>MW751824_G6P5_Field	121	TGCACACAGCGGGGTAACATTGTCTACACAATTCACCGACTACGTATCACTGAACTCAC	180
>MW751825_G6P5_Field	121	.....	180
>JF693051_G6P5_Vaccine	1211	.A.....A..G.....A.....T..G..GT...T....	1270
>MW751824_G6P5_Field	181	TAAGGTTTAGGTTTAGACTAGCAGTCGAAGAACCATCATTACGATAACTAGAACACGTG	240
>MW751825_G6P5_Field	181	.....	240
>JF693051_G6P5_Vaccine	1271	...A.....G..G..T..G.....T.....	1330
>MW751824_G6P5_Field	241	TATCAAACCTGTATGGTTTACCGCGGCAATCCAAACGGTGGAAAGGAATATTATGAAG	300
>MW751825_G6P5_Field	241	.....	300
>JF693051_G6P5_Vaccine	1331	.G.....G.....C...A..A..G..C.....C...AA..G..C.....	1390
>MW751824_G6P5_Field	301	TGGCAGGAAGATTTTCGCTAATATCATTTGGTCCCATCAAATGATGACTATCAGACGCCAA	360
>MW751825_G6P5_Field	301	.....	360
>JF693051_G6P5_Vaccine	1391	...T...G...T.C.....T..C..A.....	1450
>MW751824_G6P5_Field	361	TTATGAACTCAGTAACAGTAAGACAAGACTTGGAAAGCGCTTTAAATGAGTTGAGAGAAG	420
>MW751825_G6P5_Field	361	.....	420
>JF693051_G6P5_Vaccine	1451	.....G.....A.....	1510
>MW751824_G6P5_Field	421	AGTTTAATAACTTATCACAAGAGATAGCTGTGTACAGTTAATGACTTAGCTATGCTGC	480
>MW751825_G6P5_Field	421	.....	480
>JF693051_G6P5_Vaccine	1511	.A.....G.....C.....T...	1570
>MW751824_G6P5_Field	481	CATTAGACATGTTTTCGATGTTCTCGGGAATTGAGGGTACTGTGAACGCAGCGAAATCAA	540
>MW751825_G6P5_Field	481	.....	540
>JF693051_G6P5_Vaccine	1571	..C.....A...C...T...A.....	1630
>MW751824_G6P5_Field	541	TGGCTACCAACGTGATGAGGAAAGTTAAGAGTTCGAAACTCGCATCATCAGTGTCAATGT	600
>MW751825_G6P5_Field	541	.....T.....	600
>JF693051_G6P5_Vaccine	1631	.....T.....GT.....A.....G.....G.....	1690
>MW751824_G6P5_Field	601	TGACGGATTCTTTATCCGATGCGGCCTCATCTATCGCAAGAAGCAGTCAATACGATCGA	660
>MW751825_G6P5_Field	601	.....	660
>JF693051_G6P5_Vaccine	1691	.A....C.....T..A..G.....A.	1750
>MW751824_G6P5_Field	661	TAGGATCGACGG	672
>MW751825_G6P5_Field	661	.....	672
>JF693051_G6P5_Vaccine	1751	.....	1757

**Figure 5:** Comparison of the nucleotide sequences of partial VP4 gene of BRVA between Field strains (2 G6P5) and commercial vaccinal strain (G6P5). Dots indicate nucleotides identity with the field strains sequences.

>MW751826_G10P11_Field	1	TATGGGGCGATTACAGGCCTTCAGAAATATGGTATATGTGCGGTCGCTGAATGCGGAA	60
>LC133550_G10P11_Vaccine	1060	...T...A.....T..T.....	1119
>MW751826_G10P11_Field	61	CTAAATCAAGTGCGGTGCAGAGGAGTCACTATTCGTTTCGCACTGCCTGTAGGCTCATGG	120
>LC133550_G10P11_Vaccine	1120	.....T.....T.....	1179
>MW751826_G10P11_Field	121	CCGGTGTATGCAGGGAGGAGTGTGGTTCTAACATTCGATGGTGTAACTGTTATCTACACAA	180
>LC133550_G10P11_Vaccine	1180	.....A.....A.....T.....A..G...	1239
>MW751826_G10P11_Field	181	TTTACTGACTATGTGTCGTTAAATTCACTAAGATTCAGATTTAGATGTGCAGTAAGCGAA	240
>LC133550_G10P11_Vaccine	1240	.....G..C.....G.....	1299
>MW751826_G10P11_Field	241	CCTTCGTTTAGGGTTACCGGTACGAGGATATCAAATTTGTATGGCCTTCCAGCCGCGAAC	300
>LC133550_G10P11_Vaccine	1300	.....	1359
>MW751826_G10P11_Field	301	CCAATGGGAGACCAGCAATATTATGAGGCA 330	
>LC133550_G10P11_Vaccine	1360	.....A..... 1389	

**Figure 6:** Comparison of the nucleotide sequences of partial VP4 gene of BRVA between Field strain (G10P11) and commercial vaccinal strain (G10P11). Dots indicate nucleotides identity with the field strain sequence.

## Discussion

BRV is important cause of neonatal calf enteritis throughout the world and causes significant economic losses. DNA sequencing of BRV was done in our study for the first time in Assiut governorate. DNA sequencing and classification is very important for distinguishing one virus from another, especially for segmented viruses such as Rotaviruses [10]. Sequencing and analyzing of isolate strains (G6P5, G10P5, G10P? and G10P11) were done during this study. Sequences of our isolates were analyzed and compared the sequences with different BRV strains and vaccinal strains. The result of the current study confirms that bovine Rotaviruses are circulating in Assiut governorate. Moreover, genes, VP7 and VP4 were taken in the consideration for partial sequence analysis. Phylogenetic analysis showed that the circulating BRVA G10P5 and G10P? Isolates that were detected in our result observed close identity with G10 isolates (99.66%) of Sharkia governorate and G10P11 vaccinal strain of Scourguard vaccine (97.13%), these strains were distant from BRVA United Kingdom vaccine strain (G6P5) and USA vaccinal strain (G6P1). The phylogenetic analysis showed that circulating BRVA G6P5 isolates that were noted in our result showed close identity with VP4 Rotavirus isolate of Dakahlia governorate (98.10% %) and G6P5 vaccinal strain of United Kingdom (89.06%), these strains were distant from BRVA USA vaccine strains (G6P1 and G10P11). The phylogenetic analysis showed that circulating BRVA G10P11 isolate which was showed in our result indicated close identity VP4 Rotavirus isolate of Dakahlia governorate (98.75%) and G10P11 USA vaccinal strain (95.45%), this strain was distant from BRVA USA vaccinal strain (G6P1) and United Kingdom vaccinal strain (G6P5). The strongest identity between our field BRVA strains and previously isolated Egyptian strains may be due to VP4 and VP7 genes of BRVA

shared in nucleotide sequence. The genetically distant between field and vaccinal strain of BRV suggesting possible emergence of new genotypes which may possibly be due to genetic assortment because of segmented nature dsRNA genome of Rotavirus [10, 7]. Some studies had shown that vaccination with the genotype G6P1 of BRVA results in poor heterologous protection against BRVA G6 strains containing different P genotypes from the vaccine. Moreover, commercial vaccines containing genotype G6P1 may not be as effective, because P1 may not be the most common genotype depending on geographic region studied [1]. These findings stress the need to further investigate any change in genotype distribution and emergence of new genotypes through sequence analysis [11]. This study provides further information regarding Rotavirus G and P genotypes and sequence analysis of Rotavirus found in Egyptian calves. The results of this study show that the discrepancy between genotypes found in commercial vaccine and Rotavirus strains circulating in examined enteric calves, once more, reinforces importance of constant surveillance in order to avoid potential causes of vaccination failure [12]. Nucleotide sequences in the partial VP7 and VP4 genes of BRVA field strains were compared with those of BRVA vaccinal strains. Dissimilarity of nucleotide sequences was noted in several positions in vaccinal strains in comparison to field strains. This finding can help to distinguish the vaccinal strains from field strains of BRVA. These changes in nucleotide sequence of vaccinal strain from field strain may be attributed to RNA viruses have a high mutation rate during replication due to both lack of proofreading and post-replication error correction by RNA polymerase. These changes in nucleotide sequence can be used as “markers” of the vaccinal strains so that changes in vaccine seed strain could be monitored [13].



## Declarations

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## Competing Interests

The authors declare that they have no competing interests.

## Ethics Committee Approval

Ethics committee approval is not required for the research protocol.

## Consent for Publication

All authors are requested to consent for publication

## Authors' Contributions

AZ, AA and ZM performed the outline of the study, analyzed and interpreted the data regarding the examination, and was a major contributor in writing and revision of the manuscript. All authors read and approved the final manuscript.

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