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.

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 (Medeiros et al., 2015 and Zaitoun et al., 2018). *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) (Murphy et al., 1999). Based on VP6, Rotaviruses are classified into ten serogroup (A-J) (Hossain et al., 2020). *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 (Elkady et al., 2021). 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 (Amine et al., 2020). 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 (Fritzen et al., 2019). According to (Youssef, 2017 and Zaitoun et al., 2018), *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. Considering emergence of strains with variant G and P genotypes in a vaccinated herd, it is possible to confirm that there is homologous protection (Fritzen et al., 2019). 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 Method

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 (Appliedbiosystems, 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 (Jakel et al., 2007).

**Result**

**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, MW751826 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 Scour guard 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 Scour guard 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**
### Discussion

BRV is an 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 (Hassan, 2014). 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 vaccine 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 vaccine 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 reassortment because of segmented nature dsRNA genome of Rotavirus (Hassan, 2014 and Fritzen et al., 2019). 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 (Medeiros et al., 2015). These findings stress the need to further investigate any change in genotype distribution and emergence of new genotypes through sequence
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 (Rocha et al., 2017). 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 (Yang et al., 2011).

Conclusion

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 Scourguard vaccine containing can be used in Assiut governorate due to circulating of G6P5 and G10P11 strains of Rotavirus in Assiut.

Declarations

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Consent for publication: All authors are requested to consent for publication

Availability of data and materials:
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability: The code will be made available under request to the corresponding author.

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 the manuscript. All authors read and approved the final manuscript.

References
1. Amine, S.A.; Melloul, M.; El-Alaoui, M.A.; Boulahyaoui, H.; Loutf, C.; Touil, N. and El-Fahime, E. (2020): Evidence for zoonotic transmission of species A Rotavirus from goat and cattle in nomadic herds in Morocco, 2012–2014. Virus Genes.

2. Elkady, G.; Zhu, J.; Peng, Q.; Chen, M.; Liu, X.; Chen, Y.; Hu, C.; Chen, H. and Guo, A. (2021): Isolation and whole protein characterization of species A and B Bovine Rotaviruses from Chinese calves. Infection, Genetics and Evolution 89: 1–13.

3. Fritzen, J.T.T.; Lorenzetti, E.; Oliveira, M.V.; Bon, V.R.; Ayres, H.; Alfieri, A.F. and Alfieri, A.A. (2019): Cross-sectional study of the G and P genotypes of Rotavirus A field strains circulating in regularly vaccinated dairy cattle herds. Tropical Animal Health and Production 51:887–892.

4. Hassan, M.N. (2014): G and P typing and sequence analysis of group A Rotavirus in calves and lambs in Kashmir, PhD. Thesis, Faculty of Post-Graduate Studies Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, India.

5. Hossain, M.B.; Rahman, M.S.; Watson, O.J.; Islam, A.; Rahman, S.; Hasan, R.; Kafi, M.A.H.; Osmani, M.G.; Epstein, J.H.; Daszak, P. and Haider, N. (2020): Epidemiology and genotypes of group A Rotaviruses in cattle and goats of Bangladesh, 2009–2010. Infection, Genetics and Evolution 1:1-29.

6. Jakel, J.F.; Katz, D.L.; Elmore, J.G. and Wild, D.M.G. (2007): Epidemiology, Biostatistics and Preventive medicine. 3rd Edition. United Status of America. 183.

7. Malik, Y.S.; Sharma, K.; Vaid, N.; Chakravarti, S.; Chandrashekar, K.M.; Basera, S.S.; Singh, R.; Minakshi; Prasad, G.; Gulati, B.R.; Bhilegaonkar, K.N. and Pandey, A.B. (2012): Frequency of group A Rotavirus with mixed G and P Genotypes in bovines: predominance of G3 genotype and its emergence in combination with G8/G10 types. Journal of Veterinary Science 13(3): 271–278.

8. Medeiros, T.N.S.; Lorenzetti, E.; Alfieri, A.F. and Alfieri, A.A. (2015): Phylogenetic analysis of a G6P[5] bovine Rotavirus strain isolated in a neonatal diarrhea outbreak in a beef cattle herd vaccinated with G6P [1] and G10P [11] genotypes. Archives of Virology,160:447–451.

9. Murphy, F.A.; Gibbs, E.P.J.; Horzinek, M.C. and Studdert, M.J. (1999): Veterinary Virology. 3rd Edition. Academic Press, An imprint of Elsiever. California, United Status of America. 391-396 http://www.academicpress.com.

10. Rocha, T.G.; Silva, F.D.F.; Gregori, F.; Alfieri, A.A.; Buzinaro, M.G. and Fagliari, J.J. (2017): Longitudinal study of bovine Rotavirus group A in newborn calves from vaccinated and unvaccinated dairy herds. Tropical Animal Health and Production 49:783–790.

11. Yang, D.; Oh, Y.; Cho, S.; Kang, H.; Lee, K.; Kim, Y. and Song, J. (2011): Molecular Identification of the Vaccine Strain from the Inactivated Rabies Vaccine. Journal of Bacteriology and Virology 41(1): 47–54.

12. Youssef, Z.M.A.M. (2017): Enteric Rota and Corona viruses infection in neonatal calves, M.V.Sc. Thesis, Faculty of Veterinary Medicine, Assiut University, Egypt.

13. Zaitoun, A.M.A.; Abdel-Hakim, O. and Youssef, Z.M.A. (2018): Enteric Rota and Corona viruses infection in neonatal calves. Assiut Veterinary Medical Journal 64(156): 8–17.
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).

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 G6P1). Dots indicate nucleotides identity with the field strains sequences.
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.
| Sequence 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. |  |
|---------------------------------------------------------------|---|
| `MW751826_G10P11_Field` | `LC13550_G10P11_Vaccine` |  |
| 1 TAT GGGGCGGATT CAGAGGCCTT CAGAAATAT GGTATATG TGGGCTGCGCT GACGAA 119 | 1060  | _T_ _A_ _T_ _T_ _T_  |
| `MW751826_G10P11_Field` | `LC13550_G10P11_Vaccine` |  |
| 61 CTAATCAAGT GCCGG GCGAAGGAAGT CACTATT CATT CGCTGCACT GCTGAGGCT CATGG 129 | 1120  | _T_ _T_ _T_ _T_ _A_ |  |
| `MW751826_G10P11_Field` | `LC13550_G10P11_Vaccine` |  |
| 181 GCGGT GAT GCAAGGAGGAGTT CTGCTGCTAAGCTTT CGATGGGTT ATGCTACGTAAGGCA 180 | 1180  | _A_ _A_ _A_ _A_ _T_  |
| `MW751826_G10P11_Field` | `LC13550_G10P11_Vaccine` |  |
| 181 TTT ACT GACTATGT GT CGTTAATT CACTAAGAT TCAAGATT TACGAT GCGAGTAAGGCA 140 | 1120  | _G_ _C_ _G_ _G_ _G_  |
| `MW751826_G10P11_Field` | `LC13550_G10P11_Vaccine` |  |
| 241 CTTT GCTT TTAGGGTT ACCGTT AGGAGGAT ATCAAATTTT GTATGGCCCTTCACGGCGGAAC 300 | 1389  | _T_ _T_ _T_ _T_ _T_  |
| `MW751826_G10P11_Field` | `LC13550_G10P11_Vaccine` |  |
| 301 CCAATGGGAGACCCCCAATTTAT GAGGCA 330 | 1389  | _A_ _A_ _A_ _A_  |

**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.