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MOLECULAR ANALYSIS OF OUTER CAPSID PROTEIN (VP7) GENE OF ROTAVIRUS RECENTLY ISOLATED FROM NEWLY BORN CALVES IN EGYPT

AMANI ALI SALEH [1]; ABD EL HAMID, M.I.[2]; NADINE A. EL-SEBAY [2]; LUBNA F. FARAHAT [3] AND EMAN M.S. EL-NAGAR[2]

[1] Prof. of Virology, Veterinary Serum and Vaccine Research Institute (VSVRI), Agricultural Research Center (ARC). Cairo, 11381, Egypt.
[2] Genetic Engineering Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Agricultural Research Center (ARC). Cairo, 11381, Egypt.
[3] Department of Quality Management, Veterinary Serum and Vaccine Research Institute (VSVRI), Agricultural Research Center (ARC). Cairo, 11381, Egypt

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ABSTRACT

Rotavirus is a contagious viral disease causing acute watery diarrhea. It has a potential zoonotic and economic importance in calves under one month of age, widely. So during early January 2024**,** a total of 46 diarrheic samples were collected from dairy calves (2-4 weeks old) in El-Sharqia, Giza, New Valley (El-Kharga Oasis), and El-Beheira governorate. Tissue culturing, serological (ELISA), and molecular identification based on sequencing analysis of the VP7 gene of Rotavirus A (RVA) were done. Among the 46 diarrheic samples, only 24 samples (52%) were RVA-positive by ELISA and negative for both coronavirus and *E. coli*. RVA was successfully isolated on MDBK and VERO cells, as an obvious cytopathic effect was produced at the first and second passages of both cell lines, respectively. Haemagglutination **(**HA) activity of positive samples ranged from 1:4 to 1:16. Molecular and sequencing analysis of the VP7 gene revealed that the four sequenced rotavirus isolates belong to Bovine Rota Virus A (BoRVA), Genotype 6, with 100% identity to each other and 98.26% similarity with the local Egyptian isolates, clustered in the G6 lineage with the Argentina, USA (Scour Guard vaccine), Ireland, and Indian BoRVA strains with identity matrices of 95.42%, 93.4%, 93.31%, and 90.49%, respectively. Conclusively, the Egyptian BoRVA G6 strains are widely circulating in Upper and Lower Egypt among dairy calves. It is recommended to evaluate those strains together with G8 and G10 as vaccine candidates in the locally prepared vaccine to confer full protection against BoRVA circulating in Egypt.

Keywords: Genotype 6, MDBK, Rotavirus, VERO, VP 7 gene.

INTRODUCTION

Rotavirus A (RVA) causes extensive economic losses due to the high rates of morbidity and mortality, treatment costs, and slowed growth of infected animals; high instances of newborn calf diarrhea are related to bovine rotavirus A (BRVA) infection leading to acute diarrhea in the early stages of calves' accounts for about 75% of early calf mortality in the dairy sector. (Cho *et al.,* 2014; Wang *et al.,* 2024).

Corresponding author: Abd El Hamid, M.I.
E-mail address: mohamedvsvri@gmail.com
Present address: Genetic Engineering Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Agricultural Research Center (ARC). Cairo, 11381, Egypt.

Rotaviruses (RV) are considered the most important member of the *Reoviridae* family. The genome of rotaviruses is made up of eleven double-stranded RNA (dsRNA) segments that are encapsed in a trilaminar capsid (ESTES, 2001). RV encoded six structural proteins (VP1, VP2, VP3, VP4, and VP6) and six non-structural proteins (NSP1 to NSP6) (Matthijnssens *et al*., 2009). RV is categorized into ten groups (A-J) according to the antigen specificity of the VP6 gene (Babalola *et al.,* 2020) since the VP6 gene sequence is highly conserved and exhibits high levels of antigenicity and immunogenicity and induces the production of the specific mucosal antibodies IgG and IgA, thus making it commonly used in virus detection (Zhou *et al*., 2010). Kalica *et al.* (1983) identify the VP4 gene of RVA to be code for hemagglutination (HA) activity and protease-enhanced plaque formation. The most common group that has an impact on both people and animals is group A. Group A rotaviruses are further classified into multiple G and P types according to the molecular and antigenic characterization of the VP7 and VP4 genes, respectively (Barbosa *et al.,* 2013). Currently, there are 36 G serotypes and 51 P genotypes are determined (Rojas *et al.,* 2017; Elkady *et al.,* 2021). The most epidemiologically significant rotavirus genotypes globally are G6, G8, and G10 (linked to P1, P5, and P11) (Bertoni *et al.,* 2020 and Elkady *et al.,* 2021).Routine laboratory tests for early detection of BRV depend on virus isolation, the enzyme-linked immunosorbent assay (ELISA), and the polymerase chain reaction (PCR) (Lucchelli *et al.,* 1994; de Beer *et al.,* 1997). Since ELISA has a shorter test duration than virus isolation (VI) and is more sensitive in detecting rotavirus group A antigen (Benfield *et al.,* 1984), it is utilized in the majority of diagnostic laboratories (Verschoor *et al.,* 1990). RT-PCR is an alternate method that can be used to identify and characterize rotaviruses. In clinical specimens, the PCR approach has shown effective in detecting animal and human rotaviruses (Gouvea *et al.,* 1990). Since the genotypes of circulating RV strains can be altered over time and with the geographical area of sample collection (Badaracco *et al.,* 2013), throughout this investigation, the circulating BRVA was identified and analyzed based on the analysis of the VP7 gene isolated from calves suffering from acute diarrhea from four Egyptian governorates.

MATERIALS AND METHODS

Ethics approval:
Aside from fecal sampling of diarrhea calves, no animal studies were conducted in this study, and the sample collection work was accepted by the farm owner. During the collection of the diarrheic samples, all ARRIVE guidelines were followed with no harm to the infected calves.

Sample collection:
A total of 46 diarrheic samples were collected from dairy calves (2-4 weeks old) in El-Sharqia, Giza, New Valley (El-Kharga Oasis), and El-Behira governorate in early January 2024. All samples were transported on ice and stored at -80°C.

Cell line:
MDBK and VERO cell lines were used for virus isolation. Cells were kindly provided by the Veterinary Serum and Vaccine Research Institute. They were grown in 80 cm^2 Nunc flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) with 100 U/ml penicillin G and 100 µg/ml streptomycin. Cells were incubated at 37°C until confluence. For viral infection, cells were used in a 25cm^2 flasks in order to have cell confluences (24 h) (Ates and Yesilbag 2023).

Detection of BoRVA and co-infection with other diarrhea-causing agents using ELISA:
The 46 fecal samples from neonatal calves with diarrhea from four governments including, El-Sharqia, Giza, El-Kharga Oasis, and El-Behira, were screened for the presence

of almost all causes of diarrhea in calves using a commercially available and most reliable ELISA kit (IDEXX Rota-Corona-K99, Ag Test, Cat. No. P00603-1) to detect the presence of Bovine Rota Virus, Bovine Coronavirus, and *E. coli* K99. Samples were tenfold serially diluted using the dilution buffer supplied by the kit, and each sample was distributed in a triplicate manner against the antibodies already coated in the plate against the previously mentioned organisms that cause calf diarrhea and incubated for 30 minutes at room temperature. Then the steps were followed according to the kit manufacturer's instructions, and consequently, the positive samples of rotavirus were used for RT-PCR and sequencing (Park *et al.,* 2008; Karayel *et al.,* 2017).

Tissue culture isolation and propagation:
Three samples from each governorate (12 samples total) were isolated on tissue culture. Two ml of each diarrheic sample were added to a falcon tube containing 6 ml of sterile PBS with glass beads, centrifuged at 2500 rpm for 30 minutes, and filtered. One ml of the filtrate was inoculated into confluent monolayers of MDBK and VERO cells. After adsorption at 37°C for 1 hr., the cells were washed with plain DMEM and then overlayered with maintenance medium containing 1 µg/ml of crystalline trypsin and incubated at 37°C in a CO2 incubator. One uninoculated flask was kept as a control. Three successive passages were done for virus isolation and propagation.
(Ates and Yesilbag 2023)

Haemagglutination assay (HA):
Since most BoRVA strains can agglutinate erythrocytes, the viral isolates from the three passages (MDBK and Vero cells) were tested for their ability to agglutinate chicken red blood cells (RBCs). 50 µl of cell culture supernatant from each passage and 50 µl of 10% chicken RBCs were mixed on the HA plate and kept for 30 min at room temperature to evaluate the presence of hemagglutination character (Ennima *et al.,* 2016).

RNA extraction and reverse transcription polymerase chain reaction (RT- PCR) to detect Rotavirus VP7 gene in the fecal samples:
The viral RNA was extracted from both positive serologically and tissue culture fecal samples using a PureLink RNA Mini Kit (Thermo scientific, cat. no. 12183018A), according to the manufacturer's instructions and the extracted RNA was stored at -80ºC until use. RT-PCR was performed to detect the rotavirus VP7 gene in the fecal samples following the instructions of the one-step RT-PCR Kit (DiaStar, cat. no. DR61-K050, Solgent for Genetic Technology) using 10 pmol forward and reverse primers targeting 884 bp of VP7.VP7-F (nucleotides 49 to 71) ´5ATGTATGGTATTGAATATACCAC 3´, and VP7-R (nucleotides 914 to 933) ´5AACTTGCCACCATTTTTTCC3´ (Gómara *et al.,* 2001). The first round of amplification of the Vp7 gene consisted of cycles of 30 min at 50°C and 15 min at 95°C. The second round of amplification consisted of 35 cycles of 20 sec at 95°C, 40 sec at 52°C, and 1 min at 72°C, followed by a final extension at 72°C for 5 min. PCR products were loaded on a 1% agarose gel containing ethidium bromide (10 mg/ml) in 1x tris-acetate-EDTA buffer along with the geneRuler 1kb DNA ladder ready to use (Thermo-Scientific #SM0314) and shown on a UV transilluminator.

Specific amplicon purification and sequencing:
The PCR products of the tissue culture-isolated virus, corresponding to the identified rotavirus, were purified using the Quiaquick extraction kit (Qiagen, Cat. No. 28704) following the manufacturer's instructions. All materials needed for the sequence reaction were supplied by the GATC Company, Germany, using the ABI 3730xI DNA sequencer (Applied Biosystem, USA) and the nucleotide sequencing was performed by Sanger sequencing technique (Sanger and Coulson, 1975).

Sequence analysis and phylogeny.

The VP7 sequence was assembled using SeqMan software (version 7.0; DNASTAR Inc., USA) and compared with sequences accessible on GenBank using BLAST. The nucleotide homologies and deduced amino acid sequences were determined using the MegAlign program of DNASTAR 7.0 software (DNASTAR Inc.). Phylo-genetic trees based on amino acid sequences were constructed using the maximum likelihood method. Bootstrap analyses were accomplished based on 1000 replicates.

Nucleotide sequence accession numbers:

The VP7 nucleotide sequences data have been submitted to GenBank under the following accession numbers: PP818398, PP818399, PP818400, and PP818401.

RESULTS

Detection of BoRVA and co-infection with other diarrhea-causing agents using ELISA:

Among the 46 diarrheic samples from dairy calves, 24 (52%) were found to be BoRVA-positive by ELISA and negative for coronavirus infection and *E. coli*.

Virus isolation and HA activity:

RVA was successfully isolated on MDBK and VERO cells; obvious CPE was produced at the first, second, and third passages, respectively. The changes in MDBK and VERO cell lines were typical of rotavirus infection. Both cell lines showed vacuolation, rounded, and clumping cells at 24-48 hr post virus inoculation. Detachment of the cells was clear 72 hr post-infection in the two cell lines (Fig 1). The isolated rotavirus showed positive HA activity, and the titer of positive samples ranged from 1:4 to 1:16. However, sample number 28 from the Giza governorate didn't show any CPE on both cell lines during the first and the second passage, but at the third passage, a clear CPE was observed only on MDBK cells and was HA negative (Table 1).

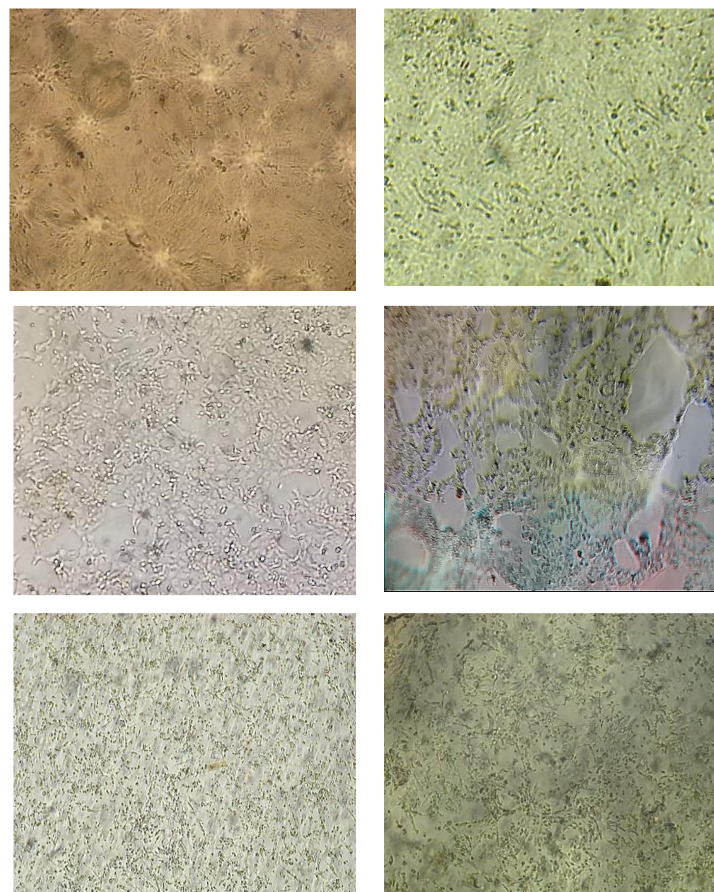

Fig .1. A) Normal MDBK, **B**) Normal VERO cells, **C, D)** MDBK and VERO monolayers 48 hr. post infection showed vacuolation and clumping of the cells, **E, F)** MDBK and VERO monolayers 72 hr. post infection showed rounded, degenered, and detached cells.

Table 1: Recorded data from 12 (3 representative samples from the four governorates) fecal samples collected during 2024.

Samples ID.	Calves age (weeks/ days)	Governorate	(HA) test	Cell culture passages (P)		
				P1 MDBK/VERO	P2 MDBK/VERO	P3 MDBK/VERO
Sh.4	3.1	El Sharqia	1:4	+ve / -ve	+ve / +ve	+ve / +ve
Sh.7	2.2	El Sharqia	1:4	+ve / -ve	+ve / +ve	+ve / +ve
Sh.11	2.1	El Sharqia	-ve	-ve / -ve	+ve / -ve	+ve / +ve
Kh.14	2.4	Kharga Oasis	1:16	+ve / -ve	+ve /+ve	+ve / +ve
Kh.19	3.2	Kharga Oasis	1:8	+ve / -ve	+ve / +ve	+ve / +ve
Kh.23	3.0	Kharga Oasis	1:8	-ve / -ve	+ve / +ve	+ve / +ve
Gi.28	2.6	Giza	-ve	-ve / -ve	-ve / -ve	+ve / -ve
Gi.29	2.2	Giza	1:16	+ve / -ve	+ve / +ve	+ve / +ve
Gi.30	2.0	Giza	1:16	+ve / -ve	+ve / +ve	+ve / +ve
Be.33	3.2	Beheira	1:4	-ve / -ve	+ve / +ve	+ve / +ve
Be.38	3.4	Beheira	1:4	+ve / -ve	+ve / -ve	+ve / +ve
Be.44	3.3	Beheira	1:16	+ve / -ve	+ve / +ve	+ve / +ve

P1: First passage, P2:2nd passage, P3: Third passage, HA: Haemagglutination activity

RT- PCR to detect the Rotavirus VP7 gene in the fecal samples:

All tested 24 samples and tissue culture-isolated virus showed positive RT-PCR targeting 884 bp of the VP7 gene as shown in Fig. (2).

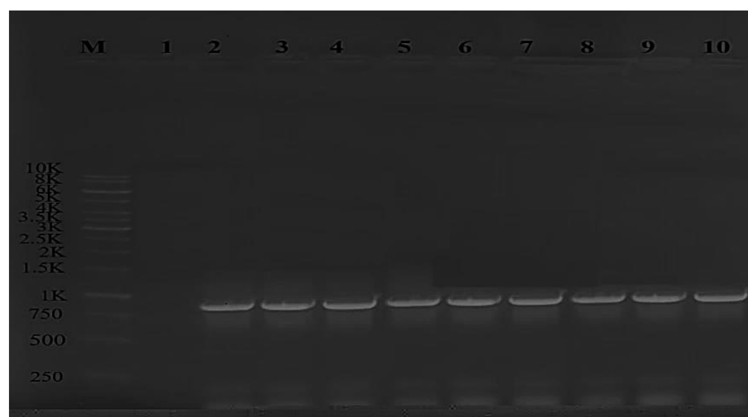

Fig. 2: Representative PCR amplification of the isolated RVA samples (lane 2 to lane 6 from diarrheic samples, lane 7 to lane 10 from tissue culture isolation) showed a clear band at 884 bp corresponding to the target primer sequence. Lane 1 control negative, M. 1Kb DNA ladder

Sequencing analysis and phylogeny:

Fig .3. Multiple alignment of the deduced amino acid sequence from residue number 1 to 150 of Vp7 outer capsid glycoprotein of the local isolates Oasis/G6/RVA/VSVRI/2024, Giza/G6/RVA/VSVRI/2024, Sharqia/G6/RVA/VSVRI/2024 and Behira/G6/RVA/VSVRI/2024, with other Egyptian isolates of the G6 of Bovine Rota Virus type.

Fig .4: Multiple alignment of the deduced amino acid sequence from residue number 151 to 274 of Vp7 outer capsid glycoprotein of the local isolates Oasis/G6/RVA/VSVRI/2024, Giza/G6/RVA/VSVRI/2024, Sharqia/G6/RVA/VSVRI/2024, and Behira/G6/RVA/VSVRI/2024, with other Egyptian isolates of the G6 of Bovine Rota Virus type.

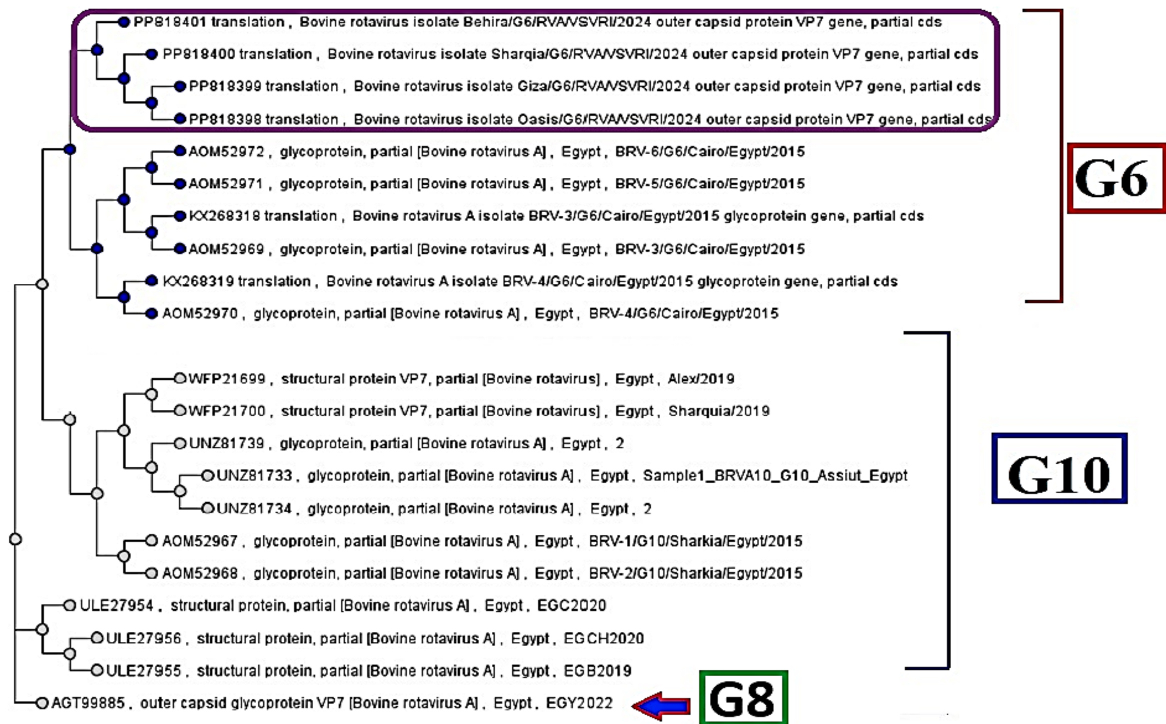

Fig. 5: Phylogenetic analysis based on genotyping of the deduced amino acid sequence of the Vp7 glycoprotein of the local isolates Oasis/G6/RVA/VSVRI/2024, Giza/G6/RVA/VSVRI/2024, Sharqia/G6/RVA/VSVRI/2024, and Behira/G6/RVA/ VSVRI/2024 using the neighbour-joining method with 1000 bootstrap replicates with other Egyptian isolates available on GenBank.

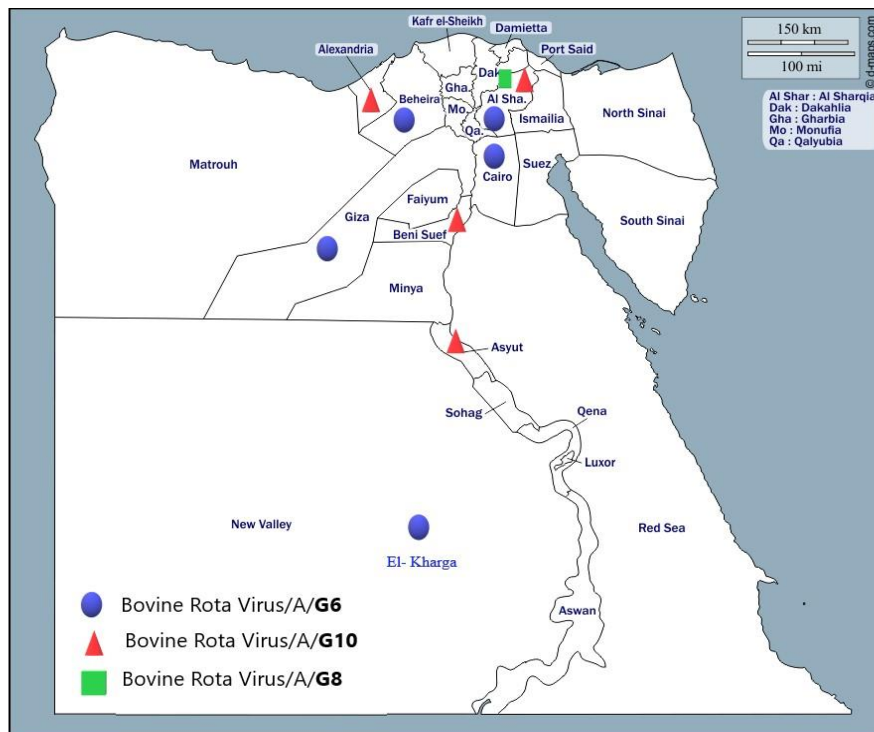

Fig. 6: Geographical distribution of Bovine Rota virus in different governorates all over Egypt is based on the amino acid sequence of the Vp7 glycoprotein of the local isolates and other Egyptian isolates according to the data available on GenBank, where the blue circles represent the G6 (representing the isolates of the current study), the red cones represent G10, and the green square represents G8.

Fig. 7: Phylogenograhtic analysis based genotyping of the deduced amino acid sequence of the Vp7 glycoprotein of the local isolates Oasis/G6/RVA/VSVRI/2024, Giza/G6/RVA/VSVRI/2024, Sharqia/G6/RVA/VSVRI/2024, and Behira/G6/RVA/ VSVRI/2024, using the neighbor joining method with 1000 bootstrap replicates with other strains available on Genbank.

	ABG35512	ABG35513	ADC80496	ADO51637	KX268318 t...	KX268319 t...	PP818398 t...	PP818399 t...	PP818400 t...	PP818401 t...	UNZ81738
ABG35512		99.69%	96.01%	92.47%	96.09%	95.70%	95.42%	95.42%	95.42%	95.42%	97.64%
ABG35513	99.69%		96.32%	92.47%	96.09%	95.70%	95.42%	95.42%	95.42%	95.42%	97.64%
ADC80496	96.01%	96.32%		90.41%	95.22%	94.53%	93.31%	93.31%	93.31%	93.31%	96.70%
ADO51637	92.47%	92.47%	90.41%		90.87%	90.23%	90.49%	90.49%	90.49%	90.49%	91.98%
KX268318 translation	96.09%	96.09%	95.22%	90.87%		99.13%	98.26%	98.26%	98.26%	98.26%	94.63%
KX268319 translation	95.70%	95.70%	94.53%	90.23%	99.13%		97.66%	97.66%	97.66%	97.66%	93.40%
PP818398 translation	95.42%	95.42%	93.31%	90.49%	98.26%	97.66%		100%	100%	100%	93.40%
PP818399 translation	95.42%	95.42%	93.31%	90.49%	98.26%	97.66%	100%		100%	100%	93.40%
PP818400 translation	95.42%	95.42%	93.31%	90.49%	98.26%	97.66%	100%	100%		100%	93.40%
PP818401 translation	95.42%	95.42%	93.31%	90.49%	98.26%	97.66%	100%	100%	100%		93.40%
UNZ81738	97.64%	97.64%	96.70%	91.98%	94.63%	93.40%	93.40%	93.40%	93.40%	93.40%	

Table 2: Showing the percentage of identity based on the deduced amino acid sequence of the Vp7 glycoprotein between the local isolates Oasis/G6/RVA/VSVRI/2024, Giza/G6/RVA/VSVRI/ 2024, Sharqia/G6/RVA/VSVRI/ 2024, and Behira/G6/RVA/VSVRI/2024 against the other G6 isolates, which are grouped in the same cluster.

DISCUSSION

Group A rotaviruses are considered one of the most important etiological agents of diarrhea in newborns of many animal species all over the world (Ates and Yesilbag, 2023). Detection of rotavirus infection in newborn calves is an important role in controlling the disease and preventing economic losses. RVA detection can be accomplished by many methods, including virus isolation in cell culture, ELISA, RT-PCR, and electron microscopy (EM) (Ennima *et al.,* 2016). During this study, ELISA and RT-PCR were successfully used for RV identification

following previous studies of (Ates and Yesilbag 2023). Those positive samples were sequenced and typed using the phylogeny analysis. VERO and MDBK cells were used for virus detection; successful isolation was obtained by a clear CPE in the 3rd and 2nd passages, respectively, and this is in accordance with Ates and Yesilbag 2023, where a clear CPE was produced at all passages in MA-104 but not with MDBK cells. Various studies are showing the hemagglutination activity of some RVA strains with erythrocytes from many species (Nakagomi *et al.,*1992). In this study, the third and the second passages of RV isolates on VERO and MDBK cells showed HA activities when evaluated with chicken RBCs, respectively, while sample number 28 from the Giza governorate didn't show any CPE on both cell lines during the first and the second passage. In the third passage, clear CPE was observed only on MDBK cells, and it was HA negative. These results go with the study of Ates and Yesilbag 2023, where they found that the fourth passage on MA-104 cells showed positive ELISA for RV and was negative for HA, and at the same time, they were detected as negative by BCoV Ag ELISA.

Vp7 plays a crucial role in virus infectivity, cell entry, and immunogenicity and is also used in the genotyping of the Bovine Rota Virus (Dennehy*,* 2008). The current study used the Vp7 gene for the genotyping of the isolated samples from different Egyptian governorates, which were assigned in GenBank as the following: Oasis/G6/RVA/VSVRI/2024, Giza/G6/RVA/VSVRI/2024, Sharqia/G6/RVA/VSVRI/2024, and Behira/G6/RVA/VSVRI/2024, with accession numbers PP818398, PP818399, PP818400, and PP818401, respectively, and revealed that all strains of interest in this study are related to G6, even though they have been isolated from different locations, either in Upper or Lower Egypt. The multiple sequence alignment of the deduced amino acid sequence of the Vp7 glycoprotein of the current local strains is typically the same, showing 100% identity either at the nucleotide or amino acid level, indicating that this isolate is the most predominant isolate during 2024, as it has been isolated from different sites all over Egypt. On the other hand, the multiple sequence alignment is based on the available deduced amino acid sequence of glycoprotein Vp7 of the G6 of other Egyptian isolates, as shown in Figs. (3, 4), confirming that they are mostly identical without any major amino acid shifts leading to antigenic differences between them, even though the a difference in spatial or periodical isolation of these strains with other Egyptian isolates. This result greatly matches the epidemiological study of (Ghonaim *et al.* 2023), where they proved that G6 was the predominant genotype in calves, followed by G10.

Geographically based phylogenetic analysis using the neighbor-Joining method of the glycoprotein Vp7 of all isolated strains in Egypt, as shown in Fig. (5), revealed that Egypt has three main genotypes (G6, G8, and G10), which are distributed in different clusters and are distributed all over the Egyptian governorates as shown in figure (6), this is according to the submitted data available in Genbank related to the amino acid sequence of the Vp7 glycoprotein of the Egyptian isolates, and as shown in the map, G6 exists in Cairo, Giza, Behira, Sharqia, and New Valley (El Kharga Oasis), while G10 exists in Alexandria, Asyut, Sharqia and BeniSuef, while G8 was recorded only in Sharqia Governorate, and as shown in fig. (6). Since previous molecular and phylogenetic studies of (Mohamed *et al.* 2017), the presence of G6 and G10 bovine rotavirus in Sharqia was emphasized and G8 as well,

From the epidemiological point of view, to assess the ancestral origin of the local isolates in the current study and the other

Egyptian isolates, a phylgenographic analysis was carried out in a wider scope to compare between the local Egyptian isolates and other isolates around the world based on the available amino acid sequences of the glycoprotein Vp7 for genotyping the Egyptian isolates of the current study and the other G6 of Egyptian isolates (Bovine rotavirus A isolate BRV-3/G6/Cairo/Egypt/2015 and Bovine rotavirus A isolate BRV-4/G6/Cairo/Egypt/2015 accession number#KX268318 and KX268319, respectively) are clustered with G6 and are showing the highest identity (98.26%), which confirms that this G6 is the most prevalent in Egypt due to the highest identity between them, while isolates from the USA accession number #UNZ81738, which is the source of the Calf Guard vaccine produced by Zoetis (a modified live virus vaccine used for routine vaccination of animals in Egypt), (Ireland Accession No# ADC80496), (Argentinean strain "B180/ Bo/Arg" accession No# ABG35512 and strain "B187/Bo/Arg" accession No# ABG35513), and the Indian strain "Bov/Ind/UP/10/ME-8(RV-A)" accession No# ADO51637 were similar to our strains with identity matrices of 93.4%, 93.31%, 95.42%, and 90.49%, respectively. So the local isolates of the current study may have an ancestral origin with the isolate of Argentina, as they are showing the highest identity percentage, 95.42%. Egypt imports live animals and animal byproducts and dairy products from these countries, which are considered to be a potent source for virus transmission, especially live animals that come by shipping and are slaughtered here. This result is emphasized by Louge Uriarte *et al.* (2023) during the Evolutionary and Spatio-Temporal Dynamics of G6 Lineage of RVA in American calves, where their study supports how bovine G6(IV) strains emerged first in the USA; on the other hand, bovine G6(III) strains emerged later in Argentina.

Regarding other RVA genotypes like genotype G8 and G10, during molecular analysis it was very clear that Egyptian G8 were clustered with isolates of South Korea and India, while Egyptian G10 clustered with isolates from Ireland and India, besides the isolated G6 RVA strains during this study that also showed clustering with both India and Ireland RVA strains, indicating that it is imperative to determine if those two countries are the ancestors of the Egyptian RVA isolates.

Conclusion

it is recommended to achieve a molecular and sequencing analysis of the other isolated strains of this study, which may reveal the presence of other genotypes other than the recorded G6, yet it is strongly recommended that the three genotypes (G6, G8, and G10) should be represented in the locally produced vaccine to confer better protection against the Bovine Rota Virus A in Egypt)

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Competing interests

The authors declare that they have no competing interests.

Author contributions

Amani and Lubna designed the study and isolated the virus and adapted it to MDBK and VERO.
Eman and Nadine performed the molecular identification by PCR, sample preparation for sequencing and writing the manuscript.
Abd El Hamid, performed VP7 gene computational analysis and Genbank submission and data interpretation.

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التحليل الجزيئي لجين البروتين الخارجي (VP7) لفيروس الروتا المعزول حديثا من العجول حديثه الولادة فى مصر

أمانى على صالح ، محمد إبراهيم عبد الحميد سيد ، نادين عادل محمد ، لبنى فرحات ، إيمان محمد سامى النجار

Email: mohamedvsvri@gmail.com Assiut University web-site: www.aun.edu.eg

فيروس الروتا هو مرض فيروسي معدي يسبب الإسهال المائي الحاد. وله أهمية حيوانية واقتصادية محتملة في العجول التي يقل عمرها عن شهر واحد، على نطاق واسع. خلال أوائل يناير ٢٠٢٤، تم جمع إجمالي ٤٦ عينة إسهال من مزارع تربية عجول الألبان واسعة النطاق (عمرها أقل من ٣ أشهر) في الشرقية والجيزة والواحة الخارجة بمحافظه الوادي الجديد ومحافظة البحيرة. عزل زراعة الأنسجة وتحديد الهوية الجزيئية والمصلية (ELISA) بناءً على التحليل الجزيئي والتسلسلي لجين VP7. من بين ٤٦ عينة إسهال، تم العثور على ٢٤ عينة فقط (٥٢٪) إيجابية لـ BoRVA بواسطة ELISA وسلبية لعدوى فيروس كورونا والإشريشيا القولونية. تم عزل RVA بنجاح على خلايا MDBK وVERO؛ تم إنتاج CPE الواضح في نوعي الخلايا الأول والثاني على التوالي. يتراوح نشاط HA وعيار العينات الإيجابية من ١:٤ إلى ١:١٦. كشف التحليل الجزيئي والتسلسلي لجين VP7 أن جميع فيروسات الروتا المعزولة تنتمي إلى النمط المصلي لفيروس الروتا A. وكشف التحليل الجزيئي والتسلسلي لجين VP7 أن جميع فيروسات الروتا الأربعة المتسلسلة تنتمي إلى فيروس الروتا البقري النمط الجيني ٦ (G6)، وأظهرت هوية ١٠٠٪ عند مستويات النوكليوتيدات والأحماض الأمينية المتبقية، كما تم تجميعها في سلالة G6 مع سلالات BoRVA الهندية والأرجنتينية وأيرلندا والولايات المتحدة الأمريكية (لقاح Scour Guard الامريكي) بتماثل 95.42% و 90.49% , 93.4 % ,93.31% على التوالي مع العترات المعزولة. وخلصت نتائج هذه الدراسة إلى أن عزلات BoRVA G6 المصرية منتشرة على نطاق واسع في صعيد مصر والوجه البحري بين عجول الألبان. يوصى بتقييم تلك العزلات مع G8 وG10 كمرشحين للقاح في اللقاح المعد محليا لمنح الحماية الكاملة ضد فيروس الروتا البقري A المنتشر في مصر.