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SHORT COMMUNICATION

Close Relationship of Group A Rotaviruses between Bovine and Human Based onVP7 Gene Sequence in Egypt

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A B S T R A C T

The study investigated the genetic relatedness between bovine and human group A rotaviruses. Ninety four human stool and calf fecal samples (47, each) were collected from different localities in Egypt and tested by rotavirus group A enzyme immunoassay. Only twenty five fecal samples (26.5%) were positive. Viral isolation and RT-PCR amplification of full length VP7 gene was performed for the reacted fecal samples. VP7 genes were sequenced for one isolate each from diarrheic human and calf. Human EGY2012 and bovine EGY2022 rotavirus strains displayed the strongest identity of their VP7 genes [95.3% nucleotide (nt), 97.6% amino acid (aa)]. The antigenic regions A, B, and C of its VP7 genes were analogue. The high homology of two studied strains explained the interspecies transmission and its zoonotic hazard. Further rotavirus P and G genotyping of human and different animal species are needed to study genetic diversity of human rotaviruses in Egypt.

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INTRODUCTION

Group A rotavirus (GARV) is one of the major causative agents of diarrhea in children (Benhafid *et al*., 2013) and in neonatal calves (Martella *et al*., 2010). In Egypt, rotavirus was successfully isolated and identified from diarrheic calves for the first time since 1981. Rotavirus usually infects calves within the first 4 weeks of age and causes economic losses associated with death and decrease in body gain. Moreover, GARVs are associated with 600,000 deaths per year in children especially in developing countries. These GARVs are transmitted mainly via fecal-oral and air-borne routes, having a zoonotic hazard (Martella *et al*., 2010). Rotaviruses are non-enveloped viruses belonging to the genus *Rotavirus* in the family *Reoviridae*. The segmented dsRNA is surrounded by a double icosahedral protein capsid and encodes VP1-VP4, VP6, VP7and NSP1-NSP6 proteins. The segmented rotavirus genome allows reassortment in mixed infection and emergence of novel serotypes. Some human rotaviruses contain genomic segments of bovine rotaviruses as a result of direct transmission to human or reassortment (El Sherif *et al*., 2011). The aim of this study was an attempt to investigate the genetic relatedness and interspecies transmission of bovine and human GARV strains based on sequence analysis of gene encoding VP7 in Egypt.

MATERIALS AND METHODS

Forty seven each of human stool and calf fecal samples were collected from different localities in Egypt. Samples were suspended in PBS containing 1000 IU penicillin, 1000 µg streptomycin, 2000 µg gentamycin and 250 µg nystatin/ml to 10% as a final concentration. The centrifugation of sample suspensions at 2000 rpm/10 min was carried out and then stored -70°C until use. The fecal samples were screened for GARV antigen using a double antibody sandwich ELISA kit (Bio-X Diagnostics, Belgique) following the manufacturer's protocol. The positive GARV antigens were adapted to grow in an Embryonic Rhesus Monkey Kidney (MA-104) cell culture (VACSERA, Egypt). The incubation of rotavirus suspensions, with 10 µg/ml trypsin, was performed for 60 minutes prior inoculation into cell cultures. The infected cell cultures were incubated at 37°C for 5 days and then examined for cytopathic effect (CPE).

Also, viral genome was extracted from 200 µl of virus-infected cell culture fluids using the GeneJET^{TM} RNA purification kit (Fermentas, USA) following the manufacturer's instructions. RT-PCR amplification was achieved using VersoTM 1-Step RT-PCR Kit (Fermentas) with aid of the manufacturer's instructions in an Eppendorf thermal cycler (MWG). Primers previously described by

(Falcone *et al*., 1999) were used to amplify the VP7 genes (forward primer: 5'-GGC TTT AAA AGA GAG AAT TTC CGT CTG G-3' & reverse primer: 5'-GGT CAC ATC ATA CAA TTC TAA TCT-3'). The negative and positive controls were included in each PCR reaction and kindly obtained from Virology Department, Faculty of Veterinary Medicine, Zagazig University. Afterwards, PCR products (5µl) were separated on 1% ethidium bromide stained agarose gel electrophoresis at 120 V for 20 min. DNA Marker (1-kbp) was used as standard and the amplicons were visualized using ultraviolet light transilluminator (Spectroline).

PCR products were purified using GeneJET PCR purification kit (Fermentas). Each purified amplicon was sequenced in both forward and reverse directions using the amplification primers. The sequencing reaction was performed in an automated sequencer (Macrogen Inc., Korea ABI 3730XL DNA analyzer). Afterwards, comparative alignment and phylogenetic analysis of the sequence identity, divergence and phylogenetic relationship was performed computationally using MEGA5 program, product version 5.1. The nucleotide sequences of the human (EGY2012) and bovine (EGY2022) GARV strains were deposited into GenBank (Table 1).

RESULTS AND DISCUSSION

The GARVs are widespread in the domestic animals and play a substantial role in the introduction of novel strains into the human population. Whereas, the close contact between animal and human promotes interspecies transmission and mixed infections. The zoonotic transmission and genetic reassortment between human and bovine GARVs have been confirmed (Martella *et al*., 2010; Mukherjee *et al*., 2013). Concerning, GARV antigens that were tested in fecal samples with a double antibody sandwich ELISA kit, twenty five (26.6%) out of 94 fecal samples were found positive. The GARV antigens were positive in diarrheic calves (34.04%; 16/47) and human (19.1%; 9/47).

The reacted 25 fecal samples were grown in MA-104 cell culture. The characteristic CPE appeared in the form of cell rounding, cell detachment and lytic foci (Fig. 1). While, the control MA-104 cells were not inoculated with rotavirus appear normal. The cell culture supernatants of 25 GRRVs isolates were subjected to RT-PCR. The full length of VP7 gene yielded a product size of 1062 bp (Fig. 2).

The VP7 sequencing and phylogenetic analysis differentiated animal strains from human rotavirus strains. Thereby, we selected one isolate each from diarrheic human and calf from the same locality for sequence analysis of full-length VP7 genes using MEGA5.1 program and compared its genomic relatedness to representative 21 GARV reference strains of human and bovine origin. Accessions numbers and similarity percentage of nucleotide and amino acid sequences of studied and reference strains were provided (Table 1). In the study, human EGY2012 and bovine EGY2022 GARV strains shared a high level of similarity to VP7 genes (95.3% nt, 97.6% aa). The VP7 sequences of current bovine and human strains displayed high homologies with EGY2295 (Holmes *et al*., 1999); EGY1850 human strains (Naficy *et al*., 1999) in Egypt and with a bovine strain 86 in India (Chitambar *et al*., 2011). Of interest, VP7 nucleotide and amino acid sequences of human EGY2295 strain (Holmes *et al*., 1999) had strongest identity (98.6% nt, 95.9% aa) with human EGY2012 and (98.7% nt, 96.9% aa) with bovine EGY2022. Otherwise, EGY2012 and EGY2022 GARV strains showed low identity with a percentage ranged from 56.1-79.8% nt and 78.1-95.3% aa comparing with bovine strains (Niigata9801, MX001, B223, NCDV, 61A, WC3, RUBV319) and human strains (PA5/89, wt/BEL/B10925). The bovine and human strains of this study shared low homologies with bovine MX001 strain in mexico (Rodríguez-Limas *et al*., 2009) and with human PA5/89 strain in Italy (De Grazia *et al*., 2011). The deduced amino acid of Niigata 9801 strain showed close relatedness (93.7, 95.3%) to EGY2012 and EGY2022 strains, respectively.

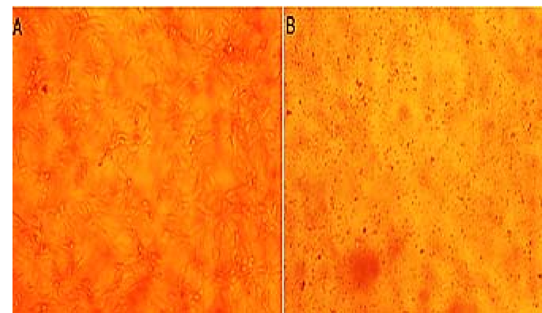

Fig. 1: Cytopathic effect of rotavirus on MA -104 cell culture. A: Normal MA-104 cell culture, showing confluent monolayer sheet. B: infected MA-104 cell culture, showing the characteristic CPE in the form of cell rounding, cell detachment and lytic foci.

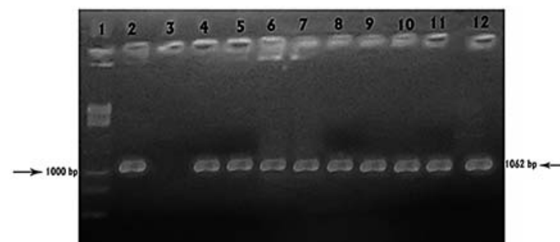

Fig. 2: Agarose gel electrophoresis of the full length VP7 gene of GARV isolates : lane 1 ;1-kbp DNA Marker; lane 2; positive control; lane 3; negative control; Lanes;4, 5, 6,7 and 8 (1062 bp full-length VP7 gene of bovine origin); lanes, 9, 10, 11and 12 (1062 bp full-length VP7 gene of human origin).

The phylogenetic relationships between two studied strains and reference GARV strains of bovine and human origin were explained using MEGA5.1 program (Fig. 3). It is interesting, the antigenic regions A, B, and C of VP7 gene in deduced amino acid sequence data were identical in the two studied strains, 86, 68, 79, 2371WCVP7C and EGY2295 strains. This was in conformity with the results reported by (El Sherif *et al*., 2011) who supported an evidence for interspecies transmission, genetic and antigenic relationship among VP7 genes for bovine and human GARV strains. Thereby, the strongest identity of human EGY2012 and bovine EGY2022 strains in this study indicated that VP7s of bovine GARV origin shared in strains of human rotaviruses. This clarified interspecies transmission of bovine GARV to human due to human in close proximity to infected farm animals in developing countries.

Table 1: Similarity Percentage of nucleotide and amino acid sequences between human EGY2012, bovine EGY2022 and representative reference GARV strains based on VP7 gene:

Rotavirus strains with accession numbers and origin				Sequence similarity (%) of virus strain			
				EGY2012		EGY2022	
Rotavirus strains	Accession number	Country	Origin	Nucleotide (nt)	Amino acid (aa)	Nucleotide (nt)	Amino acid (aa)
EGY2012	KF305320	Egypt	human	-	-	95.3	97.6
EGY2022	KF305321	Egypt	bovine	95.3	97.6	-	-
EGY1850	AF104102	Egypt	human	89.3	95	90.4	96.6
HMG035	AF359359	Nigeria	human	85.7	94	89.2	95
KY6950/02	FJ386446	Kenya	human	84.7	92.4	88.1	94
NGRBg8	AF361439	Japan	bovine	85.9	94.3	89.3	95.3
86	GU984762	India	bovine	87.2	95	90.2	95.9
68	GU984760	India	bovine	87	95.3	89.9	96.2
A5	D01054	Japan	bovine	85.3	93	87.6	94.6
79	GU984761	India	bovine	86	94.3	87.7	95.9
94H109	AB045375	Japan	human	85	94	86.1	95
2371WCVP7C	JN014000	South Africa	human	85	94	88.2	94.6
EGY2295	AF104104	Egypt	human	98.6	95.9	98.7	96.9
AU109	AB272753	Japan	human	98	94	98.1	95
Niigata9801	AB044294	Japan	bovine	76.8	93.7	79.8	95.3
MX001	FJ217204	Mexico	bovine	62	78.9	61.1	80.8
B223	X52650	U K	bovine	58.9	79.3	60.4	81.2
61A	X53403	Japan	bovine	57.1	78.1	59.3	80
PA5/89	JF793945	Italy	human	66.7	82.3	61.4	84.1
wt/BEL/B10925	EF554120.1	Belgium	human	64.8	82.6	60.6	84.1
NCDV	M12394.1	USA	bovine	57.4	80	57.4	81.2
WC3	AY050272	USA	bovine	56.1	78.9	58.5	80.8
RUBV319	EF199501	India	bovine	61.6	82.3	62.6	84.1

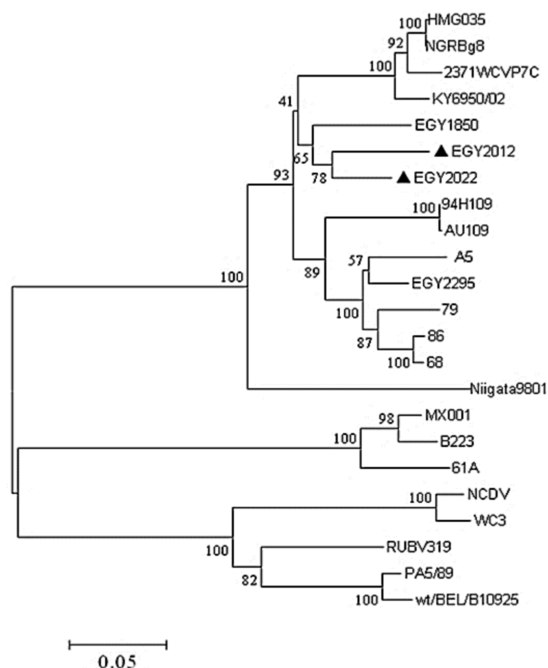

Fig. 3: Phylogenetic tree of the full length nucleotide sequences of the gene encoding VP7 (1062 bp) of GARV strains in the study, analyzed by neighbor-joining (N-J) analysis with bootstrapping (1000).

Conclusion: The strongest homology of VP7 sequences of bovine EGY2022 and human EGY2012 strains confirmed that VP7s of bovine GARV origin shared in strains of human rotaviruses. Thereby, this study evidenced an interspecies transmission of GARVs from bovine to human and supported its potential zoonotic infection. Further rotavirus VP7 and VP4 genotyping of human and different animal species are critical to emphasize the contribution of animal rotavirus to evolution and genetic diversity of human rotavirus through segment reassortment.

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