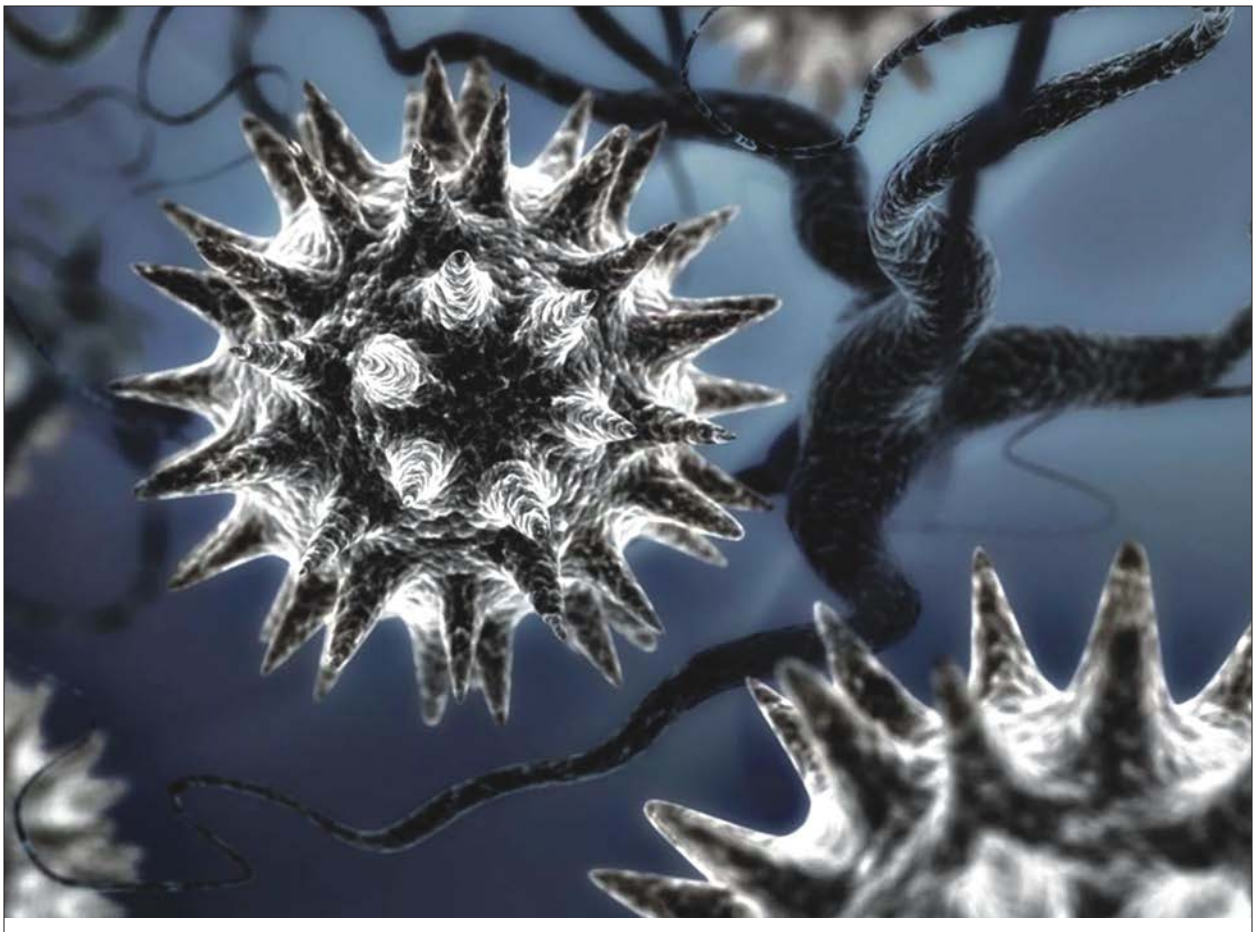

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology, 5 (1): 18-27, 2009
ISSN 1816-4900

Isolation and Antigenic and Molecular Characterization of G10 of Group A Rotavirus in Camel

[1]Eman Abo Hatab, [2]H.A. Hussein, [2]I.M. El-Sabagh and [2]M.S. Saber
[1]Department of Virology, Animal Health Research Institue, Dokki, Giza, Egypt
[2]Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

Abstract: We are here reporting the successful isolation and characterization of G10 serotype of group A rotaviruses from fecal samples collected from camel farms suffering from diarrhea in Alexandria and Esmalia Governorates. After preparation of fecal samples and inoculation on MA 104 cell line for five passages, 8 isolates were successfully isolated with a clear and reproducible CPE on the inoculated cells. The isolates were identified antigenically using *VP6*Monoclonal Antibodies (MAbs) based antigens capture ELISA that able to detect any group A rotavirus. The viral RNA was extracted from the tissue culture harvest of the propagated viruses and RT-PCR using primers specific for *VP6* and *VP7* of group A rotaviruses was employed and confirmed the molecular characterization of the isolates viruses with the correct and expected bands. The RT-PCR specific band of *VP7* gene of two selected isolates was eluted from the agarose gel and sequenced using *VP7* specific primers sequence. The obtained sequence was analyzed using computer software (BLAST) which revealed that both isolates had maximum identity to the G10 serotype of group A bovine rotaviruses ranging from 90-93%. This is the first report on the circulation of G10 serotype of group A rotaviruses in camel.

Key words: Rotavirus, characterization of G10, serotype

INTRODUCTION

Rotavirus (RV) is recognized as the single most important cause of sever acute dehydrating diarrhea in the young human and many animals species including calves (Kapikian and Chanock, 1996). High mortality and morbidity among young calves due to rotavirus disease is a serious cause of economic loss to animal farms and diary industry (Deleeuw *et al*., 1980; House, 1978; Saif and Fernandez, 1996; Woode and Bridger, 1975). The calf rotavirus infection has a worldwide distribution and associated with 40-48% of the neonatal calves (Morin *et al*., 1976; Snodgrass and Wells, 1976). The morbidity and mortality rates can reach up to 30 and 90%, respectively due to camel calf diarrhea (Schwartz and Dioil, 1992). Only few reports on viral causes of camel calf diarrhea were published (Mahin Schwers, *et al*., 1983). In general there is a lack of details study on the role of rotavirus in camel calf diarrhea.

Rotavirus is composed of triple-layered protein capsid which encloses a genome of eleven segments of double-stranded ds RNA (Estes, 1996). The genome primarily encodes six structure and six non structure protein (Estes and Cohen, 1989). VP4 and *VP7*, encoded respectively by gene segments 4 and 7, 8 or 9 depend on the strain. Specify two distinct serotype specificities termed P (protease sensitive) and G (glycoprotein) serotypes (Estes and Cohen, 1989). The intermediate capsid protein *VP6* possesses the group and subgroup specific epitopes. Rotaviruses are classified into 7 groups (A-G) based on the antigenic properties of *VP6* protein. Group A rotaviruses constitute the

Corresponding Author: Eman Abo Hatab, Department of Virology, Animal Health Research Institute, Dokki, Giza, Egypt

major pathogens in human and animals (Kapikian and Chanock, 1996). On the bases of the *VP7* and VP4 proteins and their coding nucleic acid, group A rotaviruses are classified into different serotypes and genotypes. 15 G serotypes and 16 G genotypes have been identified in diarrheic calves (Estes and Kapikian, 2007; Gulati *et al*., 2007) and there is 14 P serotypes and 27 P genotypes (Khamrin *et al*., 2007). The most predominant G serotypes in diarrheic calves in Egypt are G6 and G10 (Hussein *et al*., 1993, 1999). There is no available data on the circulating camel rotaviruses in Egypt. In this study, trial for isolation, antigenic and genetic characterization of rotaviruses in fecal samples of diarrheic camel calves was achieved.

MATERIALS AND METHODS

Fecal Samples

Eighty five fecal samples from 2 weeks till 4 months day old camel calves were collected from four different governorates in Egypt (Fayoum, Alexandria, Ismailia and Giza) during period 2004-2005. Fecal samples were tested for group A rotavirus by Mabs-based ELISA (Hussein *et al*., 1995). Positive fecal samples for RV were used for isolation trial.

Electron Microscopy

Positive fecal samples in ELISA were examined with electron microscopy (Alain *et al*., 1987) for shown the characteristic feature of rotavirus particles.

Tissue Culture

Eight positive fecal samples and tissue culture supernatant in Mabs based-ELISA were propagated after treatment with trypsin on rhesus monkey kidney (MA104) cells in the presence of 0.5 µg of trypsin per mL as described by Saif *et al*. (1988).

Extraction of Rotavirus ds RNA

The dsRNA was extracted from fecal samples using RNA extraction kit [GIBCO] according to recommended procedures that involved dissociating cells by Trizol then chloroform, isopropanol and ethanol 75% with different centrifugation then suspended the extracted RNA in nuclease free water (Chomeznski and Sacchi, 1987) the RNA suspension was kept at -85°C till used for reverse transcriptase RT-PCR.

Oligonucleotide Primers

Primers was designed according to publish database (El-Sabagh, 2006) to be used for amplification of full length *VP6* gene, the sequence of forward primer is (5'-GGCTTTTAAACGAAGTCTT CAACATGG-3') and the *VP6* reverse primer (5'-GGTCACATCCTCTCACTACGC-3') were used to amplify of 1356 bp fragment of rotavirus *VP6* gene. The *VP7* forward primer is (5'-GCGGTTAG CTCCTTTTAATGTATGG-3') and the reverse primer is (5'-GGTCACATCATATACAACCTC TAATCTAACAT-3') were used to amplify 1030 bp fragment of *VP7* gene.

RT-PCR

The RT-PCR was performed by modification according to Chang *et al*. (1996) the 30 µL of RNA were mixed with 5 µL of dimethylsulfoxide and the mixture incubated at 95°C for 5 min followed by rapid cooling on ice the denaturated ds RNA of *VP6* and *VP7* genes were amplified using one step RT-PCR kit. The first-strand cDNA synthesis was accomplish by incubating the mixture for 30 min at 47°C then at 94°C for 2 min and then 35 amplification cycles of 95°C for 45 sec (denaturation), 55°C for 45 sec (annealing), 72°C for 1.5 min (extension) and conducted followed by a (final extension

cycle) of 5 min at 72°C. The PCR products 10 µL were loaded on to agarose gel was prepared by dissolving of 1.25 g agarose gel in 100 mL (1X) TAE buffer with 0.5 µg mL^{-1} ethidium bromide. Electrophoresis was conducted for 1 h at 120 V and the gels were photographed under UV light according to Sambrook *et al*. (1989) the bands of expected correct size were cut from gel and gel slices containing DNA bands were placed in montage DNA gel extraction device. Then the eluted DNA was sent to Agricultural Genetic Engineering Research Institute (AGERI) with forward primer to be sequenced. The sequencing was analyzed by Blast computer utility of the National Center for Biotechnology and Information (NCBI) web of internet.

RESULTS AND DISCUSSION

In a total of 85 fecal samples obtained from the diarrheic calves, eight were positive for Mabs- based ELISA as shown in Table 1, then the eight positive samples were concentrated by ultracentrifugation and examined under electron microscopy as in Fig. 1.

The RT-PCR amplification of RNA extracted from fecal samples revealed a specific bands of *VP6* gene at the predicted size of 1356 bp in only one sample (No. 19) as shown in Fig. 2.

Trail for isolation of CRV from positive fecal samples of diarrheic camel calves in MA104 cell culture till 5th passage.

The propagated eight samples were identified using Mabs- based ELISA in harvested tissue culture supernatants shown in Table 2. The cytopathic behavior of the inoculated samples on MA104 cell line after five passages are shown in Table 3.

After 3rd and 5th passages, the RT-PCR using *VP6* primers was carried out on the extracted RNA from tissue culture supernatant 7 out of 8 samples produced the specific band 1356 bp as in Fig. 3 and 4.

The RT-PCR using *VP7* specific primers was carried out on the extracted RNA from tissue culture supernatant of the 5th passage. Figure 5 showing 6 out of 8 propagated samples produce the specific band of *VP7* at 1030 bp.

The RT-PCR product for *VP7* gene of two selected isolates (10, 25) was eluted from gel and sent for sequencing using *VP7* specific primers as shown in Fig. 6.

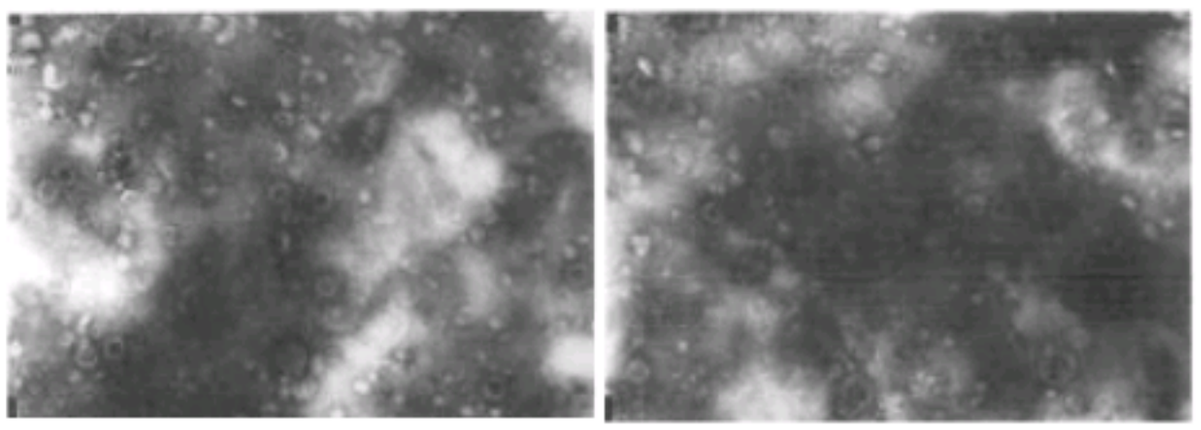

Fig. 1: The characteristic feature of rotavirus particles under electron microscopy

Table 1: Different absorbances of field fecal samples of diarrheic calves in ELISA reader

Code No. of samples	Governorate	Absorbances at wave length 450
3	Fayoum	0.219
6		0.127
10	Alexandria	1.084
19		0.453
25		1.137
33	Esmailia	0.188
34		0.227
67	Giza	0.449
Normal control		0.125

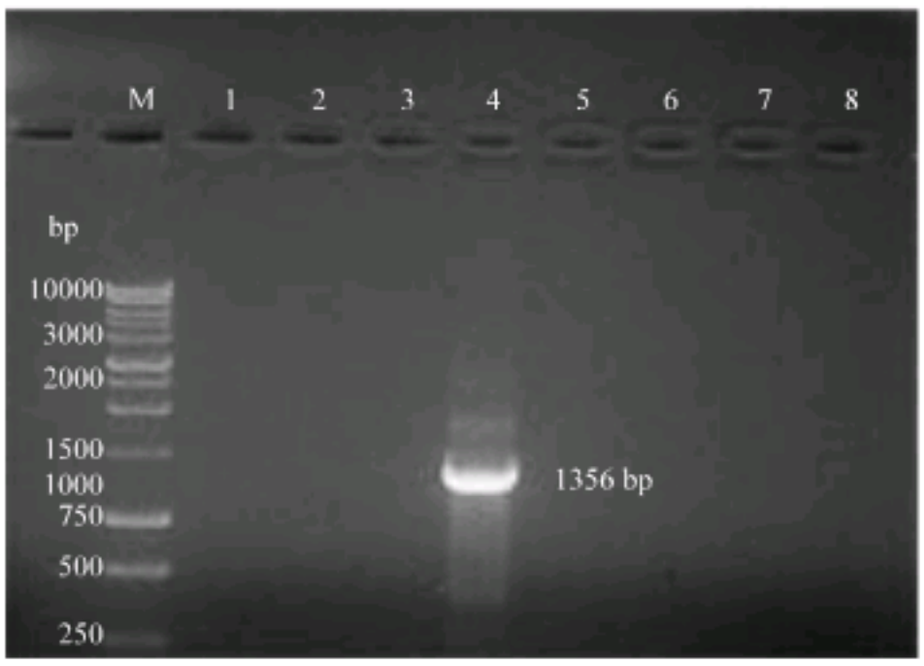

Fig. 2: Ethidium bromide stained agarose gel electrophoresis of the RT-PCR products of *VP6* gene of CRV in fecal samples along with 1 kbp DNA ladder. M: 1 kbp DNA ladder. Lane 4: The amplified product of CRV in sample No. 19. Lanes 1, 2, 3, 5, 6, 7 and 8: The negative samples for amplification

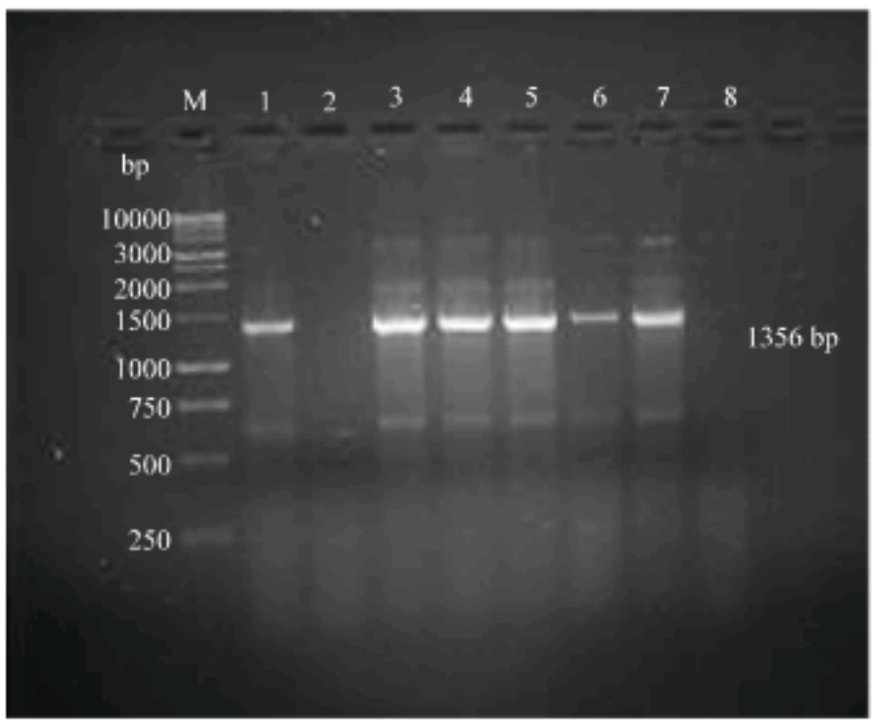

Fig. 3: RT-PCR products of *VP6* (1356 bp) of CRV isolates after 3rd passage in ethidium bromide stained agarose gel electrophoresis, along with 1 kbp DNA ladder (M) that contains 13 size bands ranged between 250 and 10.000 bp. M: Bands of molecular sizes of (10,000, 3000, 2000, 1500, 1000, 750, 500, 250 bp): Lane 1, 3, 4, 5, 6 and 7: Amplified product of correctly *VP7* gene size 1356 bp of isolates No. 3, 10, 19, 25, 33 and 34. Lane 2, 8: The negative for amplification isolates No. 6 and 67

Table 2: The optical densities of the harvested tissue culture supernatant of the eight propagated isolates in comparison with normal control

Code No. of samples	Absorbances of test samples measured by ELISA reader at wave length 450
3	0.102
6	0.112
10	0.145
19	0.111
25	0.162
33	0.115
34	0.138
67	0.203
Normal control	0.080

Table 3: The cytopathic behavior of the inoculated samples on MA104 cell line after five passages

	Degree of CPE (days post inoculutation)						
No. of samples	1st	2nd	3rd	4th	5th	6th	7th
3	+	++	+++	++++			
6	_	_	+	++	++	+++	+++
10	+	++	+++	++++			
19	+	++	+++	++++			
25	+	++	+++	++++			
33		+	++	+++	++++		
34	_	_	++	++	++	+++	+++
67	+	++	+++	++++			

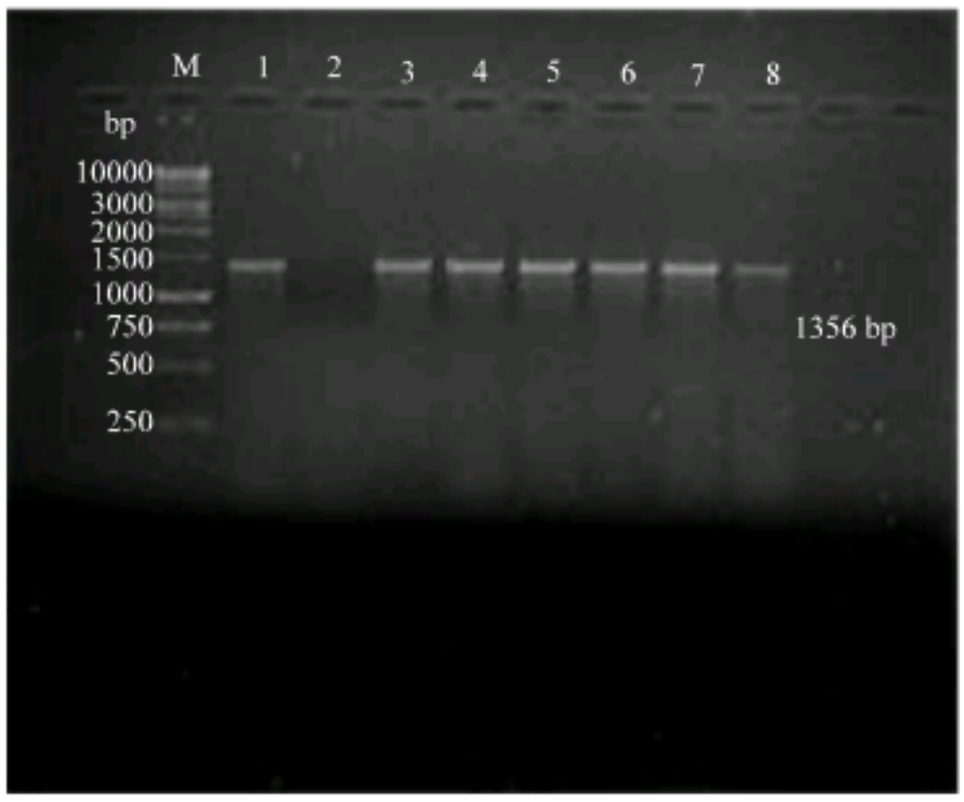

Fig. 4: RT- PCR products of *VP6* (1356 bp) of CRV propagated in tissue cell culture MA104 of 5th passage in ethiduim bromide stained agarose gel electrophoresis, along with 1 kbp DNA ladder (M). M: 1 kbp DNA ladder. Lanes 1, 3, 4, 5, 6, 7 and 8: The amplified product of *VP6* genes For the positive propagated isolates No. 3, 10, 19, 25, 33, 34 and 67. Lane 2: The negative isolates No. 6

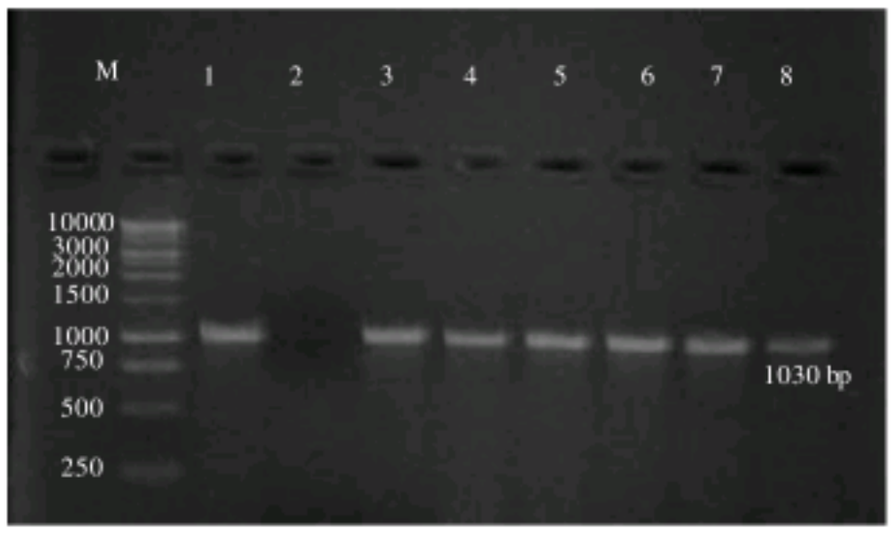

Fig. 5: RT-PCR products of *VP7* (1030 bp)of 5th passage of propagated isolates in MA104 cells of CRV in ethidium bromide stained agarose gel electrophoresis, along with 1 kbp DNA ladder M: 1 kbp DNA ladder. Lanes 1, 3, 4, 5, 6, 7 and 8: The amplified product of *VP7* genes for the seven propagated isolates No. 3, 10, 19, 25, 33, 34 and 67. Lane 2: The negative isolate No. 6

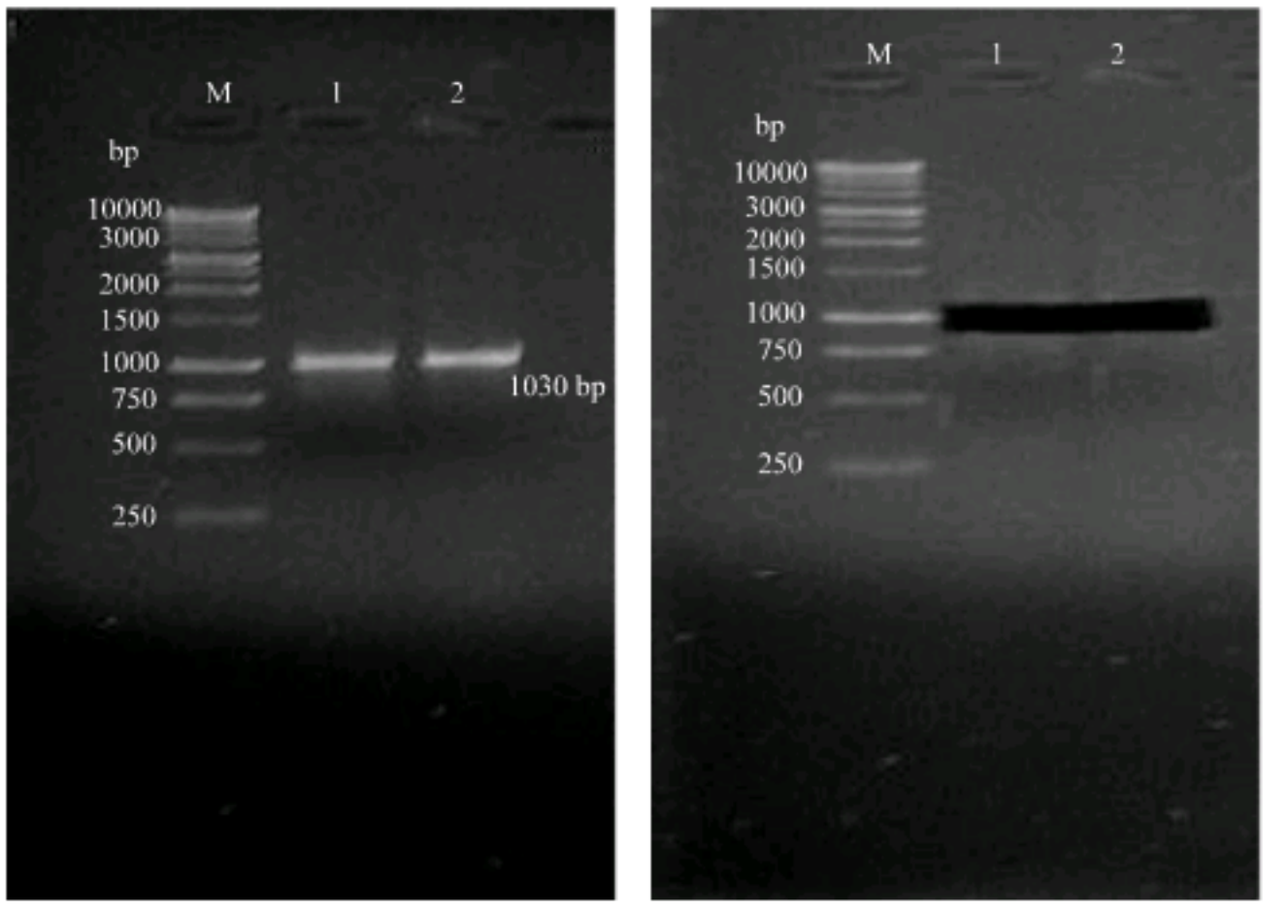

Fig. 6: Ethidium bromide stained agarose gel before and after cutting of the amplified *VP7* gene of CRV isolates coded 10 and 25 to be eluted and send for sequencing. M: 10,000 bp; Lane 1 and 2: The amplified bands of correct size (1030) of *VP7* gene of CRV

Analysis of the Nucleotide Sequence of *VP7* Gene Fragments of CRV

The purified DNA representing the outer capsid *VP7* gene of CRV was 1030bp in size only sequence of 580 bp as obtained. The obtained nucleotide sequence shown in the following box:

TTGANTCCCATCCTACTCTTGATTNTCTTATCTATGGATTCATATTAATG
AGTATAACTAGAATGATGGACTACATAATTTATAAATTTTTNGCTTATAG
TCACGATCACTTCAATTGTTGTTAACGCACAAAATTACGGTATCAATTTA
CCAGTAACTGGATCGATGGATATGTCATATGTGAACGTTACTAAAGATGA
GCCATTTCTAACATCAACATTATGTTTATACTATCCAACAGAAGCCAGAA
CAGAAATAAATGATAACGAGTGGACAAGTACGTTGTCGCAGTTGTTCCTG
ACAAAGGGATGGCCAACTGGATCCGTATACTTTAAGGAATACGATGATAT
AGCTACCTTTTCAGTAGATCCACAATTATATTGCNGACTATAACATAGTT
TTGATGAGATACAATTCGGATTTAGAACTTGATATGTCGGAATTGGCAAA
TCTAATATTAAATGAATGGCTGTGCAATCCAATGGACATTACATTATATT
ATTATCAACAAACGGACGAAGCAAACAAATGGANAGCAATGGGGCAATCA
TGTACAATAAAAGTATGTCCACTAAATCC

The obtained sequence was analyzed using computer software (BLAST) via., the Internet (www.ncbi.nlh.gov.) which revealed that the nucleotide sequence was highly related to strains of G10 bovine rotavirus in percentage range between 88 to 92% so, the CRV were related to G10 BRV lineage as shown in Table 4.

In this study, we have detected of group A rotavirus in fecal samples of diarrheic camel calves using Mabs-based ELISA and electron microscopy (House, 1978; Yolken *et al*., 1978; Herrman *et al*., 1985; Hussein *et al*., 1995). The availability of local strain of CRV was limited so, important to continue in trail to isolate CRV using MA104 cell culture (Babuik *et al*., 1977). The cytopathic effect Fig. 7 (CPE) on the inoculated cells were varied between the samples among the applied five passages (Saif and Theil, 1985).

Table 4: Homology percentage of the two highly positive samples sequenced fragment to the published group A rotavirus sequences in the gene bank (NCBI)

Accession	Description	Max. identity (%)
Camel rotavirus (10)		
L07658.1	Rotavirus sp. outer capsid protein (*VP7*) mRNA, complete CDs	90
X52650.1	Bovine rotavirus protein 7 (*VP7*), segment 8, genomic RNA	91
X57852.1	Bovine rotavirus (strain B223) *VP7* mRNA	91
M64679.1	Bovine rotavirus B11 glycoprotein (*VP7*) gene, complete CDs	91
D01056.1	Bovine rotavirus mRNA for *VP7*, complete CDs, strain:KK3	90
D01055.1	Bovine rotavirus mRNA for *VP7*, complete CDs, strain:A44	90
AY644385.1	Bovine rotavirus strain CIT10A/02 outer capsid protein gene, partial CDs	89
AF386918.1	Bovine rotavirus strain B75 outer capsid protein *VP7* gene, complete CDs	89
AF507093.1	Bovine rotavirus G10 isolate Mordva/11/99 glycoprotein (*VP7*) gene, partial CDs	93
AF507098.1	Bovine rotavirus G10 isolate Sobinka/10/99/C glycoprotein (*VP7*) gene, partial CDs	92
AF507095.1	Bovine rotavirus G10 isolate Pavlovo/04/01 glycoprotein (*VP7*) gene, partial CDs	92
AF507111.1	Bovine rotavirus G10 isolate Kavernino/01/01 glycoprotein (*VP7*) gene, partial CDs	93
Rotavirus (25) camel		
X52650.1	Bovine rotavirus protein 7 (*VP7*), segment 8, genomic RNA	91
M64679.1	Bovine rotavirus B11 glycoprotein (*VP7*) gene, complete CDs	91
X57852.1	Bovine rotavirus (strain B223) *VP7* mRNA	91
L07658.1	Rotavirus sp. outer capsid protein (*VP7*) mRNA, complete CDs	91
D01056.1	Bovine rotavirus mRNA for *VP7*, complete CDs, strain:KK3	90
U14996.1	Bovine rotavirus 2292B major capsid glycoprotein (*VP7*) mRNA, complete CDs	89
X53403.1	Bovine rotavirus strain 61A *VP7* gene RNA	89
AY644385.1	Bovine rotavirus strain CIT10A/02 outer capsid protein gene, partial CDs	89
D01055.1	Bovine rotavirus mRNA for *VP7*, complete CDs, strain:A44	89
EU221265.1	Human rotavirus A isolate CRI 1444 capsid glycoprotein (*VP7*) gene, partial CDs	88
AF507095.1	Bovine rotavirus G10 isolate Pavlovo/04/01 glycoprotein (*VP7*) gene, partial CDs	93
AF507111.1	Bovine rotavirus G10 isolate Kavernino/01/01 glycoprotein (*VP7*) gene, partial CDs	93
AF507098.1	Bovine rotavirus G10 isolate Sobinka/10/99/C glycoprotein (*VP7*)	93
AF507104.1	Bovine rotavirus G10 isolate Sobinka/04/01/A/G10 glycoprotein (*VP7*) gene, partial CDs	91

The application of Mabs- based ELISA to identify rotavirus in cell culture was of great help (Hussein *et al*., 1995).

The molecular characterization of CRV is most important in current study. The PCR has proved its efficacy in detecting rotavirus in the samples of both fecal and tissue culture (Estes and Kapikian, 2007; Gentsch *et al*., 1993; Isegawa *et al*., 1993; Gouvea *et al*., 1993, 1994) and the developed RT-PCR genotyping assay based detecting of G6 and G10 serotypes of group A bovine (Hussein *et al*., 1996) the RT-PCR based assay for genotyping of CRV was used in present study to genomic characterization of local 7 isolated stains based on extraction of ds RNA and amplification of CRV (*VP6*) gene 1356 bp and *VP7* gene 1030 bp of the RT product using specific primers complementary to full length of each *VP6* and *VP7* genes (Hussein *et al*., 1995). The obtained bands from RT-PCR were detected in the accurate size 1356 and 1030 bp for both *VP6* and *VP7* gene. Also, the original samples give one band which was detected in predicted size for *VP6* 1356 bp in Fig. 2. After 3rd passages of the inoculated samples in cell culture, RT-PCR products revealed six positive isolates bands at size 1356 bp for *VP6* gene at (Fig. 3). After the 5th passage of isolates in tissue cell culture, RT-PCR amplified products were seven positive isolates with bands at predict size 1356 bp for *VP6* gene and six positive isolates with 1030 bp of the *VP7* gene (Fig. 4, 5). Two highly positive isolates coded No. (10, 25). Extracted dsRNA which was amplified by RT-PCR for *VP7* gene. The eluted (purified) DNA after electrophoresis of the products was sent to be sequenced with the forward primer to be sequenced. The obtained sequence results of only 580 bp fragment of the amplified 1030 bp PCR product revealed the specificity of the product to rotaviruses. Nucleotide sequence analysis has indicated the high identity of the sequenced fragment of both isolates

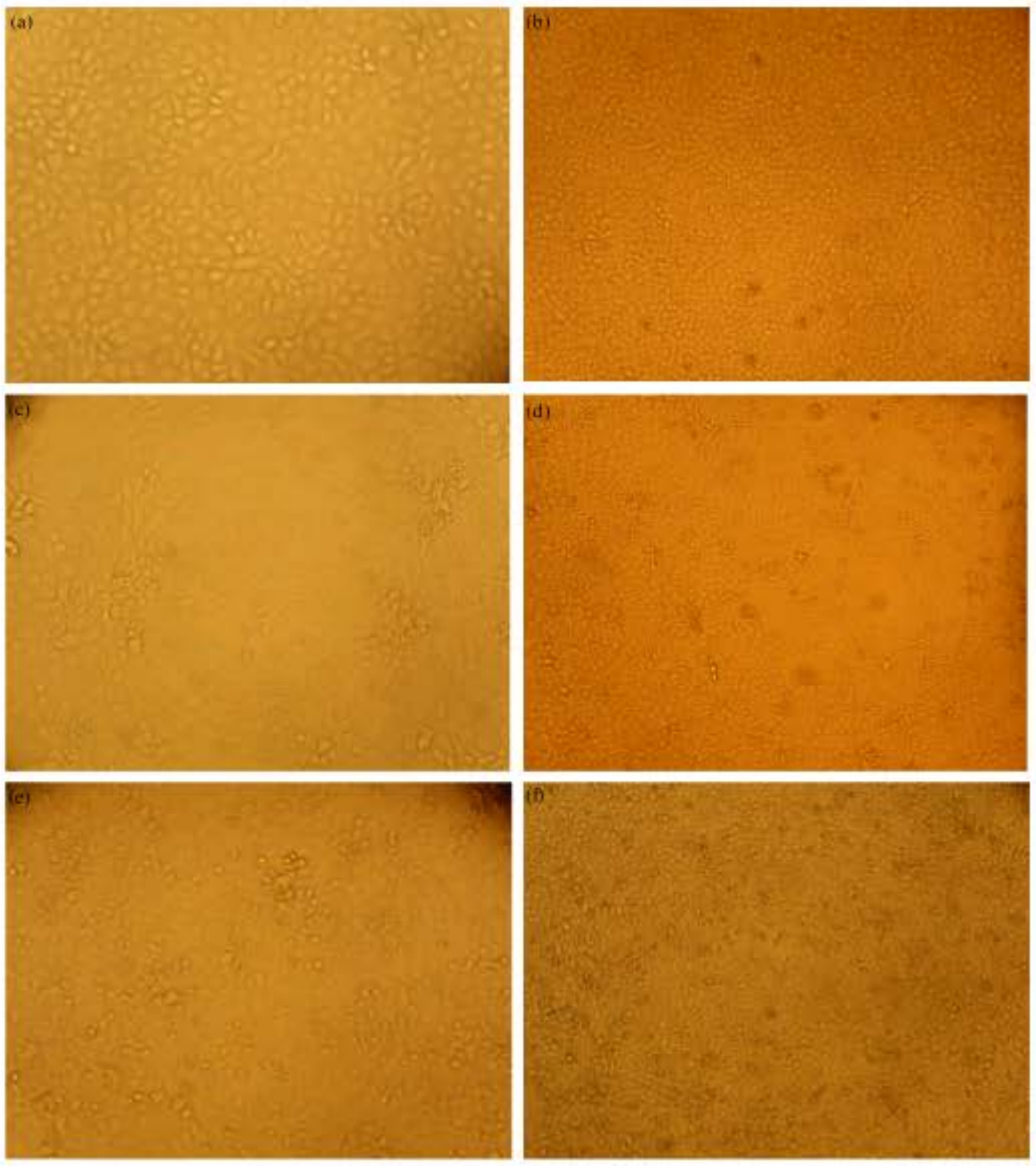

Fig. 7: Showing the sequential appearance of the characteristic CPE of rotavirus in comparison with control cells. (a) Non-inoculated cell control with high power, (b) Non-inoculated cell with low power, (c) Rounding and clumping of inoculated cells high power, (d) Cell rounding and clumping cells with low power, (e) Detachment of inoculated cells sheet with high power and (f) detachment of inoculated cells with low power

(Estes and Kapikian, 2007; Morin *et al*., 1976) to the G10 serotype of group A rotavirus. This is the first report on the existence of G10 serotype of group A rotavirus in camel. Characterization of such serotype in camel population will add more interest on gathering the diversity information of rotaviruses.

REFERENCES

Alain, R., F. Nadon, C. Seguin, P. Payment and Trudel, 1987. Rapid virus subunit visualization by direct sedimentation of samples on electron microscope gride. J. Virogical Meth., 16: 209-216.

Babuik, L.A., K. Mohammed, M. Spence, M. Fauvel and R. Petro, 1977. Rotavirus isolation and cultivation in the presence of trypsin. J. Clin. Microbiol., 6: 610-617.

Chang, K.O., A.V. Parwani and L.J. Saif, 1996. The characterization of *VP7* (G type) and VP4 (p type) genes of bovine group A rotaviruses from field samples using RT-PCR and RFLP analysis. Arch. Virol., 141: 1727-1739.

Chomeznnski, P. and N. Sacchi, 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 162: 156-159.

Deleeuw, P.W., D.J. Ellens and P.J. Staver, 1980. Rotavirus infections in calves in dairy herds. Res. Vet. Sci., 29: 135-141.

El-Sabagh, 2006. Coloning and expression of bovine rotavirus genes. Ph.D. Thesis.

Estes, M.K. and J.Cohen, 1989. Rotavirus gene structure and function. Microbiol. Rev., 53: 410-449.

Estes, M.K., 1996. Rotavirus and their Replication. In: Fields Virology, Fields, B.N., D.M. Knipe, P.M. Howely, R.N. Chanock, J.K. Melnick, T.P. Monath, B. Roizman and S.E. Straus (Eds.). Lippincott-Raven, Philadelphia, pp: 1625-1655.

Estes, M.K. and A.Z. Kapikian, 2007. Rotaviurses. In: Fields Virology, Fields, B.N., D.M. Knipe and P.M. Howley (Eds.). Wolters Kuwer Health/Lippincortt, Wiliams and Wilkins, Philadelphia, PA., pp: 1917-1973.

Gentsch, J.R., B.K. Das and B. Jiang, 1993. Similarity of the VP4 protein of human rotavirus strain 116E to that of the bovine B223 strain. Virology, 194: 424-430.

Gouvea, V., C. Ramirez and B. Li, 1993. Restriction endonuclease analysis of the *VP7* genes of human and animal rotaviruses. J. Clin. Microbiol., 31: 917-923.

Gouvea, V., N. Santos and M. do Carom Timenetsky, 1994. VP4 typing of bovine and porcine group A rotaviruses by PCR. J. Clin. Microbiol., 32: 1333-1337.

Gulati, B.R., R. Deepa, K. Singh and C.D. Rao, 2007. Diversity in Indian equine rotaviruses: Identification of G10, P6 [1] and G1 type and a new *VP7* genotype (G16) strains in diarrheic foals in India. J. Clin. Microbiol., 45: 972-978.

Herrman, J.E., N.R. Blacklow, D.M. Perron, G. Cukor, P.J. Krause, J.S. Hyams, H.H. Barrett and P.L. Ogra, 1985. Enzyme immuno assay with monoclonal antibodies for the detection of rotavirus in stool specimens. J. Infect. Dis., 152: 830-832.

House, J.A., 1978. Economic impact of rotavirus and other neonatal disease agents of animals. J. Am. Vet. Med. Assoc., 173: 573-576.

Hussein, H.A., E. Cornaglia, M.S. Saber and Y. El-Azhary, 1995. Prevalence of serotypes G6 and G10 group A rotaviruses in dairy calves in Quebec. Can. J. Vet. Res., 59: 235-237.

Hussein, H.A., A.A. EL-Sanousi, M.A. Shalaby, M.S. Saber and I.M. Reda, 1993. Stereotypical differentiation of group A rotaviruses from field cases on the basis of G-types monoclonal antibody based ELISA in Egypt. Proceedings of the TAHRP 2nd Scientific Workshop, June 9-14, Cairo and Alexandria, Egypt, pp: 1-1.

Hussein, H.A., E. Frost, B. Talbot, M. Shalaby, E. Cornaglia and Y. El-Azhary, 1996. Comparison of polymerase chain reaction monoclonal antibodies for G-typing of group A bovine rotavirus directly from fecal material vet. Microbiology, 51: 11-17.

Hussein, H.A., A.M. Samy, M.A. Shalaby, M.S. Saber and I.M. Reda, 1999. Bovine rotavirus in Egypt: Antigenic characterization of G6 and G10 serotypes within field strains of group A bovine rotavirus in dairy calves using monoclonal antibodies. Proceedings of the 5th Sci. Congress Egyptian Society for Cattle Diseases, Nov. 28-30, Assiut, Egypt, pp: 1-1.

Isegawa, Y., O. Nakagomi, T. Nakagomi, S. Ishida, S. Uesugi and S. Ueda, 1993. Determination of bovine rotavirus G and P serotypes by polymerase chain reaction. Mol. Cell. Probes, 7: 277-284.

Kapikian, A.Z. and R.M. Chanock, 1996. Rotaviruses Field's Virology. Microbiological observation made on spontaneous cases of acute neonatal calf diarrhea. Can. J. Comp. Med., 40: 228-240.

Khamrin, P., N. Maneekarn, S. Peerakoma, W. Chan-It, F. Yagyu, S. Okitsu and H. Ushijima, 2007. Novel Porcine rotavirus of genotype P[27] shares new phylogenic lineage with G2 Porcine rotavirus strain. Virology, 361: 243-252.

Mahin Schwers, L., A. Chadli, M. Maenhoudt and P.P. Pastoret, 1983. Receptivite du dromadaire (*Camelus dromedaries*) al'infection par rotavirus. Rev. Elev. Med. Vet. Pays. Trop., 36: 251-252.

Morin, M., S. Larviere and R. Lallier, 1976. Pathological and microbiological observation made on spontaneous cases of acute neonatal calf diarrhea. Can. J. Comp. Med., 40: 288-240.

Saif, L.J. and K.W. Theil, 1985. Antigentically distinct rotaviruses of human and animal origin. Processing of the Infect Diarrhea Young, 1985, Elsevier, Amsterdam, the Netherlands, pp: 208-214.

Saif, L.J., B. Rosen, S. Kang and K. Miller, 1988. Cell culture propagation of rotaviruses. J. Tissue Cult. Meth., 11: 147-151.

Saif, L.F. and F.M. Fernandez, 1996. Group a rotavirus veterinary vaccines. J. Infect. Dis., 174: S98-S106.

Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory, New York, ISBN: 0-87969-309-6.

Schwartz, H.J. and M. Dioil, 1992. Diseases of the Gastro-Intestinal System. In: The One Humped Camel in Eastern Africa, Schwartz, H.J., M. Dioli Verlag (Eds.). Josef Margraf-Scientific Books, Wekersheim, Germany, pp: 195-198.

Snodgrass, D.R. and P.W. Wells, 1976. Rotavirus infection in lambs: Studies on passive protection. Arch. Virol., 52: 201-205.

Woode, G.N. and J.C. Bridger, 1975. Viral enteritis of calves. The Vet. Record, 96: 85-88.

Yolken, R.H., B. Babbour, R.G. Wyatt, A.R. Kalica, A.Z. Kapikian and R.M. Chanock, 1978. Enzyme linked immunosorbent assay for identification of rotaviruses from different animal species. Science, 201: 259-262.