Cytopathic genotype 2 bovine viral diarrhea virus in dromedary camels

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ABSTRACT

Pestivirus infections in domesticated ruminant species and pigs are worldwide problems with severe economic impact. A wide range of wild ruminant species can also acquire pestivirus infections. We isolated several pestiviruses from Egyptian dromedary camels (Camelus dromedarius) in 1995. The camels from which these pestiviruses were isolated had signs of reproductive and congenital disease. The camel isolates were shown to experimentally infect cattle and goats. To genetically characterize these isolates, RNA extracts from two cytopathic Egyptian camel isolates, Giza4 and Giza7 were amplified by RT-PCR using a nested set of primers complementary to sequences in the E^{rns} of the pestivirus genome. The nested PCR product was characteristic of a bovine viral diarrhea virus (BVDV) type 2 for isolate Giza7 and of BVDV type 1 for Giza4. Giza7 E^{rns} nucleotide sequences were 82-88 %, 68.7-72 %, 66.5-67.2%, 68 % and 64 % homologous with BVDV-2, BVDV-1, BDV, CSFV and Reindeer-1 viruses, respectively. Giraffe-1 pestivirus sequence homology to Giza7 was 70 %, higher than homology values of BVDV-1 Oregon and BVDV-1 NADL. The predicted amino acid sequence homologies of Giza7 were 84.9-90.2 %, 77.4-81.9 %, 73.6 % and 73.6 % for BVDV-2, BVDV-1, BDV and CSFV, respectively. Giraffe-1 and Reindeer-1 isolates showed 75.1 % and 72.1 %, amino acid homology to Giza7, respectively. This is the first report of a BVDV-2 infection in dromedary camels world-wide.

Key words: *Bovine viral, Diarvhea virus, dromedary camels.*

INTRODUCTION

Pestiviruses are a group of closely related small RNA viruses belonging to the family Flaviviridae. The family also includes the genera Flavivirus and Hepacivirus (Becher *et al.*, 1999). Pestiviruses have positive stranded RNA genomes of an average length of 12.3 kb. The genome contains a large open reading frame flanked by 5' and 3' untranslated regions (UTR). The viral polypeptides and the genes encoding them are (from the N to C terminus): N^{pro}, C, E^{rns}, E1, E2, p7, NS2-3 (or NS2, NS3), NS4A, NS4B,

NS5A and NS5B (Becher *et al.,* 1999). The capsid protein C and the envelope glycoproteins E^{rns}, E1 and E2 are structural proteins. N^{pro}, P7, NS2-3 (or NS2 and NS3), NS4A, NS4B, NS5A and NS5B are nonstructural proteins.

Pestivirus infections are a primary cause of enteric, respiratory, reproductive and immune system diseases in many species including bovine, ovine, porcine, and wild ruminants (Tessaro *et al.,* 1999; Van Campen and Williams, 1995). The severity of the clinical signs in susceptible hosts depend on the virulence of the viral strain, host species (Tessaro *et al.,* 1999), physiological condition, age and immune status (Baker, 1995; Tessaro *et al.,* 1999). In utero pestivirus infections that occurs prior to the development of the fetal immune system may result in the birth of persistently infected animals that are a continuous source of virus to herd-mates (Baker, 1995).

Initially, pestivirus isolates were classified according to the species they infected into three groups: bovine viral diarrhea virus (BVDV), classical swine fever (CSFV) and border disease virus (BDV) of sheep. However, the emergence of new, genetically distinct, isolates and evidence of cross species transmission (Bolin and Ridpath, 1992; Corapi *et al.,* 1989, Pellerin *et al.,* 1994; Ridpath *et al.,* 1994) have provided the need for the development of alternative methods for pestivirus classification. Strategies for pestivirus classification based on antigenic (Paton *et al.,* 1994; Paton *et al.,* 1995) and genomic analysis have been proposed. Regions of the pestivirus genome used for genotyping have included the structural genes [C, E^{rns} and E2] as well as the nonstructural genes or regions [5'UTR, N^{pro} and NS5B] (Becher *et al.,* 1999; Giangaspero and Harasawa, 1999; Hertig *et al.,* 1995; Hofmann *et al.,* 1994; Letellier *et al.,* 1999; Paton *et al.,* 2000; Ridpath, 1996; Sullivan and Akkina, 1995; Van Gennip *et al.,* 1999; Vilcek *et al.,* 1994; Vilcek *et al.,* 1996; Vilcek *et al.,* 1999). Nucleotide phylogenetic and amino acid sequence homology analysis of complete regions of the pestivirus genome are currently used for detailed classification and determination of genetic relationships between pestiviruses (Becher *et al.,* 1995; Becher et al., 1999; Hertig *et al.,* 1995; Letellier *et al.,* 1999; Vilcek *et al.,* 1999). Nested RT-PCR using primers complementary to sequences in the E^{rns} is used for rapid detection and typing of bovine and ovine field isolates (Sullivan and Akkina, 1995). Using this E^{rns}-typing RT-PCR, Sullivan and his co-workers classified several North American bovine and ovine isolates. Bovine isolates were grouped into one of two genotypes, namely BVDV-1 and BVDV-2 whereas ovine isolates fell into one of three genotypes BVDV-1, BVDV-2 and BDV. Recently, Giraffe-1 and Reindeer-1 have been introduced as novel groups (Becher *et al.,* 1999).

The aim of this communication is to report the genetic typing of two pestiviruses isolated from Egyptian dromedary camels (Camelus dromedarius) using the nested RT-PCR for E^{rns} typing (Sullivan and Akkina, 1995). Several sero-surveys reported antibodies to BVDV in Egyptian camels but it was only in 1994 that pestiviruses were isolated from camels in our laboratory (Hegazy *et al.,* 1995). The two pestiviruses, Giza4 and Giza7, were isolated from camels showing signs of reproductive and congenital disease. Virus neutralization, Immunofluorescence and immunohistochemistry assays utilizing BVDV type 1 polyclonal antiserum initially identified the two isolates as BVDV-like, in fixed camel tissues as well as infected tissue culture samples (Hegazy *et al.,* 1995). Isolate Giza7 was able to experimentally infect cattle and goats. We also report the results of

nucleotide and amino acid sequence analysis of a region of Giza7-E^{ms}.

MATERIALS AND METHODS

Viruses, cells and GenBank sequences

The camel pestivirus isolates Giza4 and Giza7 were propagated on Madin-Darby bovine kidney (MDBK) cells obtained from the American Type Culture Collection (ATCC) (Rockville, Md.). Cells were cultured in minimum essential medium (MEM) (Gibco BRL, Rockville, Md.) supplemented with 10% irradiated-BVDV-negative fetal bovine serum (FBS) (HyClone, Logan, Utah). The media contained 100 IU/ml of penicillin, 100 g/ml streptomycin and 2.5 µg/ml fungizone (Freshney, 2000). Cell cultures were split 1:3 when needed (confluency >95%) (Freshney, 2000). Mock-infected cultures were used as negative controls. MDBK cultures were screened for the presence of noncytopathic (NCP) virus contamination using, immunofluorescence (IF), immunoperoxidase (IP) and the E^{ms}-based nested RT-PCR (Sullivan and Akkina, 1995), as described below. Both viruses showed cytopathogenicity in MDBK culture 2-3 days post infection.

GenBank pestivirus sequences used for sequence alignments, homology calculations and construction of the phylogenetic trees were: CSFV Alfort/A19 (accession number (Acc. No. U90951), CSFV Brescia (Acc. No. AF091661.1), BDV BD X818 (Acc. No. AF037405), BVDV-2 BD C413 (Acc. No. NC_002032.1), BVDV-2 890 (Acc. No. U18059), BVDV-2-28508-5 (Acc. No. 145968.1), BVDV-1 Osloss (Acc. No. M96687), BVDV-1 NADL (Acc. No. NC_001461), BVDV-1 SD-1 (Acc. No. M96751) and, BVDV-1 Oregon (Acc. No. AF041040.1). The two newly identified genotypes Giraffe-1 H138 (Acc. No. AF144617) and Reindeer-1 (Acc. No. AF144618) were also included. Giza7 sequences have been submitted to GenBank (Acc. No. AF072532). The isolates selected are representatives of the known major pestivirus types.

Oligonucleotides

Oligonucleotides were purchased from Integrated DNA Technologies, Inc., Coralville, IA. The E^{ms} typing primers P1, P2, TS1, TS2 and TS3 have been described previously by Sullivan and Akkina, 1995. Primers P1 and P2, the outer reaction consensus primers, amplify a 826-bp PCR product from ruminant pestiviruses. The nested primers TS1, TS2 and TS3 recognize specific sequences in BDV, BVDV type 2 and BVDV type 1, respectively. The specific PCR products of a nested reaction containing P2/TS1, P2/TS2 and P2/TS3 are 566-bp, 448-bp, and 223-bp, respectively (Sullivan and Akkina, 1995).

RNA extraction and PCR amplification of the pestivirus genome

Total RNA from virus-infected cultures as well as control total RNA from mock-infected cultures were extracted using the TRI-zol reagent (Gibco BRL, Rockville, Maryland, USA), according to the manufacturer's instructions. A portion of the RNA was reverse transcribed to form the first cDNA strand using the random primers provided in the GeneAmp RNA PCR kit (Perkin Elmer, Foster city, California, USA), according to the manufacturer's instructions.

RNA samples were also reverse transcribed and the P1/P2 823-bp region of the E^{ms} was amplified using the GeneAmp RNA PCR kit (Perkin Elmer, Foster city, California, USA), according to the method described by Sullivan and Akkina, 1995. Briefly, primers and viral RNA were incubated at 70 °C for 10 minutes and quickly cooled on ice for 10 min to allow annealing of the downstream primer

to the genomic RNA. The RT-PCR reaction contained 1 X PCR buffer (10 X buffer contains 500 mM KCl; 100 mM Tris-Cl, pH 8.3), 15 mM $MgCl_2$, 1mM of each deoxynucleotide triphosphate (dNTP), 20 U of RNase Inhibitor (RNase In), 2.5 U of Amplitaq, and 100 pmole from each of the P1 and P2 primers in a reaction volume of 100 l. PCR tubes were initially incubated for 35 min. at 42 °C to allow cDNA synthesis. This was followed by a five-minute hold at 95 °C to inactivate the reverse transcriptase, and then cooling at 5 °C for 5 min. The PCR reaction was carried out in 37 cycles using these parameters: template denaturation, 94 °C for 1 min; primer annealing, 55 °C for 1 min; and extension, 72 °C for 1 min. A final extension was allowed at 72 °C for 10-min. PCR products were separated by electrophoresis on 1.5 % agarose gels containing ethidium bromide stain, illuminated, and visualized using a transilluminator.

Nested PCR with type-specific primers

A nested PCR reaction was performed using the type-specific primers, TS1, TS2 and TS3. Two µl of 1:100 dilution of the first PCR product in nuclease-free distilled water were added to 98 µl of PCR master mix II composed of the same reaction components as before without RNase In or reverse transcriptase and with the substitution of P1 with 100 pmole of each of primers TS2 and TS3. Thirty-five cycles of amplification were done using the following reaction conditions: template denaturation at 94 °C for 1 min., primer annealing at 67 °C for 1 min., and extension at 72 °C for 1 min. The final extension was at 72 °C for 10 min. Fifteen l of the PCR product were electrophoresed in 2% agarose gel containing 0.5 µg/ml ethidium bromide, and visualized as before.

Nucleotide sequencing and analysis

The 448-bp product of the nested reaction of Giza7 RT-PCR was excised from the gel and the DNA fragments were purified as mentioned above. Direct nucleotide sequencing from the 448-bp purified Giza7-nested PCR product was done at the Biotechnology Center, Iowa State University, Ames, IA. The amplified fragments were sequenced twice, using the forward and reverse primers to confirm sequencing results. This was done to avoid possible mutations during cloning.

Nucleotide sequence comparisons and multiple alignments of the Giza7 isolate and reference viruses as well as the deduction of amino acid sequences were done using the BioEdit sequence alignment editor (Hall, 1999) and Clustal W software for multiple sequence alignment (Thompson *et al.*, 1994). The phylogenetic trees were constructed using Puzzle software for Tree reconstruction of sequences by quartet puzzling and maximum likelihood analysis (Strimmer and von Haeseler, 1996). Identification of homologies between nucleotide and amino acid sequences of the Giza7 and GenBank sequences was done using BLAST 2.0 and PSI- BLAST search programs (National Center for Biotechnology Information (NCBI) web site), respectively. The scores designated in the BLAST search have a well-defined statistical interpretation, making matches easier to distinguish from random background hits. BLAST uses a heuristic algorithm, which seeks local as opposed to global alignments and is therefore able to detect relationships among sequences that share only isolated regions of similarity (Altschul *et al.*, 1997).

RESULTS

Type specific PCR products

The RT-PCR of RNA extracts from two

tested isolates, Giza4 and Giza7 produced the 826-bp fragment characteristic of ruminant pestiviruses (Sullivan and Akkina, 1995). Mock-infected cultures did not produce any PCR products. The nested PCR results produced a 223-bp fragment, characteristic of BVDV-1, with the camel isolate Giza4. The camel isolate Giza7 produced the 448-bp PCR product characteristic of BVDV-2. Mock-infected cultures did not produce any PCR products (Fig. 1).

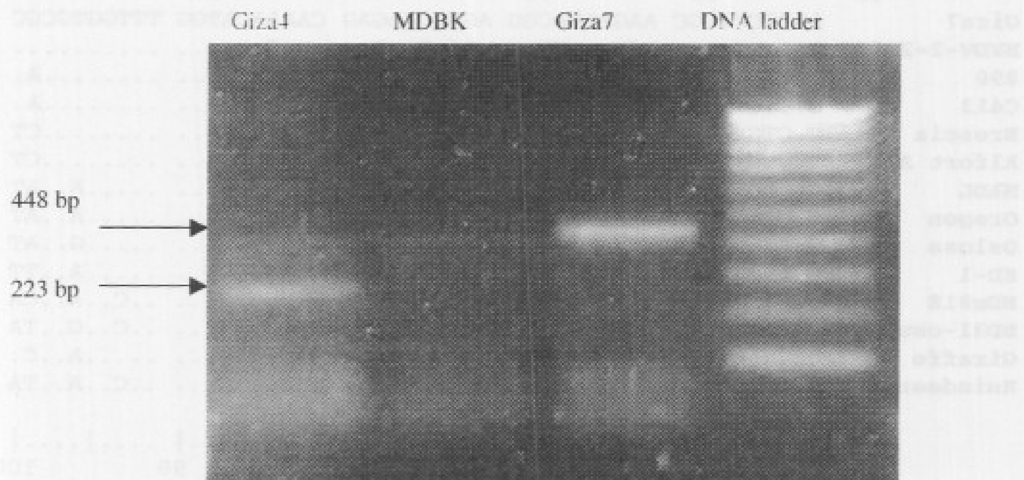

Fig. (1): Agarose gel electrophoreses analysis of DNA products with type-specific primers, TS1, TS2 and TS3 of DNA product from consensus primers P1 and P2. Giza4, a type 1 pestivirus (BVDV-1), produced a PCR product of 223 bp, lane 1. Giza7, a type 2 pestivirus (BVDV-2), produced a 448-bp PCR product, lane 3. Mock-infected MDBK cultures did not produced any PCR products, lane 2. Lane 4 is a 100-bp DNA molecular weight ladder.

Nucleotide sequence analysis of the consensus Giza7 E^{rns} PCR product

Comparison of the sequences of the Giza7 400-bp-E^{rns} core sequences encompassed by the P2 and TS2 primers with corresponding nucleotide sequences of selected isolates from the GenBank data-base (Fig. 2), revealed the distribution of the nucleotide mutations throughout the sequence. We used Clustal W multiple-alignment program to calculate the homology values between the viruses listed in Fig. 2.2. Giza7 E^{rns} nucleotide sequences showed 82-88 %, 68.7-72 %, 66.5-67.2%, and 68 % homology with BVDV-2, BVDV-1, BDV, and CSFV viruses, respectively. Surprisingly, the nucleotide sequence homology to the Giraffe pestivirus isolate was 70 %; higher than that of the BVDV-1 isolates Oregon and NADL. The lowest nucleotide sequence homology was the isolate Reindeer with 64 % nucleotide sequence homology.

Puzzle-generated nucleotide-base phylogenetic tree grouped Giza7 with BVDV-2 (Fig. 3). BVDV-2 isolates clustered in a branch separate from the genetic groups BVDV-1, BDV, CSFV, Girrafe-1 and Reindeer-1. Sequence homology differences between BVDV-2 890 and Giza7 placed the latter in a distinct subgroup with BVDV-2-

28508-5. The E^{rns} sequence information from the viruses used in phylogenetic tree construction allowed the segregation of these viruses into the six known genotypes (Fig. 3).

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             ....|....| ....|....| ....|....| ....|....| ....|....|
                     10         20         30         40         50
Giza7        AATCCCTGGC AAGAAGACGG AGAGCAAGAG CAAAACATGG TTTGGTGCGC
BVDV-2-28508 G....T.... ......TT.. .A..T..A.. ...G...... ..........
890          .....T...T ......TT.. ...AT..A.C ....G..... ........A.
C413         G....T...T ......TT.. ...AT..A.. ....G..... ........A.
Brescia CS   C.CTG.C..G ....G.TT.. ..G.T.GA.. ......C... ........CT
Alfort A19   ...TG.C..G ....G.TT.. ..G.T.GA.. ......C... ........CT
NADL         G..A.TA..A ..A...TT.. .A.A...... T..G..G... .....A..AT
Oregon       G.GATTA..A ..A..ATT.. .A.A...... ...G..G... .....A..AT
Osloss       G.AA.TA..G .....ACT.. .A.A...... T..G...... .....G..AT
SD-1         G....TA..G ..A...TT.. .A.AT..A.. ...G...... .....A..TT
BDx818       T..AATG..G ..A..ATTA. .ACA...... T.....G... ..C..A..CA
BD31-csu     T..AATG... ..A...TTA. .ACAT..... T.....T... ..C..G..TA
Giraffe      ...T.TG..T .G...ATT.. .ACAT..... T......... .....A..C.
Reindeer-1   C..AATG..A ......TT.. .ACA...A.. ......G... ..C..A..TA

             ....|....| ....|....| ....|....| ....|....| ....|....|
                     60         70         80         90        100
Giza7        ACGCAGCAAG TCCGTATTGT GATGTGGATA AGAAGATAGG TTACATCTGG
BVDV-2-28508 .......... .........C A....A..G. .......T.. ..........
890          .T........ ...A..C..C .GA.....G. G......C.. ....G.....
C413         .T........ ...A..C..C .GA.....G. .......C.. ....G.....
Brescia CS   .T..CCT.TC A..T..C... A....AACA. GT..A..... G.....A...
Alfort A19   .T..CCT.TC A..T..C... A....AACA. GC..A..... G.....A...
NADL         ....T..TTC C..T..C... .....C...C GC..A..T.. C.....A...
Oregon       .T..G..TTC C..C..C... .....T...C GA..AC.T.. C.....A...
Osloss       .T.....CTC ...C..C..C ..G..A..AC G..G.C.T.. ..........
SD-1         .T.....TTC C..T..C... .....C...C GA..A..T.. C.....A...
BDx818       .T...CA.TC A..T..C... A....AACA. GA..A..... G...G.....
BD31-csu     .T...CA.TC A..C..C... A....AAC.. GA........ G.........
Giraffe      ........TC A..A.....C ..A..AA... .....C.C.. C.....T...
Reindeer-1   .T...CAGTC C..T..C..C A.C..AACC. .A...C.G.. G..T......

             ....|....| ....|....| ....|....| ....|....| ....|....|
                    110        120        130        140        150
Giza7        TATACAAAAA ACTGCACCCC GGCTTGTCTG CCAAAAAATA CCAAGATAAT
BVDV-2-28508 .......... .T........ A.....CT.. ..GGG...C. ..........
890          .......... .......T.. A.....C..C ....G...C. ..........
C413         .......... .......T.. A.....C..T ....G...C. ..........
Brescia CS   ..C..T..C. ....T..... ......C..C ..C....... .A........
Alfort A19   ..C..T..C. .......... ......C..C ..C..G.... .A........
NADL         .......... .T........ T..C..CT.A ..C..G..C. .A..A..TG.
Oregon       .T........ .T........ T..C..CT.A ..T..G..C. .A.....T..
Osloss       ........G. .T........ T..C...T.A .......... .A.....CG.
SD-1         .T........ .......... T.....CT.. ..T..G..C. .A..A..C..
BDx818       ..C.....C. .T........ T.....C..C .....G.... .A..A.....
BD31-csu     ........C. .T........ C..A.....T ........C. ..........
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Giraffe       ..C..T..C. .......G.. ......C..T ..TGGC.... .......C..
Reindeer-1    ........C. .......... T..G...T.A ....GG..C. ........G.

....|....| ....|....| ....|....| ....|....| ....|....|
       160        170        180        190        200
Giza7         AGGGCCTGGG AAGTTCTACA CCAATGCCGA TGATGGTAAG ATACTTCATG
BVDV-2-28508  ...A..C... .....TG... .......... A........A ..........
890           ...C..C... .....TG... ....C..... A.....C..A .....C....
C413          ...C..C... .....TA... ....C..... A........A .....C....
Brescia CS    ...C..C..T ..A..TG... .T.....G.. A..C..A... ..T..C....
Alfort A19    ...C..C..T ..A..TG... .T.....G.. G..C..A... ..T..C....
NADL          C..C...... ..A..TG... .......A.. G..C..C... ...T.A....
Oregon        C..C...... ......G... .......A.. G.....C... ...T.G.G..
Osloss        T..C..C..T .G....G... .......G.. G........A .....G....
SD-1          C..C...... .....TG... .......A.. G.....C... .....G....
BDx818        ...T..G... ..A..TG... .......G.. G.....A... ..CT.G....
BD31-csu      ...T..G... ..A..TG... .......G.. G.....A... ..CT.G....
Giraffe       ...C..A... .....TG... .......T.. A......... .....C..C.
Reindeer-1    T..T..A... ..A..TG.T. ....C..A.. A.....A..A .....G....

              ....|....| ....|....| ....|....| ....|....| ....|....|
                     210        220        230        240        250
Giza7         AAATGGGGGG ACACCTATCA GAATTTGTCC TATTGTCCTT GGTAGTTATG
BVDV-2-28508  .G.....A.. .......... .......... ....A..... ...G...T..
890           .G.....A.. G.....C... .......C.. .......... ...G...C..
C413          .G.....A.. G.....C... .......... .......... ...G...C..
Brescia CS    .G........ C......... ......C.G. .GC.C..TC. ...T...C..
Alfort A19    .G.....C.. C......... ......C.G. .GC.C..TC. ...T...C..
NADL          .G........ T...T.G..G ..GG.AC.A. ..C.T..T.. A..G..GC..
Oregon        .G........ T...T.G..G ..GG.AC.A. ..C.T..T.. A..G..GT..
Osloss        .G........ C...T.G... ..GG.GC.A. ..C.C..AG. ......GC.T
SD-1          .G........ T...T.G..G ..GG.AC.A. ..C.T..T.. A.....GT..
BDx818        .G...A.... T..TA....G .....CA.T. ..C.A..T.. ......GC.A
BD31-csu      .G...AA... G..TA....G .....CA.T. ..C.A..T.. ......GC.A
Giraffe       .GC....T.. ....T..... .....CA.A. .G.....TC. ...T..GC..
Reindeer-1    .G...A.A.. T..TA....G .....CT.G. ..C.A..T.. ...G..AC.T

              ....|....| ....|....| ....|....| ....|....| ....|....|
                     260        270        280        290        300
Giza7         TCAGATTTTG CCCCAGAGAC CGCGAGTGTA ATCTACCTGG CCCTGCATTT
BVDV-2-28508  ..T......T .......A.. A........G ..A...T... T.........
890           ..T..C.... .......A.. .........C ......T... TT..A.....
C413          ..T..C.... .......A.. .......T.T ......T... TT..A.....
Brescia CS   ..T..C.... ....T..A.. A..C..C.CG T.A.....CA TTT....C.A
Alfort A19    ..T..C..C. ....T..A.. A..C..C.CG T.A.....CA TTT.T..C.A
NADL          ..C..C..C. .A..G..A.. A..T...... ..G.....AA T...A.....
Oregon        ..T..C.... .A.....A.. A..T..C.C. ..G..T..AA TT..A.....
Osloss        ..C.....C. .T........ A..C.....G G.A..TT.AA TT..A.....
SD-1          ..T..C.... .A.....A.. G..T..C.C. ..G..T...A T...A.....
BDx818        .....C..C. .A..T..A.. A..T...ACT C......... TG..A.....
BD31-csu      .....C..C. .A..T..A.. A..T..CACT C......... TA..A.....
Giraffe       .....C..C. .A..T..... A..T....CC ..T.....A. TAT.A..C.A
Reindeer-1    ..C.....C. .A..C..... G..A..CACC T.A......A TA..A..C..
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             ....|....| ....|....| ....|....| ....|....| ....|....|
                    310        320        330        340        350
Giza7        TGCAATCCCA CAGAACCATA TCAATGTAGC CACTTGTGAT AAAAACCAAC
BVDV-2-28508 ..TG...... .........G .TG....... .........C ..........
890          ...G.....G ..A.G...CG .TG......A ...A..C..C ..G.....G.
C413         ...G.....G ..A.G...CG .TG......A ...A..C..C ..G.....G.
Brescia CS   CATG..T..T ...TC....G AAG.ACCT.A AGGC..C..C .C......GT
Alfort A19   C.TG..T..T ..ATC....G AAG.ACCT.A AGGC.....C .C......G.
NADL         .T.C...... ..A.GT..CG .TG.....AT GGA....... ..G.C...GT
Oregon       .T.C.....G ....GT..CG .TG..A..A. GGAC..C..C ..G.C....T
Osloss       CT.C...... ..AGGA..C. CTG.CA..CA TGAC...... ..........
SD-1         .T.C.....G ..A.G...CG .TG..A..A. GGAC...... ..G.C...G.
BDx818       ...CT.A..T ..A.C...CG AAGT.CCTAG TGTA.....C .CC.......
BD31-csu     .A.TT.G... ..A.C.T.CG AGGTCCCTAG TGAA.....C .CC......T
Giraffe      CA.C..G..C ..A..AT..G AGGTG....G A.G...C..C .GG.....GT
Reindeer-1   CA....A..C ....G....G AGGCCCCTAG TGAG.....C .CG.....GT

             ....|....| ....|....| ....|....| ....|....| ....|....|
                    360        370        380        390        400
Giza7        TGAATCTAAC GGTTGCTACT ACTACGGCAG AAGTCATACC GGGGACAGTG
BVDV-2-28508 .....T.... .......... ..G....... .......... ..........
890          .....T.... ...C..A... ..AGTA.... .......... A.........
C413         .....T.... ...C..A... ..AGTA.... .......... A...T.....
Brescia CS   .......... A..G.AACTC .GG..T.A.. .C..A..... .TCAT....C
Alfort A19   .......... A..G.AACTC .GG..T.A.. .C..A..... .TCAT....C
NADL         ....C..C.. A..G.AGCTG ..A..A..T. .T..A..... T..AT....C
Oregon       ....C..C.. T..G.AGCTC ..A..A.... .T..A..... A...T.G..C
Osloss       .A..C..C.. C..A.GACTC ..A..A..T. ....A..... T...T....T
SD-1         ....C..C.. CA.A.AGCT. ..A..A.... .T..A..... A...T.G..C
BDx818       .A.....T.. ...CAGCTTG .GAGT..AT. .C..G..... ATCTT....A
BD31-csu     .A..C..... .....GCTTA .GAGT..AT. .C..G..... ATCTT.G...
Giraffe      .A...T.... A..GAAA..A .GAGT..A.. .C..G..C.. CA.CT....C
Reindeer-1   .A...T.... CA.C.GGTTA .GGGTA.AT. .G..GG.... ATCCT.....
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Fig. (2): Alignment of Giza7 E^{rns} nucleotide sequences with reference pestivirus sequences. Using Giza7 sequences as reference, nucleotide mutations were distributed throughout the Giza7 E^{rns} nucleotide sequences. Giza7 E^{rns} nucleotide sequences were 82-88 %, 68.7-72 %, 66.5-67.2%, and 68 % homologous with BVDV-2, BVDV-1, BDV, and CSFV viruses, respectively. The nucleotide sequence homology to the Giraffe pestivirus isolate was 70 %, higher than that of the BVDV-1 isolates Oregon and NADL. Nucleotide sequence homology with Reindeer with 64 %. Homology values were calculated using the Clustal W multiple-alignment program.

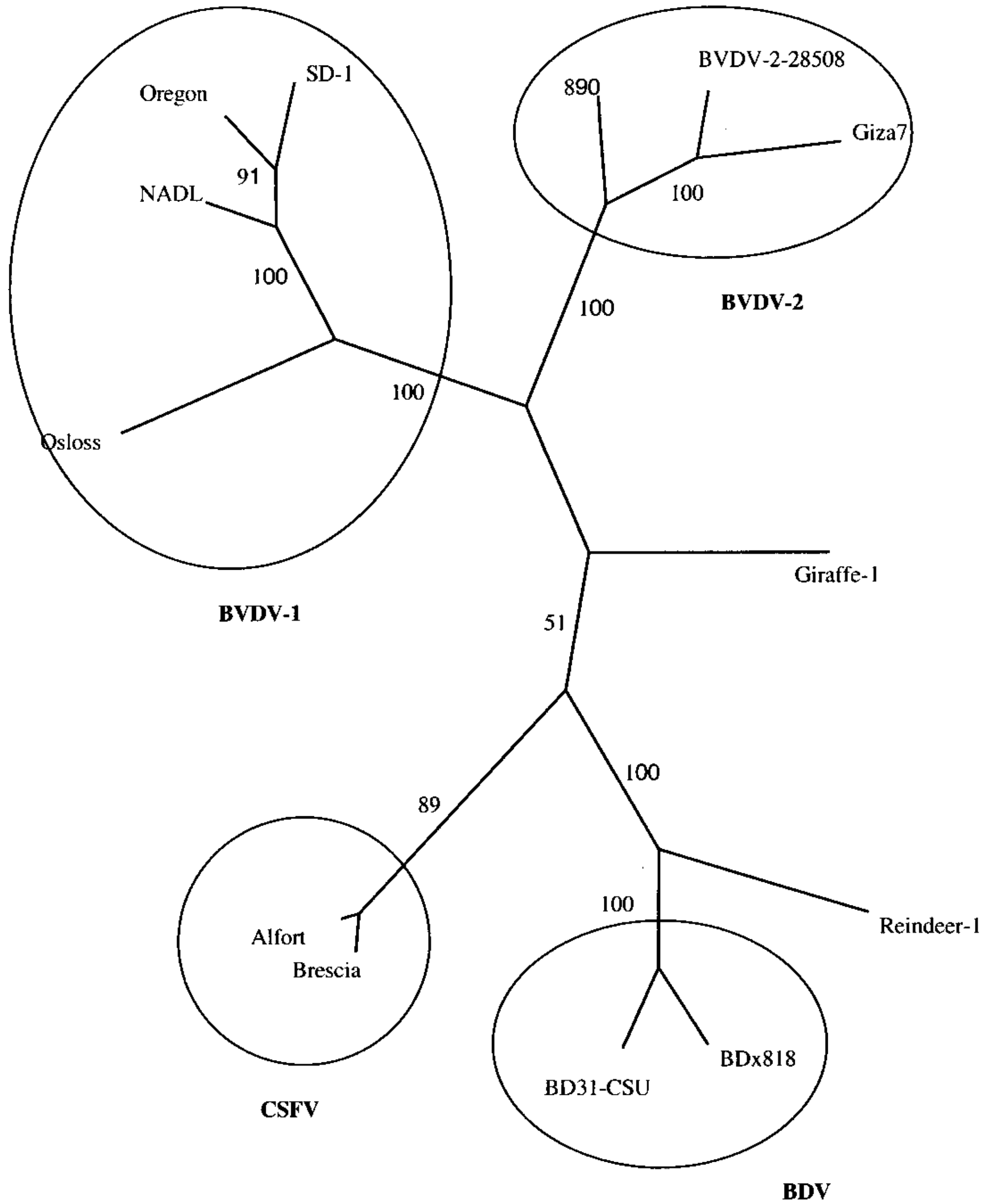

Fig. (3): Nucleotide-based phylogenetic analysis of the Giza7 E^{rns}. Puzzle-generated nucleotide-base phylogenetic tree grouped Giza7 with BVDV-2. Sequence homology differences between BVDV-2 890 and Giza7 placed the latter in a distinct subgroup with BVDV-2-28508-5.

Giza7 E^{rns} amino acid sequence analysis

The predicted amino acid sequences of Giza7, produced by BioEdit, were compared to published pestivirus sequences in the GenBank database using BLAST 2.0. The identified GenBank entries showing significant amino acid sequence alignment with Giza7 E^{rns} sequences were mostly BVDV-2 viruses followed by Giraffe-1. BVDV-1, CSFV and BDV sequences had lower sequence homology, but were not presented in a particular order.

Clustal W alignment and homology calculation of the predicted amino acid sequences of Giza7 with BVDV-2, BVDV-1, BDV and CSFV showed 84.9-90.2 %, 77.4-81.9 %, 73.6 % and 73.6 % sequence homologies, respectively. The Giraffe isolate amino acid sequences were 75.1 % homologous to Giza7, which is higher than both type 3 and 4 pestiviruses. The amino acid sequence homology between Giza7 and Reindeer was the lowest among pestiviruses (72.1 % amino acid sequence homology). However, the nucleotide blast results showed several pestiviruses belonging to CSFV and some belonging to BDV and BVDV-1 with less nucleotide sequence homology than the Reindeer isolate. Interestingly, homology to NADL sequences was higher on the amino acid level than on the nucleotide level, 81.9 % compared to 69.7 %, respectively. Most of the amino acid changes were towards the C terminus of the 133 predicted amino acids, between amino acids 125 and 131 (Fig. 4). Such mutations did not involve the predicted hydrophobic regions of this part of the E^{rns}, data not shown.

The phylogenetic tree produced by Puzzle using amino acid sequences grouped Giza7 with BVDV-2 viruses (Fig. 5). The sequence homology differences between BVDV-2 890 and Giza7 isolates confirmed the placement of Giza7 in a distinct subgroup with BVDV-2-28508-5.

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             ....|....| ....|....| ....|....| ....|....| ....|....|
                     10         20         30         40         50
Giza7        IPGKKTESKS KTWFGAHAAS PYCDVDKKIG YIWYTKNCTP ACLPKNTKII
BVDV-2-28508 .L...L.... .......... ...N.E.... .......... ....G.....
C413         .L...L.N.. .A........ ...G.E.... .V........ ....R.....
890          .L...L.N.T .A........ ...G.ER... .V........ ....R.....
NADL         .L...L.N.. ......Y... ......R... .......... .........V
SD-1         .L...L.N.. ......Y... ......R... ...F...... ..........
Osloss       KL...L.N.. ......Y... ...E.ERRL. .......... .........V
Oregon       RL...L.N.. ......Y... ......R.L. ...F...... ..........
Giraffe      .L.R.L.H.. .......... ...E.N..L. .....N.... ....G.....
Alfort A19   .A..RL.GR. ......Y.L. ...N.TS... .....N.... ..........
Brescia      TA..RL.GR. ......Y.L. ...N.TS... .....N.... ..........
BDx818       .M...L.H.. ......N.Q. ...N.TR... .V...N.... ..........
BD31-CSU     .M...L.H.. ......N.Q. ...N.TR... .....N.... ..........
Reindeer-1   .M...L.H.. ......N.Q. ...N.T..L. .....N.... ....R....V

             ....|....| ....|....| ....|....| ....|....| ....|....|
                     60         70         80         90        100
Giza7        GPGKFYTNAD DGKILHEMGG HLSEFVLLSL VVMSDFAPET ASVIYLALHF
BVDV-2-28508 .....D...E .......... .......... ..L...S... ......V...
C413         .....N...E .......... .......... ..L....... ..F...V...
890          .....D...E .......... .....A.... ..L....... ......V...
NADL         .....D...E .......... ....VL.... ..L....... ...M..I...
SD-1         .....D...E .......... ....VL.... ..L....... ..AM..I...
Osloss       ...R.D...E .......... ....VL...V ..L....... ...V..I...
Oregon       .....D...E .....R.... ....VL.... ..L....... ..AM..I...
Giraffe      .....D...E .......L.. .....I.... ..L....... ..A...V..Y
Alfort A19   .....D...E .......... .....L.... ..L....... ..AL..IF.Y
Brescia      .....D...E .......... .....L.... ..L....... ..AL..I..Y
BDx818       .....D...E ........R. .I...I.... ..L....... ..TL..V...
BD31-CSU     .....D...E ........K. .I...I.... ..L....... ..TL..V...
Reindeer-1   .....D...E ........R. .I...L.... ..L....... ..TL..I...

             ....|....| ....|....| ....|....| ...
                    110        120        130
Giza7        AIPQNHINVA TCDKNQLNLT VATTTAEVIP GTV
BVDV-2-28508 V.....VD.. .......... .......... ...
C413         ....S.VD.D .......... ....V..... .S.
890          ....S.VD.D .......... ....V..... ...
NADL         S...S.VD.M D...T..... .EL...D... .S.
SD-1         S...S.VDIT D...T..... IEL...D... .S.
Osloss       S...G.TDIH D......... .GL....... .S.
Oregon       S...S.VDIT D...T..... .EL...D... .S.
Giraffe      TM..KYEV.G S..R...... .K.RVED... SS.
Alfort A19   V...S.EEPE G..T...... .ELR.ED... SS.
Brescia      M...S.EEPE G..T...... .ELR.ED... SS.
BDx818       .L..T.EVPS V..T...... .SLRVDD... SS.
BD31-CSU     TL..TYEVPS E..T...... .GLRVDD... SS.
Reindeer-1   T...S.EAPS E..T...... IGLRVD..V. SS.
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Fig. (4): Clustal W alignment of the predicted amino acid sequences of Giza7 with reference pestivirus isolates. BVDV-2, BVDV-1, BDV and CSFV showed 84.9-90.2 %, 77.4-81.9 %, 73.6 % and 73.6 % sequence homologies with Giza7, respectively. The Giraffe-1 and Reindeer isolates showed 75.1 % and 72.1 %, amino acid homology to Giza7, respectively. Most of the amino acid changes were towards the C terminus of the 133 predicted amino acids, between amino acids 125 and 131.

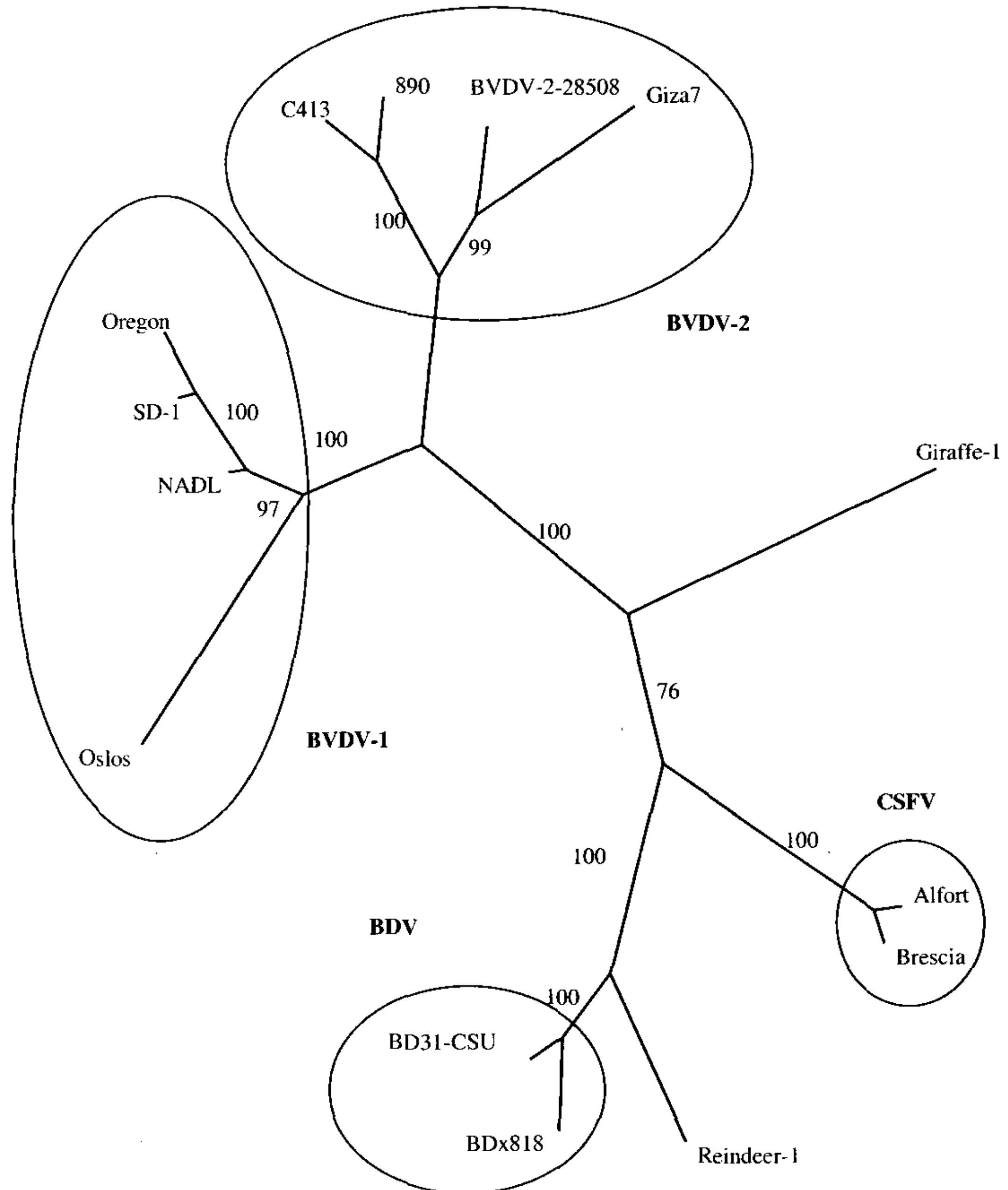

Fig. (5). Amino acid-based phylogenetic analysis of the Giza7 E^{rns}. Puzzle-generated amino acid-base phylogenetic tree grouped Giza7 with BVDV-2. Sequence homology differences between BVDV-2 890 and Giza7 placed the latter in a distinct subgroup with BVDV-2-28508-5.

DISCUSSION

Three main pestivirus types [BVDV-1, BVDV-2 and BDV] including several subtypes and strains are causes of economically important enteric, respiratory, reproductive, and immune system diseases in wild and domestic ruminant species worldwide (Baker, 1995; Bolin, 1996; Murray, 1989; Saliki, 1996). BVDV-2 was first recognized in the United States and Canada by its dramatic disease syndrome in cattle, causing severe thrombocytopenia and hemorrhaging (Corapi *et al.,* 1989; Bolin and Ridpath, 1992; Pellerin *et al.,* 1994; Ridpath *et al.,* 1994). In Egypt, BVDV was isolated from cattle as early as 1970; however, all the pestivirus isolates from cattle were serologically classified as BVDV-1 (Baz, 1992). Although local sero-surveys of camels in Egypt demonstrated the presence of BVDV antibodies in camel sera, the pathogenesis of pestiviruses in camels and the role of camel in the transmission of the disease were never investigated till 1994. A recent sero-survey found 52.5 % of Egyptian camels positive to BVDV (Zaghawa, 1998).

The two pestiviruses described in this study were isolated in Egypt from adult camels with diarrhea and from camel calves born with congenital defects (Hegazy *et al.,* 1995). E^{rns} Genotyping of the two camel pestiviruses revealed that Giza4 and Giza7 belonged to BVDV-1 and BVDV-2, respectively. Partial sequence analysis of the NS3 region using pestivirus-NS3-specific primers (Yousif *et al.,* 2002) have also allocated Giza7 with BVDV-2 viruses, data not shown. Further analysis of the biological and genetic characteristics of the genotype-2 pestivirus isolate from camels is currently underway.

It is interesting that Giza7, a BVDV-2-relative based on both the nucleotide and amino acid phylogenetic analysis, showed a higher degree of homology to BVDV-1 on the amino acid sequence level, compared to the nucleotide sequence homology. This high amino acid sequence conservation indicates a functional necessity of the pestivirus E^{rns}, especially at the N-terminus of the region examined.

The observation that Giza7 had a higher nucleotide but not amino acid sequence homology with Giraffe-1 compared to BVDV-1 strains Oregon and NADL may indicate a role for this part of the E^{rns} in host tropism. Our research team has previously reported that Giza7 can infect susceptible caprines (Hegazi *et al.,* 1995).

Giza7 could have originated from one of two sources, import or evolution from an unknown African pestivirus. Giza7 is closely related to a subgroup of BVDV-2 viruses that have been reported in the US. The adaptation to camels could have been due to interspecies transmission. In the Egyptian countryside, cattle and camels or sheep and camels owned by small farmers and herdsmen share the same facilities and fields, thus interspecies transmission of pestiviruses is a real threat. Genotyping and phylogenetic analysis of more isolates from both cattle and camels will prove extremely valuable for explaining the role of camels in maintaining the pestivirus cycle in the Egyptian environment.

The second possible origin of Giza7 is evolution from an unknown African pestivirus. This possibility arises from the high nucleotide sequence homology with the Giraffe-1 isolate. Some Egyptian camel breeds are imported from Sudan and other African countries into Egypt. During this trip, contact with wild ruminant species can occur. Quarantine measures at the borders will probably not identify infected animals because of the subtle clinical signs exhibited by adult camels (Hegazy *et al.,* 1995). A mutant African

pestivirus that has established dromedary camel infection could have been the origin of this Giza7.

To our knowledge, genotyping of Giza7 as BVDV-2 represents the first BVDV-2 infection in African dromedary camels, and the first report of a BVDV type 2 infection in Africa. This finding demonstrates the need for developing multi-type pestivirus vaccination programs that include camel populations in contact with cattle and other farm animals.

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REFERENCES

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nuc. Acids Res. 25: 3389-3402.

Baker, J.C. (1995). The clinical manifestations of bovine viral diarrhea infections. In: Baker, J.C., Houe, H., (Eds.), Bovine Viral Diarrhea Virus. The Veterinary Clinics of North America, 11 (3): 425-446.

Baz, T.I. (1992). Bovine virus diarrhea-mucosal disease and border disease in Egypt. Rev. Sci. Tech., 11(3): 867-872.

Becher, P., Konig, M., Paton, D.J. and Thiel, H.J. (1995). Further characterization of border disease virus isolates: Evidence for presence of more than three species within the genus pestivirus. Virology, 209: 200-206, doi:10.1006/viro.1995.1243.

Becher, P., Orlich, M., Kosmidou, A., Konig, M., Baroth, M. and Thiel, H.J. (1999). Genetic diversity of pestiviruses: identification of novel groups and implications for classification. Virology, 262: 64-71, doi: 10.1006/viro.1999.9872.

Becher, P., Orlich, M., Paton, D.J. and Thiel, H.J. (1998). Complete genomic sequence of Border Disease Virus, a pestivirus form sheep. J. Virol., 72(6): 5165-5173.

Becher, P., Shannon, A.D., Tautz, N. and Thiel, H.J. (1994). Molecular characterization of border disease virus, a pestivirus from sheep. Virology. 198: 542-551.

Bolin, S.R. (1996). Vaccination for bovine viral diarrhea virus. International Symposium. Bovine Viral Diarrhea Virus, a 50 year review, p. 16.

Bolin, S.R. and Ridpath, J.F. (1992). Differences in virulence between two noncytopathic bovine viral diarrhea viruses in calves. Am. J. Vet. Res., 53: 2157-2163.

Corapi, W.V., French, T.W. and Dubovi, E.J. (1989). Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhea virus. J. Virol., 63: 3934-3943.

Donis, R.O. (1995). Molecular biology of bovine viral diarrhea virus and its interactions with the host. In: Baker, J. C., Houe, H., (Eds.), Bovine Viral Diarrhea Virus. The Veterinary Clinics of North America, 11(3): 393-423.

Freshney, I.R. (2000). Culture of Animal Cells. A manual of basic technique. Book. John Wiley and Sons Inc. New York., 1: 77-193.

Giangaspero, M. and Harasawa, R. (1999). Ovine pestiviruses: their taxonomic status revealed by plindromic nucleotide substitutions. Vet. Microbiol., 70: 33-39.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41: 95-98.

Hegazy, A.A., Lotfia S. Fahmi, Saber, M.S., Aboellail, T.A., Yousif, A.A. and Chase, C.C.L. (1995). Bovine virus diarrhea virus infection causes reproductive failure and neonatal deaths in dromedary camel. In: International Symposium. Bovine Viral Diarrhea Virus A 50 Year Review, p.205.

Hertig, C., Stalder, H. and Peterhans, E. (1995). Genetic heterogeneity within the coding regions of E2 and NS3 in strains of bovine viral diarrhea virus. Gene, 153: 191-195.

Hofmann, M.A., Brechtbuhl, K. and Stauber, N. (1994). Rapid characterization of new pestivirus strains by direct sequencing of PCR-amplified cDNA from the 5' noncoding region. Arch. Virol. 139: 217-229.

Kummerer, B.M., Stoll, D. and Meyers, G. (1998). Bovine viral diarrhea virus strain Oregon: a novel mechanism for processing of NS2-3 Based on point mutations. J. Virol. 72: 4127-4134.

Letellier, C., Kerkhofs, P., Wellemans, G., Vanopdenbosch, E. (1999). Detection and genotyping of bovine diarrhea virus by reverse transcription-polymerase chain amplification of the 5' untranslated region. Vet. Microbiol. 64: 155-167.

Meyers, G., Tautz, N., Dubovi, E.J. and Thiel, H.J. (1996). Origin and diversity of cytopathogenic pestiviruses. International Symposium: Bovine Viral Diarrhea Virus; A 50-Year Review p.24-34.

Murray, R.D. (1989). A field investigation into causes of bovine abortion in dairy cattle. Vet. Rec. 219.

Paton, D.J., McGoldrick, A., Greiser-Wilke I., Parchariyanon, S., Song, J.Y., Liou, P.P., Stadejek, T., Lowings, J.P., Bjorklund, H. and Belak, S. (2000). Genetic typing of classical swine fever virus. Vet. Microbiol. 73(2-3): 137-157.

Paton, D.J., Sands, J.J. and Edwards, S. (1994). Border disease virus: delineation by monoclonal antibodies. Arch. Virol. 135: 241-252.

Paton, D.J., Sands, J.J., Lowings, J.P., Smith, J.E., Ibata, G. and Edwards, S. (1995). A proposed division of the pestivirus genus using monoclonal antibodies, supported by cross neutralization assays and genetic sequencing. Vet. Res. 26: 92-109.

Pellerin, C., Hurk, J.V.D., Lecomte, J. and Tijssen, P. (1994). Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high moralities. Virology. 203(2): 260-268 (doi:10.1006/viro.1994.1483).

Ridpath, J.F. (1996). Sequence diversity and genotyping. International Symposium, Bovine Viral Diarrhea Virus. A 50-Year Review. p. 39-42.

Ridpath, J.F. and Bolin, S.R. (1995). Delayed onset postvaccinal mucosal disease as a result of genetic recombination between genotype 1 and genotype 2 BVDV. Virology 212: 259-262, doi:10.1006/viro.1995.1480.

Ridpath, J.F., Bolin, S.R. and Dubovi, E.J. (1994). Segregation of bovine viral diarrhea virus into genotypes. Virology 205: 66-74. doi:10.1006/viro.1994.1620.

Saliki, J.T. (1996). Bovine Viral Diarrhea: Clinical picture in South Central United States. International Symposium. Bovine Viral Diarrhea Virus, a 50-year review. p. 159.

Strimmer, K. and von Haeseler, A. (1996). Quartet puzzling: A quartet Maximum likelihood method for reconstructing tree topologies. Mol. Biol. Evol. 13: 964-969.

Sullivan, D.G. and Akkina, R.K. (1995). A nested polymerase chain reaction assay to differentiate pestiviruses. Virus Res. 38: 231-239.

Tessaro, S.V., Carman, S. and Deregt, D. (1999). Viremia and virus shedding in Elk infected with type 1 and virulent type 2 bovine viral diarrhea virus. J. Wild. Dis. 35(4): 671-677.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting. position-specific gap penalties and weight matrix choice. Nuc. Acids Res. 22: 4673-4680.

Van Campen, H. and Williams, E.S. (1995). Wildlife and bovine viral diarrhea virus. International Symposium, Bovine Viral Diarrhea Virus. A 50-Year Review. p. 167-175.

Van Gennip, H.G., Widjojoatmodjo, M.N., de Smit, A.J. and Moormann, R.J. (1999). An internal duplication in the 5' noncoding region of strain H: a bovine viral diarrhoea virus (BVDV) isolated from pigs. Virus Genes, 19: 45-49.

Vilcek, S., Drew, T.W., McGoldrick, A. and Paton, D.J. (1999). Genetic typing of bovine pestiviruses from England and Wales. Vet. Microbiol. 69: 227-237.

Vilcek, S., Herring, A.J., Herring, J.A., Nettleton, P.F., Lowings, J.P. and Paton, D.J. (1994). Pestivirus isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Arch. Virol. 136: 309-323.

Vilcek, S., Stadejek, T., Ballagi-Pordany, A., Lowings, J.P., Paton, D.J. and Belak, S. (1996). Genetic variability of classical swine fever virus. Virus Res. 43: 137-147.

Windisch, J.M., Scneider, R., Wiland, E., Meyers, G. and Thiel, J.H. (1996). RNase of classical swine fever virus Biochemical characterization and inhibition by virus neutralizing monoclonal antibodies. J. Virol. 70(1): 352-358.

Yousif, A.A., Braun, L., Brock., K.V., Hennings, J.C., Hurley, D.J., Benfield, D.A., Ridpath, J.F. and Chase, C.C.L. (2002). NS3 typing of pestiviruses: a reference point for simultaneous detection and tying of bovine pestiviruses using molecular beacons. Chapter 3 in "Ruminant Pestiviruses Genetic Diversity: The Key to Detection and Control". Ph.D. Dissertation. South Dakota State University. Brookings. SD, USA: 138-157.

Zaghawa, A. (1998). Prevalence of antibodies to bovine viral diarrhoea virus and/or border disease virus in domestic ruminants. Zentralbl Veterinarmed. 45(6): 345-351.

الملخص العربي

فيروس الإسهال الفيروسي في الأبقار ذو النوع السيتوباثولوجي الجيني ٢ في الجمال ذات السنام الواحد

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العدوى بفيروسات مجموعة البستي في الحيوانات المجترة المستأنسة والخنازير تسبب مجموعة مشاكل ذات تأثير اقتصادي شديد عالميا. كما أن العديد من المجترات غير المستأنسة يمكن أن تصاب بعدوى مجموعة فيروسات البستي. ولقد عزلنا عدة فيروسات من نوع البستي من الجمال المصرية أحادية السنام في ١٩٩٥. وكانت الجمال التي تم منها العزل قد أظهرت أعراضا تناسلية وعيوب خلقية . كما أظهرت تجربة سابقة قدرة هذه المعزولات على إصابة الماعز و الأبقار. ولتوصيف هذه المعزولات جينيا تم إكثار الحمض النووي الريبوسي للمعزولتين ،جيزة ٧ و جيزة ٤ باستخدام منظومة معششة (Nested) من البادئات (Primers مكملة لتتابعات في جينات ال Erns) و ال NS3 لفيروسات البستي عن طريق الانساخ العكسي للحمض النووي الريبوسي المتبوع نتفاعل البلمرة المتسلسل (RT-PCR). ولقد أظهرت نتائج التفاعل أن الحمض النووي لفيروس جيزة ٧ متطابق مع المجموعة الجينية ٢ من فيروسات البستي ،وأن الحمض النووي لفيروس جيزة ٤ متطابق مع المجموعة الجينية ١ لفيروسات البستي. وأظهر تحليل تتابعات النيوكليوتيدات لفيروس جيزة ٧ في جين ال Erns أن هناك تشابه بنسب ٨٢ ــ ٨٨ % ،و ٦٨,٧ ــ ٧٢ % ،و٦٦,٧ ــ ٦٧,٢ % ،و ٦٨ % ،و ٦٤ % مع مجموعات فيروس البستي ٢ ،و ١ و فيروس مرض الحدود (BDV) ،و فيروس حمى الخنازير الكلاسيكي (CSFV) ،و فيروس الريندير-١(Reindeer-1) على التوالي. و لقد كانت نسبة التشابه على مستوى تتابعات النيوكليوتيدات بين جيزة ٧ و عترة الزراف الأفريقية الوحيدة من نوعها (70% (Giraffe-1 و هي نسبة تشابه أعلى من تلك التي بين جيزة ٧ و العترات المرجعية اوريجون (Oregon) ونادل (NADL) التى تنتمي للمجموعة الجينية ١ من فيروسات البستي. أما على مستوى تتابعات الأحماض الأمينية المستنبطة ،فان نسبة التشابه بين جيزة ٧ و الفيروسات الأخرى كانت كالتالي : ٨٤،٩- ٩٠،٢ % مع فيروسات المجموعة الجينية ٢ ،و ٧٧,٤-٨١,٩ % مع فيروسات المجموعة الجينية ١ ،و ٧٣,٦ % مع فيروسات المجموعتين BDVو CSFV 77, 81.9 % ،على التوالي. و قد أظهرت فيروسات ال Giraffe-1 و ال Reindeer-1 نسبة تشابه ٧٥,١ % و ٧٢,١ % مع فيروس جيزة ٧ ،على التوالي. هذا ،وقد أظهرت المقارنات بين التتابعات النيوكليوتيدية لأجزاء قصيرة من جين ال NS3 لفيروس الجيزة ٧ و فيروسات البستي الأخرى باستخدام BLASTn وجود درجة عالية من التشابه مع فيروسات المجموعة الجينية ٢. ويعتبر هذا أول ذكر لعدوى فيروس الإسهال الفيروسي في الأبقارمن النوع الجيني ٢ في الجمال أحادية السنام.