# Genetic characterization of the Egyptian vaccinal strain "Abu-Hammad" of bovine herpesvirus-1

*(Received: 15.04.2006; Accepted: 05.06.2006)*

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## ABSTRACT

*The local Egyptian vaccinal strain "Abu-Hammad" of bovine herpesvirus-1 (BHV-1) was genetically characterized based on two main molecular approaches; the Hind III endonuclease for genomic fingerprinting of the local BHV-1 compared to that of the reference strain "Cooper 1" of BHV-1 subtype 1 (BHV-1.1), and the analyses of nucleotide (nt) sequences, deduced amino acid (aa) sequences and phylogeny of the major viral immunogen, glycoprotein D (gD) of the local BHV-1 versus its counterparts of other related alpha(α)-herpesviruses. The resulted sizes and electrophoretic patterns of the Hind III viral DNA fragments (A-to-M) revealed close identity between the local BHV-1 and reference BHV-1.1. Both nt and deduced aa sequence alignments revealed variable degrees of similarity of the local BHV-1 gD, which was high with BHV-1.1 and BHV-1.2; moderate with bovine herpesvrius-5; low with caprine herpesvirus-1 and suid herpesvirus-1 (pseudorabies virus); or very low with human herpesvirus-1 and herpesvirus-2. A possible mutational frame shift at nt 509 and 615 was observed toward the carboxyl-terminus of the local BHV-1 gD. The gD nt and deduced aa sequence data enabled phylogenetic characterization of the local BHV-1 and correlated with the results of genomic fingerprinting after restriction endonuclease cleavage. Based on phylogenetic analyses, the Egyptian vaccinal BHV-1 was grouped as a BHV-1 subtype 1 in a distinguished branch within the phylogenetic tree, together with BHV-1.1. Determined conservation of five cysteine residues and the glycosylation domains in the amino (N-) terminal half emphasized the importance of the N-terminus for immunological and biological function of gD among α-herpesviruses. Presence of the most divergent domain of 17 aa residues at positions 168-184 and an additional cysteine residue at position 178 could be used as tools to distinguish the local Egyptian BHV-1 from other related herpesviruses. Findings of this work showed that genomic fingerprinting, based on endonuclease Hind III cleavage, and direct sequencing of the gD gene-derived PCR amplicons were relevant tools for genetic characterization of BHV-1 strains / isolates. The comparative genetic analyses conducted were useful to trace conservation of the local BHV-1 among related α-herpesviruses and to establish genetic tools for national-wide epidemiological studies and development of novel efficient BHV-1 vaccines.*

***Key words***: *Bovine herpesvirus 1 (BHV-1)-Infectious bovine rhinotracheitis (IBR)-Egyptian vaccinal strain – Alphaherpesviruses - Glycoprotein D (gD).*

## INTRODUCTION

Bovine herpesvirus 1 (BHV-1), an important contagious viral pathogen of domestic and wild bovines, is distributed worldwide exerting an economic impact on livestock industry. BHV-1 is associated with a broad-spectrum of disease manifestations including: severe respiratory infection (infectious bovine rhinotracheitis), conjunctivitis (pink eye), vulvovaginitis, balanopostitis, shipping fever and systemic infection in neonate calves (Gibbs and Rweyemamu, 1977; Wyler, *et al.*, 1989; and Tikoo *et al.*, 1995). In Egypt, since 1960s, attention was drawn to BHV-1 (IBRV) as one of the most significant causes of great economic losses in feedlot and dairy farms; mainly due to deaths from pneumoenteritis, mainly in cattle and buffalo calves, abortions, prolonged feeding periods with weight loss, costs of treatment and prevention programs. (Hafez and frey, 1973 and Aly *et al.*, 2003). The local Egyptian vaccinal strain of BHV-1, namely Abu-Hammad strain, was isolated during an outbreak in Sharqia (Hafez *et al.*, 1976).

BHV-1, an enveloped DNA virus, is currently classified as a member of the genus *Varicellovirus* of the sub-family *Alphaherpesvirinae* within the family *Herpesviridae*, which is a large family containing more than 100 different members, 8 human herpesviruses and at least one for each animal species (Studdert, 1999). All herpesviruses share a common overall genome structure, but differ in the fine details of genome organization, at levels of nucleotide sequence and biological properties. BHV-1 genome consists of a linear double-stranded DNA molecule of about 136 Kilobases (Kb) long which is subdivided into a unique long segment ($U_L$, 104 Kb) and a short segment, containing a unique short region ($U_S$, 10 Kb) flanked by internal and terminal inverted repeats ($IR_S$ & $TR_S$, 11 Kb long each) with alternative orientations of $U_S$ relative to the fixed $U_L$ (Schwyzer and Ackermann, 1996). Based on restriction endonuclease analysis of BHV-1 genomic DNA, virus strains were classified into subtypes 1, 2a and 2b (Metzler *et al.*, 1985). BHV-1 subtype 1.1 is associated with respiratory infections whereas; BHV-1 subtype 1.2 is associated with genital infections in cattle (Wyler *et al.*, 1989). Recently, this classification has been extended, according to individual fragment numbers or sizes produced by each enzyme with two main groups consisting of fragments A to I and J to L, to subtypes with numeric codes such as 1.1.I, 1.1.II, 1.1.III, and 1.2.Iva on using the *Hind III* enzyme. Although subtype 1 is likely more virulent than subtype 2b, only one antigenic type of BHV-1 has been recognized to date (Wyss *et al.*, 2000). The BHV-1 nucleotide sequence comprises 67 unique genes and two genes, both duplicated in the inverted repeats, encoding for at least 69 proteins among which 40 proteins are structural. These proteins are grouped according to their presumed function or location in the virion into: Glycoproteins, Envelope, Tegument, Capsid, Cleavage, Packaging, DNA replication, Enzymes, Regulatory, and BHV-1 specific proteins (Schwyzer and Ackermann, 1996). The BHV1 genome contains at least 10 genes with the potential to encode glycoproteins, namely, gB, gC, gD, gI, gE, gH, gL, gG, gK and gM. They all share important roles in pathogenicity, virulence and replication in host cell. Glycoproteins gB, gC and gD of BHV-1 have been the major viral immunogens recognized by sera from infected cattle (Collins *et al.*,

1985 and Van Drunen Little-van den Hurk and Babiuk, 1986). Glycoprotein gD is essential for virus replication and is responsible for inducing the strongest immune response, reduced virus replication and shedding in the host (Tikoo *et al.*, 1995). The gD gene is well studied and proved highly conserved among herpesviruses. It is located in the $U_S$ region between map units 0.892 and 0.902 of the BHV-1 genome, encoding a 71 kDa glycoprotein of 417 amino acids, containing both N- and O- linked oligosaccharides with a signal sequence cleavage site between amino acids 18 and 19 (Tikoo *et al.*, 1990 and Schwyzer and Ackermann, 1996). All these properties of gD, as a very essential and major BHV-1 gene, made it an excellent candidate as a target for genetic characterization of our local vaccinal strain "Abu-Hammad" of BHV-1. Several microbial genome sequences have been published that indicated the value of comparisons at the genomic level for better campaigns against pathogens regarding pathogenesis, host range and cross-immunity among related pathogens (Van Oirschot, 1999 and Thomson *et al.*, 2003).

The objective of this endeavor is to genetically characterize our local vaccinal BHV-1 strain (Abu-Hammad), at the genomic level, by fingerprinting of the whole viral genome using restriction endonucleases and comparative sequence analysis of its major immunogen glycoprotein D (gD), *versus* its counterparts in genomes of other related herpesviruses. This may determine the genetic type of our local vaccinal *versus* other reference and vaccinal strains of BHV-1 and reveals potential targets useful for development of new vaccines, BHV-1 diagnostic realm, epizootiological studies and better control programs.

## MATERIALS AND METHODS

### Virus and cells

The local vaccinal BHV-1 "Abu-Hammad" strain (Hafez *et al.*, 1976) and the reference Cooper 1 strain of BHV-1 (National Veterinary Services Laboratory, Animal and Plant Health Inspection Services, Ames, IA, USA) were used in this study. Viral stocks were prepared by infecting MBDK cells at a multiplicity of infection (MOI) of 0.1 from plaque-purified viruses and were subsequently titrated on Madin Darby Bovine Kidney (MDBK) cell cultures. The MDBK cells were grown and maintained in minimum essential medium with Earle's salts (MEME) supplemented with heat-inactivated 10% bovine calf serum (BCS), 100 U/ml penicillin and 100 µg/ml streptomycin.

Prior to experimental work, both MDBK cells and BCS were tested free of BHV-1 by indirect immunofluorescence. Further, the virus identity of both local and reference strains of BHV-1 was proved by their unique strong reaction with the local and reference (Central Vet. Lab., Wybridge, England) anti-BHV-1 polyclonal antibody using indirect immunofluorescence in MDBK cells (Van Donkersgoed and Babiuk, 1991).

### Extraction of viral DNA

The DNA was extracted following the procedure described in Vilcek *et al.* (1994) after some modifications. Briefly, a 25 ml of each crude virus in culture supernatant from the BHV-1 (Abu-Hammad and Cooper 1 strains) infected MDBK cells was clarified by centrifugation at 6000 rpm / 4°C for 20 minutes. The clarified viruses were ultracentrifuged at 40,000 rpm at 4°C for 2 hr then, the supernatants were discarded. The virus pellets were dissolved in 0.5 ml of 2% SDS, mixed with 0.4 mg/ml proteinase K and incubated at 56°C for 1 hr with intermittent shaking. The

mixture was then extracted with equal volume of phenol : chloroform : isoamyl alcohol reagent (25:24:1, vol/vol/vol, equilibrated to pH 8.0). DNAs in the aqueous phases were precipitated with 2 volumes of cold absolute ethanol. The DNAs were pelted by 30 min centrifugation at 14000 rpm and 4°C. The DNA pellets were washed in cold 70% ethanol, re-precipitated by centrifugation, dried, dissolved in 25 µl of nuclease-free water and stored at -20°C until used. Concentration and purity of the BHV-1 genomic DNA were measured following the procedure described by Sambrook and Russell (2000).

**Restriction endonuclease analysis**

The restriction endonucleases *Hind III* and *BamH I* were used to cleave the genomic DNAs of both local "Abu-Hammad" and reference "Cooper 1" strains of BHV-1, following standard protocols described in Sambrook and Russell (2000). Electrophoretic patterns of the resulting viral genomic DNA fragments were analysed by 0.7% agarose gel electrophoresis. The DNA bands were visualized after gel staining with ethidium bromide (0.5 µg/ml).

**Polymerase chain reaction (PCR) assay**

The oligonucleotide primers used in this study were selected from highly conserved sequences encoding the gD gene of Cooper 1.1 strain of BHV-1 genome (GenBank Accession No. NC_001847). Sense 5'-GCGAACAT-GCAAGGGCCGACATTG -3' Anti sense5'-CACGGCGTCGGGGGCCG-CGGGCGT -3'

This primer set was used in the PCR assay to partially amplify the gD gene (a full length gene lacking only to about 0.2 Kb fragment encoding for the transmembrane anchor) of BHV-1 genome. PCR reaction was applied in a total volume of 50 µl containing: 1 X PCR buffer (20 mM Tris HCl pH 8.4 and 50 mM KCl); 1.5 mM $MgCl_2$; 0.2 mM deoxynucleotides triphosphates mixture; 100 pmol of each primer; 2.5 units (U) thermus aquaticus (Taq) polymerase; 0.1µg of extracted viral DNA and nuclease-free sterile double distilled water up to 50.0 µl. Then, the resulting mixture was subjected to precise thermal profile in a programmable thermocycler as follows: One cycle: 96°C for 2 min.; 35 cycles: 96°C for 50 sec, 58°C for 50 sec, and 72°C for 1 min.; One cycle: 72°C for 10 min.

**Analysis of PCR amplification products (amplicons)**

The resulting PCR amplicons were analyzed by 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2000). The DNA bands were visualized after gel staining with ethidium bromide (0.5 µg/ml). The PCR amplicons of the proper predicted size (about 1.1kb) were gel purified using DNA gel purification kit (ABgene, Germany) and quantitated according to the procedure described by Sambrook and Russell (2000).

**Direct Sequencing of PCR amplicons**

The PCR DNA amplicons of local vaccinal "Abu-Hammad" strain of BHV-1 were purified using microcon columns (Amicon, USA) and directly sequenced in both directions with the same primers used to generate PCR amplicons. Sequencing was done in an ABI PRISM system using the dideoxy chain-termination method (Sanger *et al.*, 1977), based on the incorporation of fluorescent-labeled dideoxynucleotide terminators. The primer walking strategy was used and identity of each nucleotide was verified at least twice.

**Computer assisted Sequence and Phylogenic analyses**

The resulted nucleotide and deduced amino acid sequence data of the selected region of the gD gene of "Abu-Hammad"

strain of BHV-1 were compiled and submitted to the GenBank (Accession No. AY690484). These sequence data were compared to those of other related alphaherpesviruses accessed via the GenBank, including: BHV-1.1 "Cooper 1" (Accession No. NC_001847), BHV-1.2 ST (Accession No. AY437088), Bovine herpesvrius-5 (BHV-5 "TX89"; Accession No. U14656), Caprine herpesvirus-1 (CHV-1, "E/CH"; Accession No. AY437088), Suid herpesvirus-1 "Kaplan", Pseudorabies virus, Accession No. AJ271966), Human herpesvirus-1 "KHS2" (HHV-1, Herpes simplex virus type 1; Accession No. AF487902), and Human herpesvirus-2 "CAM4B" (HHV-2, Herpes simplex virus type 2; Accession No. U12180). The nucleotide sequences were aligned by the Clustal W (1.82) program of European Bioinformatics Institute (EBI). Clustal W is a fully automatic program for global multiple alignment of DNA and protein sequences. Phylogenetic correlation and tree construction were done using the PHYLIP and Treeview 32 (1.6.6) programs.

## RESULTS

### Restriction endonuclease analysis (Finger-printing)

The electrophoretic profiles of the BHV-1 genomic DNA digested with restriction endonuclease *Hind III*, revealed an identical DNA fingerprints for both (Abu-Hammad) and reference (Cooper 1) strains of BHV-1 (Figure 1). While, no fingerprint could be observed on repeated cutting in genomic DNA of both local and reference BHV-1 by the *BamH I* enzyme (data not shown).

### Analysis of PCR amplification products (amplicons).

Agarose gel electrophoretic analysis of the PCR amplicons indicated that amplified DNA fragment encoding the glycoprotein D (gD) from the (Abu-Hammad) and reference (Cooper 1) strains of BHV-1 were correspondent to the expected size of about 1.1 Kb. The amplified DNA bands were of the same size for both local and reference BHV-1 strains (Fig. 2).

### Sequence and phylogenetic analyses of the BHV-1 gD gene

Analysis of the nucleotide sequence of PCR amplicons, from the local Egyptian vaccinal strain "Abu-Hammad" of BHV-1, revealed a single open reading frame (ORF). This ORF was 1.083 nucleotides long, starting from the first ATG at nucleotide (nt) 7 and extending upstream to nt 1089 in the sequence. Homology search revealed sequence similarity between this ORF and the published gD gene of alpha (α-) herpesviruses. Therefore, the present sequenced gene fragment of "Abu-Hammad" strain was identified as a BHV-1 gD gene. Since the location of gD gene is conserved through out the sub-family (α-herpesviridea, there was no need to further locate consensus sequences of other transcriptional regulatory elements specially the endogenous promoter TATA box or polyadenylation signal. Nucleotide composition of the ORF sequence was calculated to be A 17.26%, T 13.13%, C 35.26% and G 34.16%, with a G+C content of 69.42%.

Nucleotide sequence alignment of the local BHV-1 gD and other related *Alpha-(α-) herpesviruses* showed variable percentages of homology (7% - 98%) as illustrated in table (1). The highest gD sequence identity was recorded with the reference "Cooper 1" strain of BHV-1.1 (98%), followed by the ST strain of BHV-1.2 (97%), the TX89 strain of BHV-5 (84%), E/CH strain of caprine herpesviruses -1 (69%) , and Kaplan strain of suid herpes-

viruses (bovine pseudorabies virus, 59%). In contrast, human herpesviruses types 1 and 2 (HSV-1 and HSV-2) scored extreme gD sequence divergence of 7% and 16%, respectively, with the local BHV-1 gD (Table 1 and figure 4). Comparison of the local BHV-1 gD sequence with other *α-herpesviruses* showed a single or triplet mismatches or substitution, mainly at nt 476, 509, 557, 567-569, 588, 598 and 615 (data not shown). The phylogenetic analysis of aligned gD sequences of these α-herpesviruses (Fig. 4) revealed close ancestral genetic relation among these viruses.

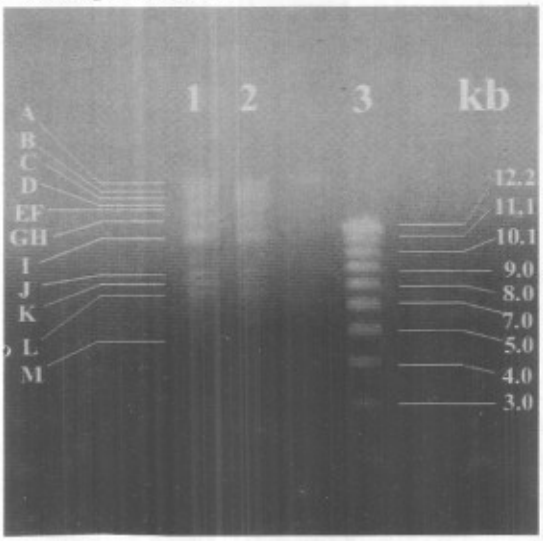


***Fig.(1) : Agarose gel electrophoresis of the genomic viral DNA cut with restriction endonucleases, separated on 0.7% agarose gel and stained with ethidium bromide. Lanes: (1)"Abu-Hammad" strain of BHV-1 cut with HindIII; (2) Reference "Cooper 1" strain of BHV-1.1 cut with HindIII; (3) 1 Kb DNA ladder (Gibco, BRL). Lines indicate the BHV genomic DNA fragments from A to M..***

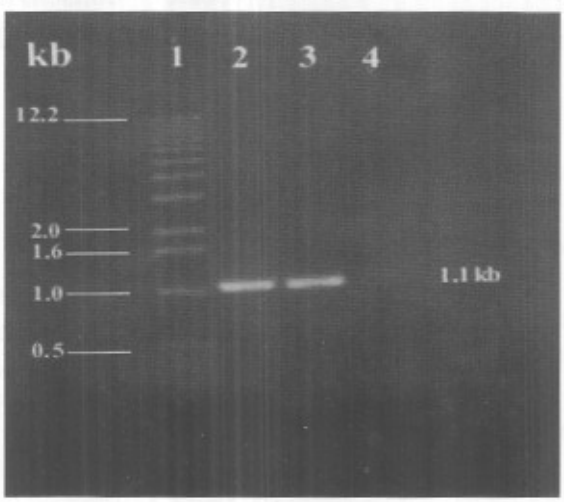


***Fig. (2): Agarose gel electrophoresis of the PCR-derived amplicons of BHV-1 gD gene, separated on 1.5% agarose gel and stained with ethidium bromide. Lanes : (1) 100 bp DNA ladder (Gibco, BRL); (2) PCR amplicons of the"Abu-Hammad" strain of BHV-1; (3) PCR amplicons of the reference "Cooper 1" strain of BHV-1; (4) non-infected MBDK cell control.***

**Amino acid sequence analysis and comparison of the local BHV-1 gD**

The deduced amino acid (aa) sequence of the local BHV-1 gD (Abu-Hammad strain) was compared to those of related glycoproteins of reference BHV-1.1 "Cooper 1" (Goltz *et al.*, 2006), BHV-1.2 "ST" (Leung-Tack *et al.*, 1994) BHV-5 "TX89" (Abdelmagid *et al.*, 1995), CHV-1 "E/CH" (Keuser *et al.*, 2006), SuidHV-1 "Kaplan" (Brack *et al.*, 2000), HSV-1 "KHS2" (Kim *et al.*, 2002), and HSV-2 "CAM4B" (Waston, 1983). As shown in table (2) and figure 3, the reference Cooper 1 strain of BHV-1.1 scored the highest gD sequence identify (90%) with the local BHV-1 gD, followed by BHV-1.2 (88%), BHV-5 (73%), CHV-1 (51%) and suid HV-1 (27%). No gD aa sequence identify could be recorded with gD of either HSV-1 or HSV-2 (table 2 and figure 3). All cysteine residues but one in the gD sequence of local BHV-1 were conserved without deletions or substitution at residue position 75, 114, 126, 135 and 215. Only one cysteine residue at position 178 of the local BHV-1 gD had no match in all other gD sequences in the current alignment (figure 3). In particular, the amino (N-) terminus including the N- linked glycosylation domains at position 40-42 and 102-104 were highly conserved in the gD of local BHV-1 "Abu-Hammad", BHV-1 "Cooper 1", BHV-1.2 "ST", CHV-1 "E/CH" and BHV-5 "TX89" (figure 3). On comparison, the major gaps of aa mismatches or substitution in the local BHV-1 gD sequence were observed in aa residues at positions 157, 158, 160-164, 168-184, 188, 195, 196 and 198-202 (figure 3). The most divergent domain was noticed at the segment 168-184 in the local gD consensus aa sequence might be useful for gD specificity of each *α-herpesvirus*. However, the resulting translation contains a possible frame shift at nts 508 and 614 compared to other BHV gD genes. It was realized that frequent mutations within the coding region may result in frame shifts or premature stop cordons.

***Table (1): Score table of multiple nucleotide sequence alignment of the local Egyptian vaccinal BHV-1 "Abu-Hammad" strain (Seq. A) Versus other related alpha-herpesviruses (Seq. B) using CLUSTAL W (1.82) program.***

| Seq. A Name | Len. (nt) | Seq. B Name | Len. (nt) | Score (%) |
|---|---|---|---|---|
| Abu-Hammad | 1089 | BHV-1.1 Cooper1 strain | 1254 | 98 |
| Abu-Hammad | 1089 | BHV-1.2 ST strain | 1254 | 97 |
| Abu-Hammad | 1089 | BHV-5 TX89 strain | 1254 | 84 |
| Abu-Hammad | 1089 | CHV-1 E/CH strain | 1230 | 69 |
| Abu-Hammad | 1089 | Suid HV-1 (Pseudorabies virus) Kaplan strain | 1203 | 59 |
| Abu-Hammad | 1089 | HHV-1 (herpes simplex 1) KHS2 strain | 1125 | 7 |
| Abu-Hammad | 1089 | HHV-2 (herpes simplex 2) CAM4B strain | 1274 | 16 |

Seq. = sequence Len. (nt) = length in nucleotides Score (%) = sequence homology percent

***Table (2): Score table for the deduced amino acid sequence identity of the local Egyptian vaccinal BHV-1 "Abu-Hammad" strain (Seq.A) Versus other related alpha-herpesviruses (Seq. B) using CLUSTAL W (1.82) program.***

| Seq. A Name | Len. (aa) | Seq. B Name | Len. (aa) | Score (%) |
|---|---|---|---|---|
| Abu-Hammad | 361 | BHV-1.1 Cooper 1 strain | 417 | 90 |
| Abu-Hammad | 361 | BHV-1.2 ST strain | 417 | 88 |
| Abu-Hammad | 361 | BHV-5 TX89 strain | 417 | 73 |
| Abu-Hammad | 361 | CapHV-1 E/CH strain | 407 | 51 |
| Abu-Hammad | 361 | Suid HV-1 (Pseudorabies virus) Kaplan strain | 400 | 27 |
| Abu-Hammad | 361 | HHV-1 (herpes simplex 1) KHS2 strain | 394 | 17 |
| Abu-Hammad | 361 | HHV-2 (herpes simplex 2) CAM4B strain | 393 | 14 |

Seq. = sequence Len. (aa) = length in amino acid Score (%) = sequence homology percent

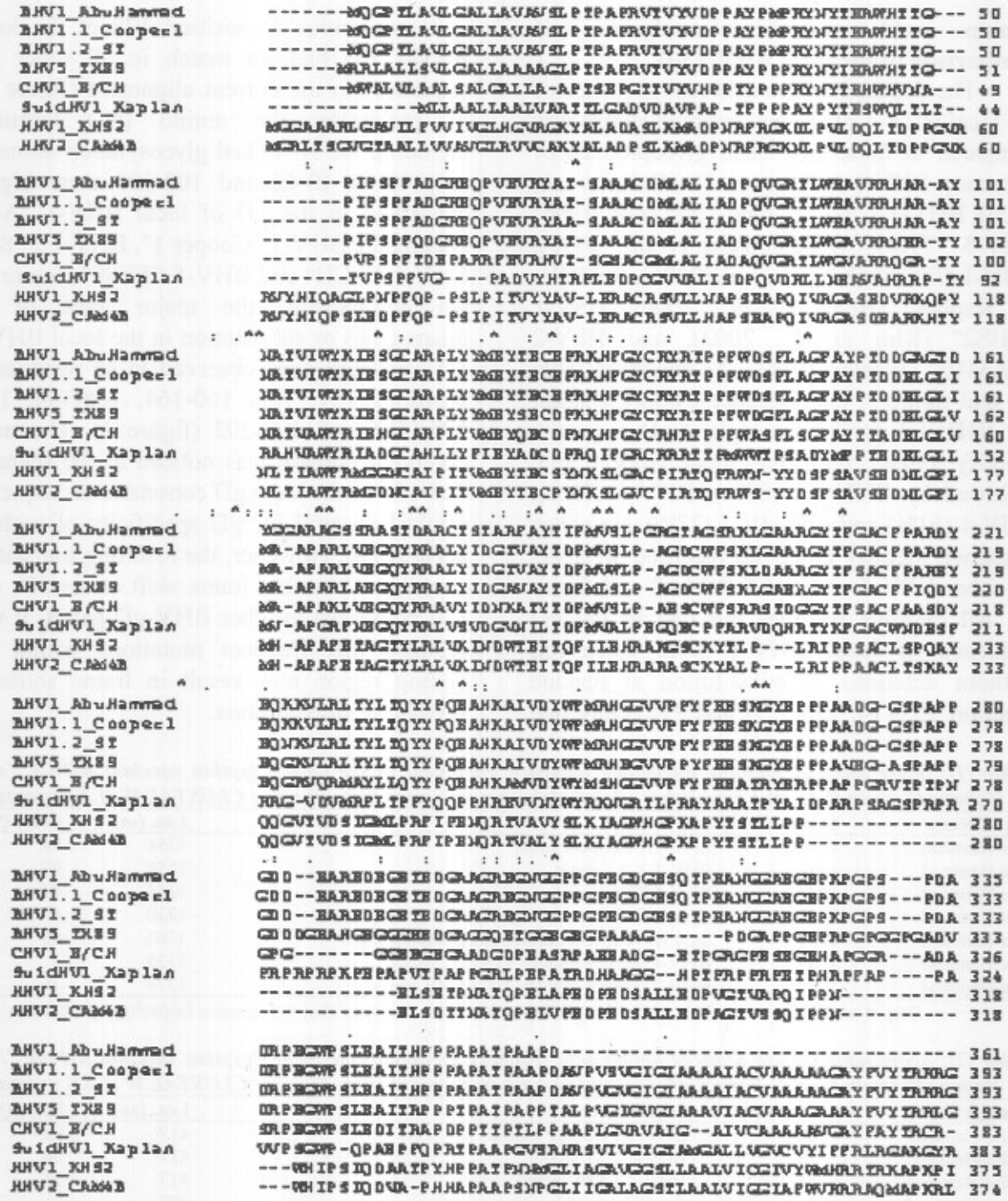

*Fig (3): Deduced amino acid (aa) sequence alignment of BHV-1 (Abu-Hammad) versus other related alphaherpesviruses . Numbers of the sequence indicate positions of amino acids in the gD protein relative to the GenBank data for each virus. Dots mean that conserved ( : ) or semi-conserved ( .) substitutions are observed whereas, stars (*) indicate that amino acid residues in that column are identical in all sequences.*

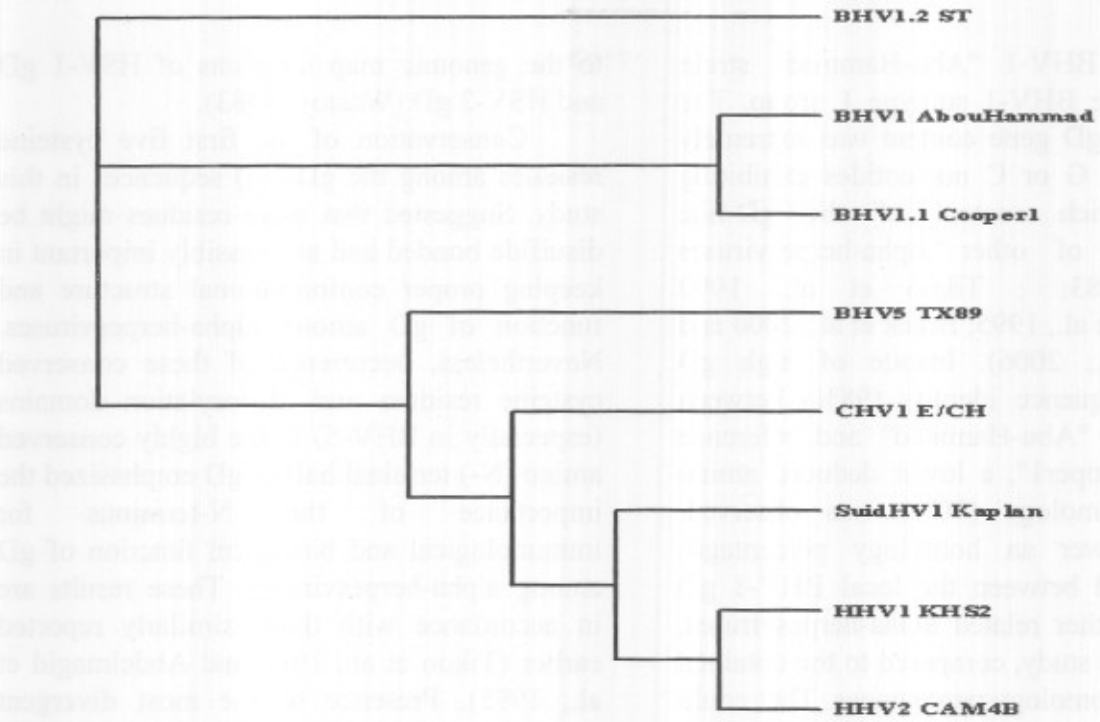


***Fig. (4): Phylogenetic tree of BHV-1 (Abu-Hammad) strain and other related alpha herpesviruses. Generated from nucleotide sequences encoding for glycoprotein D (gD) of the analyzed viral genomes.***

## DISCUSSION

Herpesviruses have been a major cause of pneumoenteritis, abortions and deaths among livestock animals specially cattle and buffalo calves (Gibbs and Rweyemamu, 1977; Wyler, et al., 1989; Tikoo et al., 1995; and Aly et al., 2003). Recently, the molecular virology of ruminant herpesviruses has achieved a considerable progress in terms of genomic sequence analyses laying a good basis for further studies of BHV-1 and related viruses (Schwyzer and Ackermann, 1996).

The genetic characterization of the local Egyptian vaccinal strain "Abu-Hammad" of BHV-1, based on application of two main approaches. First approach utilized the genomic fingerprint of the local BHV-1 compared to that of the reference strain "Cooper1" of BHV-1 subtype 1 (BHV-1.1). The second approach relied on analysis of the nucleotide sequences, deduced amino acid sequences and phylogeny of the major viral immunogen, glycoprotein D (gD) of the local BHV-1 versus other related herpesviruses.

Genomic fingerprinting, based on sizes and electrophoretic patterns of the viral DNA fragments (A-to-M), observed after Hind III endonuclease cleavage, revealed close identity between the "Abu-Hammad" BHV-1 and the "Cooper1" BHV-1.1. The resulted size and pattern of the Hind III fragments K and L in both local and reference strains are very characteristic finger prints to viral genomes of BHV-1 subtype 1 (Mayfield et al., 1983; Metzler et al., 1985 and Wyss et al., 2000). It has been reported that the endonuclease Hind III was the enzyme of choice that most clearly reflected differences existing among BHV-1 strains or isolates (Metzler et al., 1985).

Accordingly, BHV-1 "Abu-Hammad" strain fits clearly the BHV-1 subtype 1 group. The local BHV-1 gD gene content was extremely biased to use G or C nucleotides exhibiting similar GC-rich content of the gD-like glycoproteins of other alpha-herpesviruses (Watson, 1983; ; Tikoo et al., 1990 Abdelmagid et al., 1995; Brack et al., 2000 and Keuser et al., 2006). Inspite of high gD nucleotide sequence identity (98%) between local BHV-1 "Abu-Hammad" and reference BHV-1.1 "Cooper1", a lower deduced amino acid (aa) homology (90%) was observed. Similarly, lower aa homology percentages were recorded between the local BHV-1 gD and that of other related alpha-herpesviruses, aligned in this study, compared to the obtained nt sequence homology percentages. That could be attributed to the occurrence of a possible mutational frame shift at nucleotides (nt.) 509 and 615 which was biased toward the carboxyl-terminus of BHV-1 gD. Bovine herpesvirus-5 (BHV-5) is the causative agent of a fatal meningeo-encephalitis in calves and is closely related to BHV-1 (Whitbeck et. al., 1999). Also, other related herpesviruses selected for comparison in this endeavor are neurotropic mammalian alphaherpesviruses (Studdert, 1999).

Both nucleotide and deduced amino acid sequence alignments revealed variable degrees of sequence homology with the local BHV-1 gD which was high (BHV-1.1 Cooper 1) and BHV-1.2, moderate (BHV-5), low (CHV-1 and suid HV-1 or pseudorabies virus), or very low (HSV-1 and HSV-2). It has been established that BHV-1 gD gene is located in the US region of the viral genome between map units 0.892 and 0.902 (Tikoo et al., 1990), which approximately collinear with the gD-like glycoproteins of other alpha-herpesviruses (; Abdelmagid et al., 1995; Brack et al., 2000 and Keuser et al., 2006), but inverted relative to the genomic map locations of HSV-1 gD and HSV-2 gD (Waston, 1983).

Conservation of the first five cysteine residues among the gD (aa) sequences in this study. Suggested that these residues might be disulfide bonded and are possibly important in keeping proper conformational structure and function of gD among alpha-herpesviruses. Nevertheless, occurrence of these conserved cysteine residues and glycosylation domains (especially in BHV-5) in the highly conserved amino (N-) terminal half of gD emphasized the importance of the N-terminus for immunological and biological function of gD among alpha-herpesviruses. These results are in accordance with those similarly reported earlier (Tikoo et al., 1990 and Abdelmagid et al., 1995). Presence of the most divergent domain of 17 aa residues at positions 168-184 and the additional cysteine residue at position 178 could be used as a tool to distinguish the "Abu-Hammad" strain from other related herpesviruses.

The gD nucleotide and deduced amino acid sequence data enabled phylogenetic characterization of the BHV-1 "Abu-Hammad" strain and correlate with the results of genomic finger printing after restriction endonuclease cleavage. Phylogenetic trees based on either gD nucleotide sequences or deduced amino acid sequences were consistently similar, in the present work. Three major branches were apparent in the constructed trees, revealing variable range of genetic differences among alpha- herpesviruses. Important is that "Abu-Hammad" and reference BHV-1.1 "Cooper 1" strains represented two close lineages lying in the same branch of the phylogenetic tree, suggesting a unique antigenic subtype BHV-1.1 for both strains. Phylogenetic reconstruction of herpesvirus evolution is generally founded on amino acid sequence comparisons of specific proteins (Karlin *et al.*,

1994). It has been established in the dilemma of phylogeny that nucleotide substitution, insertion, deletion, or duplication events in the sequences are manifested as differences in branch lengths or absence in the phylogenetic trees, proportional to the genetic change (Felsenstein, 2001). The results obtained in this study correlate clearly to the reported antigenic differences among alpha-herpesviruses, with particular considerations to viral glycoproteins gB, gC, gD and gH (Metzler *et al.*, 1986; Friedli and Metzler, 1987; Tikoo *et al.*, 1990; Lyaku *et al.*,1992; Collins *et al.*, 1993; Karlin *et al* ., 1994; Meyer *et al.*, 1999; and Whitbeck *et al.*, 1999). Moreover, these results coincide with recent reports regarding the importance of the gD gene based molecular assays for pathogenesis and epidemiological studies of BHV-1 infections (Tikoo *et al.*, 1990; Wiedmann *et al.*, 1993; Vilcek *et al.*, 1994; Rocha *et al.*, 1998; Fuchs *et al.*, 1999; Van Oirschot, 1999; and Wyss *et al.*, 2000). Similar positive reaction with the local and reference anti-BHV-1 polyclonal antiserum in the VNT indicated high antigenic identity between the local (Abu-Hammad) and reference (Cooper 1) strains of BHV-1. In spite of considerable degree of sequence conservation among antigenically related ruminant alpha herpesviruses (Engels *et al.*, 1987, Vanderplaschen *et al.*, 1993 and Studdert, 1999), some herpesviruses react poorly with antibodies made against antigenically heterologous viral strains (Studdert, 1999 and Van Oirschot, 1999).

In conclusion, Findings of this endeavor showed that genomic fingerprinting, based on endonuclease *Hind III* cleavage, and direct sequencing of the gD gene-derived PCR amplicons were relevant tools for genetic characterization of BHV-1 strains. Phylogenetic analyses indicated that the local vaccinal strain (Abu-Hammad) belongs to BHV-1 subtype 1. Thus, Egyptian BHV-1 vaccinal strain was grouped as a BHV-1.1 in a distinguished branch within the phylogenetic tree, together with the reference (Cooper 1) strain of BHV-1.1. The comparative genetic analyses conducted in this endeavor were useful not only to trace conservation of the local BHV-1 among related *α-herpesviruses* but also to establish genetic tools for national-wide epidemiological studies. Nevertheless, it is highly recommended to use of the locally isolated viruses is the best choice for local vaccine preparation to avoid viral escaped neutralization by antibody raised against heterogonous variants and to obtain efficient BHV-1 vaccines.

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الملخص العربي

## التوصيف الوراثي لعترة اللقاح المصرية ( أبو حماد ) لفيروس الهيربس البقري -1

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** المركز القومي للبحوث – شعبة البحوث البيطرية – قسم الطفيليات وأمراض الحيوان – الدقي – الجيزة .

في هذا البحث إجرى التوصيف الوراثي لعترة اللقاح المصرية ( أبو حماد ) لفيروس الهيربس البقري –1 باستخدام التوصيف الجزيئي في تحديد البصمة الوراثية لجينوم عترة الفيروس المصرية مقارنة بالعترة المرجعية ( كوبر –1) النوع الوراثي 1.1 للفيروس باستخدام إنزيم قطع الحمض النووي ( هند III ) .في التقنية الثانية تم تحليل التتابع النيوكلوتيدي والأحماض الأمينية المستنتجة وكذلك التشعب الوراثي لجين البروتين الرئيسي المحدث للمناعة ضد الفيروس وهو الجليكو بروتين الغلافي "دي" ( جي دي ) لعترة الفيروس المصرية ومقارنتها مع مثيلاتها في فيروسات نفس مجموعة الألفا هيربس وقد أوضحت النتائج تطابق البصمة الوراثية بإستخدام الفصل الكهربائي للجينوم في كل من عترة الفيروس المصرية والعترة المرجعية من النوع الوراثي 1.1 .كذلك وجد تماثل بنسب مختلفة في التتابع النيو كلوتيدي والأحماض الأمينية المستنتجة لجين (جى دى ) بين عترة الفيروس المصرية و مثيلاتها من مجموعة الالفا لفيروس الهيربس البقرى –1 من النوع الوراثى 1.1 و 1.2 و متوسطة مع فيروس الهيربس البقرى –5 و ضعيفة مع فيروس الهيربس الماعزى –1 و فيروس الهيربس الخنزيرى –1 و لكنها كانت ضعيفة جدا مع فيروسات الهيربس الادمى نوعى 1 ، 2. وهناك إحتمال حدوث طفرة فى إطار التتابع النيوكلوتيدى خاصة عند القاعدتين النوويتين رقم 509 و 615 فى اتجاة الطرف الكربوكسيلى لجين (جى دى ) فى عترة اللقاح المصرية. وطبقا لتحليل التشعب الوراثى فقد تم تصنيف عترة اللقاح المصرية لفيروس الهيربس البقرى –1 من النوع الوراثى 1.1 فى فرع منفصل من الشجرة الوراثية بين الفيروسات المقارنة من مجموعة فيروس الفا هيربس .

وقد اتضح ان هناك اهمية للوظائف البيولوجية و المناعية للطرف الامينى لجين (جي دى) فى هذه المجموعة لفيروسات ألفا هيربس نظرا لوجود خمسة احماض امينية من النوع سيستيين و مواقع هيدروكربونية (إدماج السكريات ) فى شكل تتابع محفوظ فى جميع هذة الفيروسات . وقد وجد ان أكثر ما يميز عترة الفيروس المصرية هو وجود اختلاف كلى لعدد 17 حمض امينى عند المواقع من 168 – 184. و كذلك وجود موقع إضافى من السيستيين عند الحمض الامينى 178 و قد يمكن استخدام هذه الاختلافات لتمييز العترة المصرية عن مثيلاتها من فيروسات الهيربس الاخرى . وقد اثبت هذا العمل ان تطبيق تقنية بصمة الجينوم الوراثى باستخدام انزيم القطع (هند III) و تقنية التحليل المباشر للتتابع النيوكلوتيدى للجين (جى دى ) الناتج من تفاعل البلمرة المتسلسل هى ادوات جيدة للتوصيف الوراثى لعترات و معزولات فيروس الهيربس البقرى –1، مما يؤتى فائدة كبرى فى دراسة وبائية المرض عالميا و تطوير لقاحات فعالة لهذا الفيروس .