Dept. of Animal Medicine (Infectious Diseases),
Fac. of Vet. Med., Kafr Elsheikh University, Kafr Elsheikh 33516, Egypt.

DNA SEQUENCING AND COMPARATIVE SEQUENCE ANALYSIS OF GENE 15 (UL 45) OF JAPANESE AND EXOTIC STRAINS OF EQUIDAE HERPESVIRUS 1 STRAINS

(With 3 Tables and One Figure)

By

Y.M. GHANEM; M.I. MOURAD*; T. MATSUMURA** and H. FUKUSHI***

* Dept. of Animal Medicine (Infectious Diseases), Fac. of Vet. Med., Assuit University, Assuit, Egypt.

** Epizootic Research Station, Equine Research Institute, The Japanese Racing Association, Tochigi 329-04, Japan.

*** Laboratory of Veterinary Microbiology, Dept. of Applied Vet. Sciences, Fac. of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan.

(Received at 1/10/2010)

تحليل ومقارنة التركيب النيوكليوتيدى للجين رقم (15) لفيروس الفصائل الخيلية هربس(1) فى الفصائل اليابانية والبرية لعترات الفيروس

ياسر محمد غانم ، مراد اسماعيل مراد ، توميو ماتسومورا ، هيديتو فوكوشى

الجين رقم 15 عبارة عن 659 نيوكليوتيدة ويقع فى المكان بين النيوكليوتيدة رقم 21146 ورقم 20487 من تركيب الحمض النووى الخاص بفيروس الخيول الربس رقم 1 والذى يحتوى على 219 كودون وذات وزن جزيئ 23798 كيلو دالتون والذى يعتبر من التكوين البروتينى للفريون. بعض الباحثين قاموا بازالة هذا الجين من الفصائل المعروفة للفيروس راك ال 11 وراك اتش ووجدوا ان هذا الجين غير ضروري لتكاثر الفيروس فى المزارع الخلوية. دراساتنا الحديثة لتحليل التركيب النيوكليوتيدى لفيروس الخيول الهربس رقم 9 والذىوجد انة يرتبط بصلة وثيقة بفيروس الخيول الهربس رقم 1 ولكنة اقوى من ناحية تاثيرة على الجهاز العصبى للخيول. دلت الدراسة على تماثل فى الجين رقم 15 بنسبة 88% بينهما بالرغم من نسبة تماثل اكبربينهما بنسبة 92% فى معظم الجينات الاخرى. 1فى هذة الدراسة وجدنا ان التسلسل النيوكليوتيدى للجين رقم 15 متماثل بدرجة كبيرة فى ستة فصائل يابانية وثلاثة فصائل برية للفيروس والتى تختلف فى قوتها على احداث المرض من غير ضار الى ضعيف الضراوة الى ضار.

SUMMARY

Gene 15 (UL 45) is 659 bp in length and positioned in 21,146 to 20,487 bp in the genome of EHV-1 Ab4p, which genes contain 219 codons with Mr 23,798 KD. The product is regarded as virion tegument. Some researchers deleted gene 15 (UL45) in RacL11 and in RacH strains of EHV-1 and found that this gene is non essential for virus replication in culture cells. Our recent study analysis of the genomic sequence of Equine herpesvirus 9 which is a closely related to EHV-1 but has stronger neurovirulence than EHV-1 indicated that the identity of gene 15 between EHV-9 and EHV-1 is only 88% in spite of greater than 92% of other most genes. At the present study we reported that the nucleotide sequence of agene 15 is highly conserved in six Japanese strains and three exotic strains which vary in virulence from non virulent to low virulent and virulent.

Keywords: *EHV-1, ORF15, DNA sequence, phylogenic tree*

INTRODUCTION

Equids serve as hosts for six alphaherpesviruses which are EHV-1, EHV-3, -4, -6, -8, and -9. Isolation of EHV-1 from captive and wild equids has been reported (Montali *et al.,* 1985; Chowdhury *et al.,* 1986; Wolff *et al.,* 1986). It is well known that herpesviruses closely related to EHV-1 can infect not only different captive species of zoo equids (Przewalski's wild horse, Damara zebra, Grant's zebra, Burchell's zebra, onager and domestic ass) but also non-equid species (fallow deer, cattle, blackbuck, alpacas, llamas and Thomson's gazelle) (Chowdhury *et al.,* 1988; Crandell *et al.,* 1988; Rebhun *et al.,* 1988).

The equine herpesvirus 1 (EHV-1) is an important pathogen of horses. (EHV-1 causes rhinopneumonitis, late-term abortions, and neurological disorders (Allen and Bryans, 1986). The mechanism by which the virus cause diseases are not yet completely understood and need to be investigated. The EHV-1 genome has been characterized as a linear double-stranded DNA molecule with an estimated base composition of 56 or 57 % G+C (Darlington and Randall, 1963; Soehner *et al.,* 1965). The complete DNA sequence was determined of a pathogenic British isolate of equine herpesvirus 1, Ab4p (Telford *et al.,* 1992). The genome is 150,223 bp in size, and contain 80 open reading frames. Since four open reading frames are duplicated in the major

inverted repeats, the genome is considered to contain 76 distinct genes. The genes are arranged collinearly with those in the genomes of the two previously sequenced alphaherpesviruses, Varicella-Zoster virus and herpes simplex virus type-1, and comparison of the predicted amino acid sequences allowed the functions of many equine herpes virus 1 proteins to be assigned, However, only few genes and gene products that determine the virulence of the individual strains have been identified. At least 76 proteins are encoded by EHV-1 (Telford *et al.,* 1992), of which about 15 distinct genes in the genome of EHV-1 considered tegument and virion protein genes. Twelve EHV-1 glycoproteins have been described and are named in accordance with membrane glycoproteins of herpes simplex virus 1 (HSV-1). These glycoproteins homologs are glycoproteins B, C, D, E, G, H, I, K, L, and M whereas glycoprotein 1/2 is unique for EHV-1 (Osterrieder *et al.,* 1999). Another glycosylated protein (gp 10), the homolog of HSV-1 VP 13/14 and products of UL45, is not part of the viral envelop but is a component of the tegument (Wittaker *et al.,* 1991). In addition, several open reading frames (ORFs) in alphaherpesvirinae have been identified that could encode for additional membrane-associated proteins, some of which are carrying glycosylation consensus sites. These ORFs which are homologs of the HSV-1 UL 45 have been described in HSV-2 (Cockrell and Muggeridge, 1998), several avian herpesviruses such as Marek 's disease virus, and herpesviruses of turkeys but also in feline herpesvirus 1 FHV-1 (Willemse *et al.,* 1994) and in EHV-1 and EHV-4 (Telford *et al.,* 1998). However no UL 45 homologous ORFs have been detected in the genomes of such Varicelloviruses as Varicella-Zoster virus (Davison and Scott, 1986), bovine herpes virus 1 (Schwyzer *et al.,* 1996), and pseudorabies virus 1 PrV (Bras *et al.,* 1999). Information on the structure and the function of several UL 45 homolog has been acquired, but the proteins functions are not yet fully understood. The amino acid homology between the predicted UL 45 gene product is moderate, but they all contain a large internal hydrophobic domain (McGeoch *et al.,* 1988; Ihara *et al.,* 1989; Willemse *et al.,* 1994; Ziemann *et al.,* 1998). The gene 15, UL45 homolog, is classified as a true late gene. UL45 homolog product was detected as early as 4 h p.i. in HSV-2 infection (Cockrell and Muggeridge, 1998). The function of the EHV-1 UL 45 homolog (ORF 15 is known to be expressee as a late-2 gene that encodes a 0.9 kb m RNA that is 3-coterminal with glycoprotein C transcript (Matsumura *et al.,* 1993). EHV-1 ORF15 homologue in HSV-1 is UL45 which encodes a

18-kDa protein with a possible transmembrane domain (McGeoch *et al.,* 1988; Visalli and Brandt, 1993).

Insertion of a marker gene into the UL 45 genes of HSV-1 or FHV-1 demonstrated that the protein is non essential for replication of these viruses (Visalli and Brandt, 1991; Willemse *et al.,* 1994). Experiments to analyse the product of the equine herpesvirus type 1 (EHV-1) UL 45 homolog were conducted and demonstrated that the EHV-1 UL 45 protein represents a type 11 membrane glycoprotein. Deletion of the UL 45 gene in RacL 22 and Rac H showed that reduction of the virus release was observed although no significance influence was noticed either on plaque size or on the syncytial phenotype of the EHV-1 strain Rac H. (Oettler *et al.,* 2001).

The HSV-1 UL45-negative mutant forms plaques smaller in size than those of the wild-type, and shows an altered plaque morphology. HSV-1 normal UL45 expression was required to allow for cell fusion induced by gB syn mutant, hence, the UL45 product may be a mediator of fusion events (Haanes *et al.,* 1994). However no UL45 homologous ORFs have been detected in the genomes of such Varicelloviruses as VZV (Davison and Scott, 1986), and in bovine herpes virus 1 (Schwyzer *et al.,* 1996). No study was applied on EHV-1 ORF15 except for DNA sequencing on strain KyA by Matsumura *et al.* (1993).

In this paper we report the complete DNA sequence of gene 15 (UL 45) of six different Japanese strains of EHV-1 and three exotic American strains of EHV-1.

MATERIALS and METHODS

Viruses used and propagation

We used six EHV-1 isolated in Japan, which were 90c16(1P) non virulent strain, 89c103 (1P) low virulent strain, 89c104 (1B) virulent strain, 97c5 (1B) non virulent, 97c9 (1B) non virulent strain and 98c16 (1B) non virulent strain (Matsumura *et al.,* 1996), Virulence characters were investigated in various EHV-1 Japanese field isolates in hamster model by Pagamjav *et al.* (2005), and three exotic EHV-1 isolates including T616 virulent strain from aborted fetus of Zebra (Wolff *et al.,* 1986), T-gazelle virulent strain (94-137) from encephalitis of Thomson s gazelle in USA (Kennedy *et al.,* 1996), and T-529 from an onager (*Equus hemionus onager*) fetus which was aborted after 10 months of gestation (Montali *et al.,* 1985). Ab4p was kindly provided by Dr. A. J. Davison, Glasgow University, UK. All strains were passaged twice in fetal horse

kidney (FHK) cells in our laboratory after arrival and the strains were propagated as described previously (Pagamjav *et al.,* 2005).

DNA Extraction

Total DNA was extracted from infected FHK cells by the phenol chloroform method as described previously (Pagamjav *et al.,* 2005). Viral DNA was extracted as follows. Virus was inoculated to FHK cells at low multiplicity of infections. Cells showing almost complete cytopathic effects (CPE) were treated with 1% sodium dodecyl sulphate and 0.1 mg/ml of proteinase K in 0.01 M Tris-HCl, 0.1M NaCl, 0.5 mM EDTA, pH 8.0, at 37 ºC for overnight. DNA was extracted with phenol, phenol-chloroform and chloroform followed by ethanol precipitation and then dissolved in 0.01 M Tris-HCl, 0.1 mM EDTA, pH 8.0. DNA concentration and purity were assayed with Gene Quant II (Amersham Pharmacia, Japan).

PCR Amplification

The PCR amplification primers were designed (Table 1) and sent to Dragon Genomics (Yokkaichi, Mie, Japan). The PCR reaction mixture and PCR program were standardized to specifically amplify gene 15 of interest which is 659 bp in length without presence of any unspecific PCR product as follows. Each genomic DNA sample was amplified by PCR using 1 µ1 of DNA in 50 µ1 reaction mixture consisting of 13.5 µl distilled water, 8 µ1 dNTP mixture, 25 µ1 2x GC buffer 1, 1 µ1 of each primer (100 pmol), LA-Taq enzyme 0.5 µ1 (TAKARA Bio INC., JAPAN). The amplification program was denaturation at 95 ^{0}C for 1 minute followed by 30 cycles of denaturation at 94 ^{0}C for 20 seconds, annealing at 53 ^{0}C for 30 seconds and extension at 72 ^{0}C for 45 seconds (TAKARA Biomedicals). Each Amplified product was precipitated by ethanol, resuspended in 30 µ1 of T.E. buffer and stored for at least 24 hours at 4 ^{0}C until use. DNA was quantified by gel electrophoresis using a 100 bp DNA ladder markers (TAKARA Bio INC., JAPAN- code no. 3407A) in 0.9%.agarose gel Tris-phosphate –EDTA buffer.

Table 1: Primers used for sequence of ORF15

Sequenced tegument genes	Position	Sequence	Tm ºC	GC%
PCR primers of ORF 15				
Forward primer	20,471-20,492	CGC ATC GGT TTC TCT ATT ACC G	59	50
Reverse primer	21,159-21,180	GTA AAG CAA CAT GGC AGG AGA C	59	50

DNA molecular cloning

We used pGEM-T-Easy vector system (Promega, 2800 woods Hollow Road Madison) for cloning of gene 15 (UL45). A total volume of 10 µ1 of ligation mixture is consisted of 5 µ1 of 2x rapid ligation buffer, 1 µ1 pGEM-T-Easy vector solution, 1 µ1 T4 DNA ligase and 3 µ1 of purified PCR product solution. The mixture was incubated at 4 ^{0}C for 24 h for maximum transformation efficiency, then add 50 µ1 of DH5 alpha competent cells to 3 µ1 of ligation mixture to be incubated in ice for 20 minutes. The mixture was incubated at 42 ^{0}C for 50 seconds, immediately return into ice for further 2 minutes. Then 950 µ1 of SOC medium was added the mixture and incubate at 37 ^{0}C with vigorous shaking for 1 h. The solution was plated LB agar plate containing Ampicillin, IPTG and x-gal and incubate in 37 C for 16 hours. white clones were picked up for further analysis. Plasmid DNA was extracted from overnight culture of a white colony by using boiling method according to Holmes and Quigley (1981). Five ml of LB broth containing Ampicillin was inoculated with a single white colony, was incubate at 37 ^{0}C for 12 hrs with vigorous shaking (200 rpm). Pouring 1.5 ml of the culture into an Eppendorf tube, which was then centrifuged for 1 minute at room temperature with speed 13,000 rpm, stored the remainder of overnight culture at 4 ^{0}C. The medium was removed from Eppendorf tube after centrifugation by aspiration, leaving the bacterial pellet as dry as possible. Then the bacterial pellet was resuspend in 0.35 ml of STET solution (8% sucrose, 0.5% Triton X-100, 50 mM EDTA, 10 mM Tris.Cl PH 8.0), then, place the tubes was kept in boiling water bath for 40 seconds then centrifuge immediately for 10 minutes at room temperature at 13,000 rpm and removed the pellet by using tooth picks. The supernatant was added 40 µ1 of 2.5 M sodium acetate and 420 µ1 of isopropanol and mixed by vortexing to store for 15 minutes in a dry ice/ethanol bath. Then the tubes were centrifuged again at 4 ^{0}C with speed 13,000 rpm. The supernatant was discarded to dry the pellet, which were then resuspended in 50 µ1 of TE buffer (PH 8.0) and store at 4 ^{0}C overnight. Extracted plasmid DNA was digested by restriction enzyme for confirmation of the presence of insert using ScaI restriction enzyme (TOYOBO), which would give two bands.

Sequencing of positive insert clones

Positive insert clones were stored in – 80 C as 1 m1 of 20% glycerol culture. For sequencing, 96 well plate was prepared as including at least three positive insert colonies derived from one amplified product. Each colony was put in two wells of 100 µ1/well for forward and reverse

sequencing. The plates was sent with dry ice to Dragon Genomics (Yokkaichi, Mie, Japan). Two computer programs for sequence analysis were GENETYX-MAC/ATSQ and GENETYX –MAC.

Fig. (1) Phylogenetic tree of ORF 15 according to amino acid sequence comparison

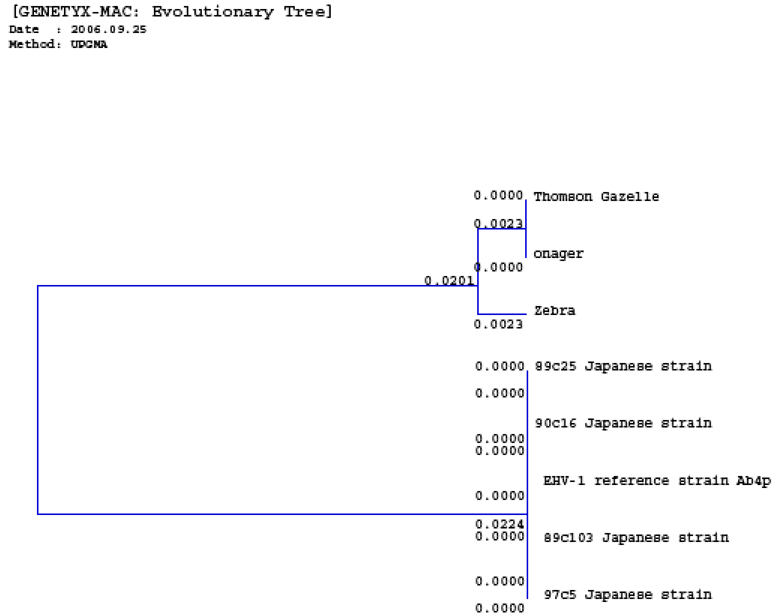

Figure 1. Phylogenic trees of virion protein gene showed that the exotic non horse strains are gentically distinct from that of horse isolates and situated in separate phylogenic branch from horse strains used in this study and other strains from data bank of sequence. ORF15 sequence showed that zebra is different from onager and gazelle isolates and form a unique branch from both of them.

Fig. 1: Phylogenetic tree of ORF 15 according to amino acid sequence comparison

Table 2: Amino acid variation of ORF 15 among EHV-1 examined

Position Viruses	8	9	16	19	38	73	114	124	166	177
Ab4p	Q	L	A	P	Q	I	A	V	D	S
97c5	Q	L	A	P	Q	I	A	V	D	S
90c16	Q	L	A	P	Q	I	A	V	D	S
89c103	Q	L	A	P	Q	I	A	V	D	S
89c25	Q	L	A	P	Q	I	A	V	D	S
T-616	K	M	V	S	P	M	T	M	N	G
T-529	K	M	V	S	P	M	T	M	N	S
94-137	K	M	V	S	P	M	T	M	N	S

Table 3: ORF 15 (218 aa) Number of amino acid differences among studied strains

	strains							
Strains	Ab4p	97c5	90c16	89c103	89c25	Onager	T.gazelle	Zebra
Ab4p		0	0	0	0	9	9	10
97c5	0		0	0	0	9	9	10
90c16	0	0		0	0	9	9	10
89c103	0	0	0		0	9	9	10
89c25	0	0	0	0		9	9	10
Onager	9	9	9	9	9		0	1
T.gazelle								
Zebra	10	10	10	10	10	1	1	

RESULTS and DISCUSSION

EHV-1 has a large genomic DNA and various strains have been isolated until now. However, the structure and functions of the genome was not fully understood and still need to be investigated. Also, investigation of the genetic relatedness and genomic DNA diversity between non equid strains (onager, zebra and gazelle) and equid strains of EHV-1 by sequence comparison of several glycoproteins and tegument protein genes as this can provide some guide about the origin and pathway of infection, range of pathogenicity and interspecies transmission of EHV-1 infection. In this study, the molecular diversity and genetic relatedness were investigated between exotic EHV-1 isolated from animals other than horses (onager, zebra, and gazelle) with that of EHV-1 isolated from horses in Japan which vary in virulence and Ab4p in the analysis of gene 15 nucleotide and amino acid sequence. Little information is available about the correlation between genomic structure and expression of specific pathogenicity in various EHV-1 strains. The pathogenicity of EHV-1 exotic non equid strains were evaluated in hamster model (Ibrahim *et al.,* 2007). However, the pathogenic range, origin of the exotic non equid virus strains, and the genetic relatedness together with the molecular diversity between the non equid and horse isolates of EHV-1 are not yet fully understood and need to be investigated. The virulence of the B and P types of EHV-1 Japanese isolates in hamsters were previously tested (Pagamjav *et al.,* 2005) and it was found that all EHV-1 B strains were non virulent while EHV-1P strains were virulent, mildly virulent or non virulent. The determinants of virulence and pathogenicity of EHV-1 various isolates are still unknown and need to be investigated. I addressed the possibility of involvement of virion tegument gene 15 for expressing specific pathogenicity in EHV-1 infection by nucleotide and amino acid sequence comparison. Sequence comparison indicated that isolates (T-529 and 94-137) from onager and gazelle were identical and closely related while the zebra isolate (T-616) was slightly different from them but distinct from horse isolates. The small amount of observed diversity supported a phylogenic separation of the three isolates, and separated them from the horse isolates. All of the glycoproteins of EHV-1 have been suggested to be virulence and pathogenicity determinant factors (Papp-Vid and Derbyshire, 1978; Stokes, *et al.,* 1989; Osterrieder, *et al.,* 1996; Matsumura, *et al.,* 1998; Frampton, *et al.,* 2002; Zhang *et al.,* 2003; Von Einem, *et al.,* 2004) which lead us to believe that some specific sequence differences related

to causing pathogenicity. However, we only found highly conserved or identical sequences among all strains examined. The findings indicated that EHV-1 isolates from onager, zebra and gazelle (T-529, T-616 and 94-137, respectively) were distinguishable from horse isolates. We found that gene 15 (UL 45) nucleotide sequence of the six Japanese strains were completely identical; to that of Ab4p. Also the gene 15 sequence of the three exotic strains of T616 of Zebra T-gazelle of Thomson 's gazelle was 97% identical to that of Ab4p (Figure 1). Putative amino acid sequence were also conserved among EHV-1 examined. (Table 2,3). Phylogenic trees of virion protein gene showed that the exotic non horse strains are gentically distinct from that of horse isolates and situated in separate phylogenic branch from horse strains used in this study and other strains from data bank of sequence. ORF15 sequence showed that zebra is different from onager and gazelle isolates and form a unique branch from both of them (Figure 1). Oettler *et al.* (2001) studied the replication of the virus after deletion of UL45. They reported that the UL 45 –deleted virus was able to efficiently replicated but was affected egress step by showing reduced extracellular virus titres up to 250 fold lower compared to that of wild type of EHV-1. The functional characterization of the UL 45 negative EHV-1 in vitro indicated that the UL 45b facilitate virus egress. However, they don't evaluate virulence of the UL 45 deleted virus in experimental infection of animals. Therefore alteration of virulence should be observed on the UL45 deleted EHV-1 to evaluate the role of UL45 in EHV-1 infection. The present results indicated that there is no relation between the sequence of gene 15 and the virulence character of EHV-1. High conservation of this gene indicated a possible essential role of gene 15 product in virus replication and infection.

REFERENCES

Allen, G.P. and Bryans, J.T. (1986): Molecular epizootiology, pathogenesis, and prophylaxisi of equine herpesvirus 1 infections. Prog. Vet. Microbiol. Immunol., 2: 78-144.

Bras, F.; Dezelee, S.; Simonet, B.; Nguyen, X.; Vende, P.; Flamand, A. and Masse, M.J. (1999): The left border of the genomic inversion of pseudorabies virus contains genes homologous to the UL46 and UL47 genes of hepes simplex virus type 1, but no Ul45 gene. Virus Res., 60: 29-40.

Chowdhury, S.I.; Kubin, G. and Ludwig, H. (1986): Equine herpesvirus type 1 (EHV-1) induced abortions and paralysis in a lipizzner stud: a contribution to the classification of equine herpesviruses. Arch. Virol. 90:273-288.

Chowdhury, S.I.; Ludwing, H. and Buhk, H-J. (1988): Molecular biological characterization of equine herpesvirus type 1 (EHV-1) isolates from ruminant hosts. Virus Res. 11: 127-139.

Cockrell, A.S. and Muggeridge, M.I. (1998): Herpes simplex virus 2 UL45 is a type 11 membrane protein. J. Virol., 72: 1759-1816.

Crandell, R.A.; Ichimura, H. and Kit, S. (1988): Isolation and comparative restriction endonuclease DNA fingerprinting of equine herpesvirus-1 from cattle. Am. J. Vet. Res. 49: 1807-1813.

Darlington, R.W. and Randall, C.C. (1963): The nucleic acid content of equine abortion virus. Virology, 19: 322-327.

Davison, A.S. and Scott, J.E. (1986): The complete DNA sequence of varicella-zoster virus. J. Gen. Virol., 67: 1759-1816.

Frampton, A.R. Jr.; Smith, P.M.; Zhang, Y.; Matsumura, T.; Osterrieder, N. and O'Callaghan, D.J. (2002): Contribution of gene products encoded within the unique short segment of equine herpesvirus 1 to virulence in a murine model. Virus Res. 90: 287-301.

Haanes, E.J.; Nelson, C.M.; Soule, C.L. and Goodman, J.L. (1994): The UL45 gene product is required for herpes simplex virus type 1 glycoprotein B-infected fusion. J. Virol. 68: 5825-5834.

Holmes, D.S. and Quigley, M. (1981): A rapid boiling method for the preparation of bacterial plasmids. Anal Biochem. 114 (1): 193-7.

Ibrahim, E.S.; Kinoh, M.; Matsumura, T.; Kennedy, M.; Allen, G.P.; Yamaguchi, T. and Fukushi, H. (2007): Genetic relatedness and pathogenicity of equine herpesvirus 1 isolated from onager, zebra and gazelle. Arch Virol. 152(2): 245-55.

Ihara, T.; Kato, A.; Ueda, S.; Ishihama, A. and Hirai, K. (1989): Comparison of the sequence of the secretory glycoprotein A (gA) gene in Md5 and BC-1 strains of marik s disease virus type 1. Virus genes, 3: 127-140.

Kennedy, M.A.; Ramsay, E.; Diderrich, V.; Richman, L.; Allen, G.P. and Potgieter, L.N.D. (1996): Encephalitis associated with a variant of equine herpesvirus 1 in a Thomson's gazelle (*Gazella thomsoni*). J. Zoo and Wild Life Med. 27:533-538.

Matsumura, T.; Kirisawa, R.; Kondo, T.; Sugita, S. and Kamada, M. (1996): Molecular epizootiological studies on respiratory and foetal strains of equid herpesvirus-1 isolated in Japan. Equine Infectious Diseases VII: 243-249.

Matsumura, T.; Kondo, T.; Sugita, S.; Damiani, A.M.; O'Callaghan, D.J. and Imagawa, (1998): An equine herpesvirus type 1 recombinant with a deletion in gE and gI genes is avirulent in young horses. Virology 242: 68-79.

Matsumura, T.; Smith, R.H. and O. Callaghan, D.J. (1993): DNA sequence and transcriptional analysis of the region of the equine herpesvirus type 1 Kentucky A strain genome encoding glycoprotein C. Virology, 193: 910-923.

McGeoch, D.J.; Dalrymple, M.A.; Davison, A.J.; Dolan, A.; Frame, M.C.; Mcnab, D.; Perry, L.J.; Scott, J.E. and Tylor, P. (1988): The complete DNA Sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol., 69: 1531-1574.

Montali, R.J.; Allen, G.P.; Bryans, J.T.; Phillips, L.G. and Bush, M. (1985): Equine herpesvirus type 1 abortion in an onager and suspected herpesvirus myelitis in a zebra. J. Amer. Vet. Med. Ass. 187: 1248-1249.

Oettler, D.; Kaaden, O.R. and Neubauer, A. (2001): The equine herpes virus 1 UL45 Homolog encodes a glycosylated type 11 Transmembrane protein and is involved in virus Egress. Virology, 279: 302-312.

Osterrieder, N.; Holden, V.R.; Brandmueller, C.; Neubauer, A.; Kaaden, O.R. and O'Callaghan, D.J. (1996): The equine herpesvirus 1 IR6 protein is nonessential for virus growth in vitro and modified by serial virus passage in cell culture. Virology 217: 442-451.

Osterrieder, N.; Kaaden, O. and Neubauer, A. (1999): Structure and function of equine herpesvirus glycoproteins-a review in *proceedings of the eighth international conference on equine infectious diseases* (U. Wernery, J.F. Wade, J.A. Mumford and O.R. Kaadan, Eds.), pp. 111-118. R + W Publications, Newmarket.

Pagamjav, O.; Sakata, T.; Matsumura, T.; Yamaguchi, T. and Fukushi, H. (2005): Natural recombinant between equine herpes virus 1 and 4 in the ICP4 gene. Microbiol. Immunol. 49: 167-179.

Papp-Vid, G. and Derbyshire, J.B. (1978): The protective antigens of equine herpesvirus type 1. Can. J. Comp. Med. 42: 219-226.

Rebhun, W.C.; Jenkins, D.H.; Riis, R.C.; Dill, S.G.; Dubovi, E.J. and Torres, A. (1988): An epizootic of blindness and encephalitis associated with a herpesvirus indistinguishable from equine herpesvirus I in a herd of alpacas and llamas. J. Am. Vet. Med. Assoc. 192: 953-956.

Schwyzer, M.; Styger, D.; Vogt, B.; Lowery, D.E.; Simard, C.; LaBoissiere, S.; Misra, V.; Vicek, C. and Paces, V. (1996): Gene contents in a 31-kb segment at the left genome end Of bovine herpesvirus-1. Vet. Microbiol., 53: 67-77.

Soehner, R.L.; Gentry, G.A. and Randall, C.C. (1965): Some physiochemical characteristics of equine abortion virus nucleic acid. Virology. 26: 394-405.

Stokes, A.; Allen, G.P.; Pullen, L.A. and Murray, P.K. (1989): A hamster model of equine herpesvirus type 1 (EHV-1) infection; passive protection by monoclonal antibodies to EHV-1 glycoproteins 13, 14, and 17/18. J. Gen. Virol. 70: 1173-1183.

Telford, E.A.; Watson, M.S.; McBride, K. and Davison, A.J. (1992): The DNA sequence of equine herpesvirus-1. Virology, 189: 304-316.

Visalli, R.J. and Brandt, C.R. (1991): The HSV-1 UL45 gene Product is not required for growth in vero cells. Virology, 185: 419-423.

Visalli, R.J. and Brandt, C.R. (1993): The HSV-1 UL45 18 kDa gene product is a true late protein and a component of the virion. Virus Res. 29: 167-178.

Von Einem, J.; Wellington, J.; Whalley, J.M.; Osterrieder, K.; O'Callaghan, D.J. and Osterrieder, N. (2004): The truncated form of glycoprotein gp2 of Equine herpesvirus 1 (EHV-1) vaccine strain KyA is not functionally equivalent to full length gp2 encoded by EHV-1 wild-type strain RacL11. J. Virol. 78: 3003-3013.

Whittaker, G.R.; Riggio, M.P.; Halliburton, I.W.; Killington, R.A.; Allen, G.P. and Meredith, D.M. (1991): Antigenic and Protein sequence homology between VP13/14, a herpes Simplex virus type 1 tegument protein, and gp 10, A glycoprotein of equine herpes virus 1 and 4. J. Virol., 65: 2320-2326.

Willemse, M.J.; Chalmers, W.S.; Cronenberg, A.M.; Pfundt, R.; Strijdveen, I.G. and Sondermeijer, P.J. (1994): The gene downstreamof the gC homoilogue in feline herpes virus type 1 is

involved in the expression of virulence. J. Gen. Virol., 75: 3107-3116.

Wolff, P.L.; Meehan, T.P.; Basgall, E.J.; Allen, G.P. and Sudberg, J.P. (1986): Abortion and perinatal foal mortality associated with equine herpesvirus type 1 in a herd of Grevy's zebra. J. Amer. Vet. Med. Ass. 189: 1185-1186.

Zhang, Y.; Smith, P.M.; Frampton, A.R.; Osterrieder, N.; Jennings, S.R. and O'Callaghan, D.J. (2003): Cytokine profiles and long-term virus Specific antibodies following immunization of CBA mice with equine herpesvirus 1 and viral glycoprotein D. Viral. Immunol. 16: 307-320.

Ziemann, K.; Mettenleiter, T.C. and Fuchs, W. (1998): Gene Arrangement within the unique long genome region of Infectious laryngotracheitis virus is distinct from that of other alphaherpesviruses. J. Virol., 72: 847-852.