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Genetic relatedness and pathogenicity of equine herpesvirus 1 isolated from onager, zebra and gazelle

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Summary. Equine herpesvirus 1 was isolated from an onager in 1985, a zebra in 1986 and a Thomson's gazelle in 1996 in USA. The genetic relatedness and pathogenicity of these three viruses were investigated based on the nucleotide sequences of the glycoprotein G (gG) gene, experimental infection in hamsters, and comparison with horse isolates. The gG gene sequences of EHV-1 from onager and zebra were identical. The gG gene sequences of the gazelle isolate showed 99.5% identity to those of onager and zebra isolates. The gG gene sequences of EHV-1 isolated from horses were 99.9–100% identical and 98, 98 and 97.8% similar to gG from onager, zebra and gazelle isolates, respectively. Hamsters inoculated with onager, zebra and gazelle isolates had severe weight loss, compared with hamsters inoculated with horse isolates. The histopathological findings were related to the virulence of each isolate. The results indicated that EHV-1 isolates from onager, zebra and gazelle differ from horse EHV-1 and are much more virulent in hamsters.

Introduction

Equine herpesvirus 1 (EHV-1), a member of the alphaherpesvirus subfamily, is a major pathogen of horses all over the world. EHV-1 infection is associated with respiratory disease, epizootic abortion and neurological disorders [1, 9, 24].

Isolation of EHV-1 from captive and wild equids has also been reported [4, 23, 30]. EHV-1 infections are usually limited to equine species; however, infections in non-equine animals have been reported [5, 10, 20, 21, 26]. The clinical manifestations of EHV-1 in captive equid and non-equid animals differ. EHV-1 was isolated from an onager (*Equus hemionus onager*) fetus which was aborted after 10 months of gestation [23], from an aborted Grevy's zebra (*Equus grevyi*) fetus [30], and from a captive Thomson's gazelle (*Gazella thomsoni*) that died after an acute neurological illness characterized by depression, recumbency, and seizures [10]. While monoclonal antibody analysis identified the onager, zebra and gazelle isolates as EHV-1 [20], DNA restriction endonuclease analysis indicated that those isolates were genetically distinct from EHV-1 from horse [23, 30]. However, there is little information about the molecular characteristics of EHV-1 isolated from these animals. Moreover, the pathogenicity of these isolates has not been evaluated.

The glycoproteins of herpesvirus play important roles in the infection process, mediating attachment, entry of the virion into the host cell, egress, and cell-to-cell spread [11]. Because of their locations in the viral envelope and on the surfaces of infected cells, glycoproteins are targets for host immune responses [8]. The envelope glycoprotein G (gG) gene of EHV-1 has been shown to be nonessential for growth in cell culture [2, 17]. However, gG binds a broad range of chemokines with high affinity and blocks chemokine activity by preventing their interaction with specific receptors [3]. The gG homologue has been examined in EHV-1 and related viruses [6, 7, 16]. The gG homologue is relatively divergent among alphaherpesviruses. Therefore, the gG gene is used as a marker for the molecular relatedness among alphaherpesviruses [13, 16, 17].

Pathogenicity and virulence of EHV-1 have been evaluated in small rodent models. Syrian hamsters are known to be susceptible to EHV-1. We have shown that an experimental hamster model is useful for evaluating the pathogenicity of EHV-1 [14]. EHV-1 isolates could be classified as virulent, intermediate virulent, or nonvirulent according to the virulence in hamsters. EHV-1 virions associated with neurological illness in horses were classified as virulent. Therefore, the pathogenicity and virulence of EHV-1 in hamsters might reflect those in horses.

In this study, we investigated the genetic relatedness of EHV-1 isolated from horse, onager, zebra and gazelle by analyzing the nucleotide and predicted amino acid sequences of the gG gene. In addition, the pathogenicity of these isolates was investigated by infection of hamsters. The results indicated that EHV-1 isolated from onager, zebra and gazelle might be distantly related to EHV-1 horse isolates and much more virulent.

Materials and methods

Viruses and cells

The EHV-1 isolates used in this study were T-529, from an aborted onager fetus [23], T-616, from an aborted zebra fetus [30], 94-137, from a Thomson's gazelle that died after acute neurological illness [20], and 90c18, 94c19 and 97c7, from horses with respiratory disease

in Japan (Matsumura et al. unpublished). Based on our previous studies, isolate 90c18 was virulent, and isolates 94c19 and 97c7 were nonvirulent [18, 25]. Viruses were propagated in fetal horse kidney (FHK) cells. Virus titrations were in Madin-Derby bovine kidney (MDBK) cells as described previously [18]. The FHK and MDBK cells were grown in Eagle's minimum essential medium (Eagle's MEM) (Nissui, Japan) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5% fetal bovine serum (FBS).

DNA extraction

Viral DNA was extracted as follows. FHK cells were inoculated with virus at low multiplicity of infection. Cells showing almost complete cytopathic effects (CPE) were treated with 1% sodium dodecylsulphate and 0.1 mg/ml of proteinase K in 0.01 M Tris–HCl, 0.1 M NaCl, 0.5 mM EDTA, pH 8.0, at 37 °C overnight. DNA was extracted with phenol, phenol-chloroform, and then chloroform, followed by ethanol precipitation and resuspension in 0.01 M Tris–HCl, 0.1 mM EDTA, pH 8.0. DNA concentration and purity were assayed with GeneQuant II (Amersham Pharmacia, Japan).

DNA amplification and sequencing

The gG gene was amplified with primers Fo-1 and Re-5 (Table 1) using a Takara LA PCR kit (TaKaRa Biomedicals, Japan) and the shuttle PCR program (30 cycles of 98 °C for 10 sec and 65 °C for 5 min) with a thermal cycler (GeneAmp PCR System 9600, Perkin-Elmer-Cetus, USA). Amplified products were treated with exonuclease I (USB, USA) and shrimp alkaline phosphatase (USB, USA) at 37 °C for 15 min. The reaction was stopped by incubation at 80 °C for 15 min. The treated DNA was used as the template for nucleotide sequencing. The sequenced region corresponded to nucleotide numbers 127606–129020 of the EHV-1 Ab4p strain genome sequence (GenBank accession no. M86664) [28]. The sequence was read using 13 sequencing primers (Table 1) with Thermo Sequenase Cy5.5 Terminator Sequencing Kit (Amersham Pharmacia, Japan). Cycle sequencing was performed using 40 cycles of 95 °C denaturation for 30 sec, annealing for 20 sec (annealing temperatures were adjusted according to the melting temperature of each primer), and 72 °C extension for 1 min. The products were

Table 1. Primer sequences used in this study

Primer	Sequence	T_m (°C)	Position in Ab4p genome
Fo-1	5′-AAA GTT GGT CTG CTT TCA AGC CCT G-3′	60.6	127606–127630
Fo-a	5′-ACA GTG CCA CCA ACT GTA AAG CG-3′	60.2	127641–127663
Fo-2	5′-CAA TTG CGA TTA AAG CTG AAT CCA-3′	55.3	127837–127860
Fo-3	5′-GGA GTT TAC CAT CGT AA CAT GAG-3′	57	128075–128098
Fo-b	5′-TGG CAT GAT TCA TGG ACT CAC TGC-3′	58.7	128219–128242
Fo-4	5′-CAG ACT GTC ACT CGT GG AGT CA-3′	62	128419–128441
Fo-5	5′-AAC GGC ATC CAG GAC TGT GAC AGT-3′	62.1	128721–128744
Re-1	5′-GTC AAA AAA CCA AGC AAT GGA CGC-3′	58.4	127952–127929
Re-2	5′-ACT TCC AAC GTT ACA CGT CCT GT-3′	60	128194–128172
Re-3	5′-GCG CCA AGA TTC TGA CTC CAA C-3′	58.7	128523–128502
Re-4	5′-CTA TCA ACC CTA TCA TGG CAC ATG-3′	53.9	128814–128791
Re-a	5′-CCA CAC TCA GTT ATT ACA GAC-3′	60.4	128974–128954
Re-5	5′-TGT ATG GGG CTA TTT CCG GAA TTG C-3′	60.6	129020–128996

purified by ethanol precipitation, then dissolved in 6 μl of formamide loading dye. The labeled DNA products were sequenced with Gene Rapid (Amersham Pharmacia, Japan).

Sequence data were analyzed by using GENETYX-MAC/ATSQ 4.0.2 and GENETYX-MAC ver.10.1 (Software development co., ltd. Japan). The gG gene sequences were submitted to the DNA Data Bank of Japan (DDBJ, National Institute of Genetics, Japan). Accession nos. are AB187029–AB187033. Other DNA sequences were obtained from DDBJ for the gG gene of EHV-1 Ab4p (accession no. M86664) [28], EHV-8 (asinine herpesvirus 3, AHV-3) (accession no. U24184) [17], and EHV-9 (GHV-1) (accession no. D85905) [16]. Sequence alignments were performed using CLUSTAL X [19]. The phylogenic trees were constructed by the neighbor-joining method [27].

Experimental animal infection

Hamster inoculation was as described previously [15]. Three-week-old specific-pathogen-free (SPF) male Syrian hamsters (SLC, Japan) were used. Food and water were freely available to the hamsters during the course of the experiment. Hamsters were observed for 3 days before inoculation. All experiments were conducted under the guidelines for animal experiments at Gifu University with approval by the Committee of Animal Care and Welfare, Faculty of Applied Biological Sciences, Gifu University. Hamsters were divided into 4 animals per group, anesthetized by 2.5 mg/head of ketamine hydrochloride (Sankyo, Japan), and inoculated intranasally with 25 μl of viral suspension containing 1×10^3 pfu/g of hamster body weight into each nasal cavity. Control hamsters were inoculated with the same volume of Eagle's MEM. Hamsters were weighed and monitored for general behavior twice a day. All animals were euthanized after 10 days post-inoculation with injection of an excess amount of sodium pentobarbital.

Histopathology and immunohistochemistry

Organs including olfactory bulbs, brain, lungs, liver and kidneys were sampled for histopathological examination. Tissue samples were fixed in 4% paraformaldehyde in 0.01 M phosphate buffer pH 7.0. Tissues were dehydrated and embedded in paraffin wax using routine techniques. Then, tissues were sectioned at 5 μm and stained with haematoxylin and eosin (HE). Viral antigens were detected by immunostaining using the standard avidin-biotin-complex (ABC) immunoperoxidase method with ABC kits (Vector Laboratories, Burlingame, CA, USA) using rabbit EHV-9 antiserum as described previously [31]. It was known that there is a serological cross-reaction between EHV-1 and EHV-9 [16].

Results

Phylogenic analysis of glycoprotein G gene

Genetic relatedness of T-529, T-616, 94-137, 90c18, 97c7 and other EHV-1-related viruses was investigated using the gG nucleotide sequence. The segment examined was 1233 bp in length and encoded a 411-residue open reading frame. The nucleotide sequences of gG of onager isolate T-529 and zebra isolate T-616 were identical. The nucleotide sequence of the gG gene of the gazelle isolate 94-137 showed 99.6% (1228/1233) identity with those of the onager and zebra isolates. The nucleotide sequences of the gG gene of 90c18 and Ab4p were identical, whereas the gG gene of 97c7 showed 99.8% (1231/1233) identity with those of Ab4p and 90c18. The nucleotide sequence of the gG of T-529

Table 2. Amino acid variation of glycoprotein G among EHV-1 examined

Viruses	Position								
	175	205	305	311	319	326	335	336	392
T-592	D	P	H	V	L	N	G	H	D
T-616	D	P	H	V	L	N	G	H	D
94-137	D	S	N	V	L	N	G	H	D
Ab4p	D	P	H	A	S	S	V	N	N
90c18	D	P	H	A	S	S	V	N	N
97c9	N	P	H	A	S	S	V	N	N

and T-616 showed 98% (1208/1233) homology with that of Ab4p and 90c18 and 97.9% (1207/1233) homology with 97c7. The gG gene of 94-137 showed 97.9% (1207/1233) homology with those of Ab4p and 90c18 and 97.8% (1206/1233) with that of 97c7. These nucleotide variations affected some substitutions in the predicted glycoprotein G amino acid sequences (Table 2).

The nucleotide sequences of T-529, T-616, 94-137, 90c18, 97c7, Ab4p, EHV-8 and EHV-9 were used to construct a phylogenic tree, which showed that gG of T-592, T-616 and 94-137 form a new branch that is closely related to EHV-1 in the horse (Fig. 1).

Pathogenicity of T-529, T-616 and 94-137 in hamsters

The pathogenicity and virulence of T-529, T-616 and 94-137 were evaluated in hamsters. EHV-1 isolated from horses, including 90c18, 94c19 and 97c7, were also used for comparison. Hamsters inoculated with T-529, T-616, and 94-137 showed nervous manifestations, namely restlessness, lack of coordination, fighting and aggressiveness, and lethargy, which started on day 3 post-inoculation in the case of 94-137 and T-529 and on day 5 post-inoculation in the case of T-616 (Table 3).

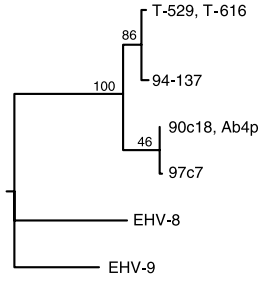

Fig. 1. A phylogenic tree constructed from nucleotide sequences of the gG of T-529 (onager), T-616 (zebra), 94-137 (gazelle), Ab4p (horse), 90c18 (horse), 97c7 (horse), EHV-8 (accession no. U24184), and EHV-9 (accession no. D85905). EHV-9 was used as an out-group in the construction of the phylogenic tree. Bootstrap values are shown

Table 3. The nervous manifestations of hamsters inoculated with T-529, T-616, 94-137 and 90c18

Viruses	Days post-inoculation										
	0	1	2	3	4	5	6	7	8	9	10
T-529	–	–	–	4/4	4/4	4/4	4/4	3/3*	2/2*	1/1*	Died
T-616	–	–	–	–	–	4/4	4/4	–	–	–	–
94-173	–	–	–	4/4	4/4	3/4	2/4	2/3*	1/3	–	–
90c18	–	–	–	–	–	–	3/4**	3/4**	–	–	–

– No nervous manifestations such as salivation, convulsion, motor disorder and hyper-reactivity to outer stimulation, were observed

*A hamster died

**One out of 4 showed salivation, convulsion, motor disorder and excitation. Other two out of 4 seemed to be lighter hyperreactive against outer stimulation. The other hamster did not show any changes

Nervous signs disappeared at day 7 post-inoculation with T-616 and at day 9 post-inoculation with 94-137. One of the four 94-137-inoculated hamsters died on day 6 post-inoculation. Nervous signs were severe and persisted until the end of the observation in the case of T-529. Moreover, T-529-inoculated hamsters died on days 5, 6 and 7 post-inoculation. On the other hand, hamsters inoculated with EHV-1 horse isolates had few nervous symptoms. One of 4 hamsters inoculated with 90c18 showed salivation, convulsions, motor disorder and excitation. Two others were slightly hyper-reactive to external stimulation. The fourth hamster did not show any changes. In addition to these symptoms, body weight loss was observed (Fig. 2). In the case of 94-137 and T-616, the maximum weight loss was on day 7 post-inoculation, and hamsters did not gain their original weight until the end of observation period. Hamsters inoculated with 94c19 and 97c7 did not show any changes.

Histopathological findings in hamsters

While gross pathological changes were not found in all animals at necropsy, non-suppurative encephalitis and interstitial pneumonia were found to a varying degree in all animals inoculated with viruses. Severe degeneration and necrosis of the neurons were found in the brains of the T-529- and 94-137-inoculated hamsters (Table 4). Extensive lymphocytic cell infiltration and perivascular cuffing were observed in the olfactory bulbs, anterior olfactory nucleus, olfactory tubercle, and pyriform cortex. Glial proliferation was also observed. Histopathological findings in the brains of the T-616-inoculated hamsters included slight to moderate degrees of degeneration and necrosis of the neurons with diffuse gliosis in olfactory bulbs, anterior olfactory nucleus, olfactory tubercle, and pyriform cortex. Lymphocytic cell infiltration and perivascular cuffing were observed in the olfactory bulbs, anterior olfactory nucleus, olfactory tubercle, and pyriform cortex (Table 4).

Table 4. The distribution of the pathological lesions in the brain of hamsters inoculated with EHV-1 isolates

Viruses	Necrosis							Gliosis	Microglial reaction
	Olfactory bulb	Cerebra		Hippocampus		Cerebellum	Medulla oblongata		
		Cortex	Medulla	Cornu ammonis	Dentate gyrus				
T-529	++	++	–	–	+	–	+	–	medulla oblongata
T-616	+	+	–	+	+	+	–	frontal lobe, temporal lobe	temporal lobe
94-137	+	++	–	+	+	–	+	frontal lobe, temporal lobe	frontal lobe, temporal lobe
90c18	–	++	–	+	+	++	+	olfactory bulbs, frontal lobe, parietal lobe, cornu ammonis	olfactory bulbs, temporal lobe, occipital lobe, cornu ammonis
94c19	++	–	–	++	++	+	–	–	–
97c7	++	+	–	+	+	+	+	frontal lobe	frontal lobe

– No pathological lesions
+ Moderate pathological lesions
++ Severe pathological lesions

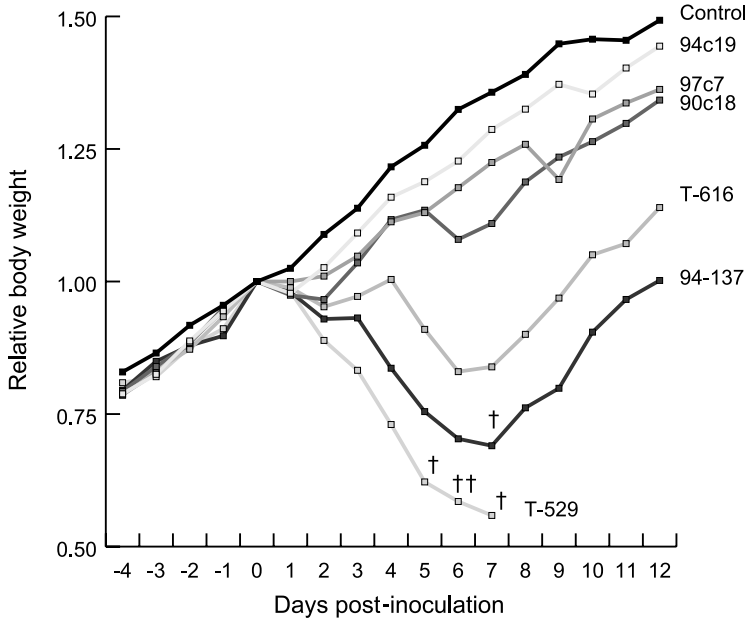

Fig. 2. Body weight curves of hamsters after intranasal inoculation with T-529, T-616, 94-137, 90c18, 94c19 and 97c7. The weight loss started on day 2 post-inoculation in groups of hamsters inoculated with T-529, T-616 and 94-137. In the case of the T-529-inoculated group, weight loss was severe, and hamsters died by day 7 post-inoculation. In the case of 94-137- and T-616-inoculated groups, the weight loss was also severe. One hamster in the 94-137-inoculated group died on day 6 post-inoculation. The body weight ratios were determined as follows. The body weight of a hamster each day was divided by its body weight on day 0. The mean of the body weight ratios of each hamster in a group was plotted day by day. Variability was less than 0.02 and is not shown in order to avoid complexity. †Died

Various degrees of neuronal degeneration and necrosis with moderate gliosis and lymphatic infiltration in the olfactory bulb were observed in the brains of hamsters inoculated with 97c7 and 90c18. Immunohistochemistry confirmed the presence of viral antigens in these lesions (data not shown).

Discussion

In this study, the genetic relatedness of EHV-1 isolates from equids was investigated by comparing nucleotide and predicted amino acid sequences of the gG gene. Virulence variation was determined using the hamster infection model. 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 also used the gG gene as an indicator of phylogeny in EHV-1. Other researchers reported that the gG gene is a variable gene related to phylogenic relationship among equine herpesviruses [13, 16, 17]. Sequence comparison indicated that isolates (T-529 and T-616) from onager and zebra were closely related, and the gazelle isolate (94-137) was slightly different. The small amount of observed diversity did not support a phylogenic separation of these three

isolates, but did separate them from the horse isolate. Thus, other methods such as genomic restriction fragment polymorphism and sequence comparison of other genes should be carried out in order to know the phylogenic relatedness among these viruses and other EHV-1.

The onager isolate, T-529, caused lethal infection in hamsters, while EHV-1 isolates from horse were not always lethal, as shown in the present study and in our previous results in which we classified EHV-1 strains into virulent, intermediate and nonvirulent groups according to their neurological virulence in hamsters [14]. In the present study the onager isolate T-529 belonged to the virulent group. The zebra, T-616, and gazelle, 94-137, isolates belonged to the intermediate group.

One of the three EHV-1 isolates, 94-137, was isolated from a non-equine species, Thomson's gazelle. The clinical manifestations of 94-137 in Thomson's gazelle were mainly neurological [20]. Although the native hosts of the gazelle isolate have not been clarified, it was reported that a zebra mare aborted a fetus on the farm where the affected gazelle had been kept [20]. Our present results indicate a close relatedness between T-616 and 94-137. It is well known that some herpesviruses can cause severe neurological diseases in atypical hosts, such as monkey B virus in humans and swine pseudorabies virus in various animals [29]. Therefore, a zebra might have been the source of infection in the gazelle.

The present results indicate that T-529, T-616 and 94-137 EHV-1 variants are distinguishable from EHV-1 from domestic horses. Thus, natural barriers such as varied habitat and ecology have separated the domestic horse from exotic equids. The natural barriers may have prevented the transmission of viruses among wild equids and domestic horses in the past. However, the increase of capturing, displaying and keeping wild animals in close contact with domestic animals may facilitate the transmission of viruses between wild and domestic animals beyond the natural barriers. EHV-1 variants could be a threat to horse industry.

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References

1. Allen GP, Bryans JT (1986) Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus-1 infection. Prog Vet Microbiol Immunol 2: 78–144
2. Balan P, Davis-Poynter N, Bell S, Atkinson H, Browne H, Minson T (1994) An analysis of the in vitro and in vivo phenotypes of mutants of herpes simplex virus 1 lacking glycoproteins gG, gE, gI, or the putative gJ. J Gen Virol 75: 1245–1258
3. Bryant NA, Davis-Poynter N, Vanderplasschen A, Alcami A (2003) Glycoprotein G isoforms from some alphaherpesviruses function as broad-spectrum chemokine binding proteins. EMBO J 22: 833–846
4. Chowdhury SI, Kubin G, Ludwig H (1986) Equine herpesvirus type 1 (EHV-1) induced abortions and paralysis in a Lipizzaner stud: a contribution to the classification of equine herpesviruses. Arch Virol 90: 273–288

5. Chowdhury SI, Ludwing H, Buhk H-J (1988) Molecular biological characterization of equine herpesvirus type 1 (EHV-1) isolates from ruminant hosts. Virus Res 11: 127–139
6. Colle CF, Flowers CC, O'Callaghan DJ (1992) Open reading frames encoding a protein kinase, homolog of glycoprotein X of pseudorabies virus, and a novel glycoprotein map within the unique short segment of equine herpesvirus type 1. Virology 188: 545–557
7. Crabb BS, Nagesha HS, Studdert MJ (1992) Identification of equine herpesvirus 4 glycoprotein G: a type-specific, secreted glycoprotein. Virology 190: 143–154
8. Crabb BS, Studdert MJ (1993) Epitopes of glycoprotein G of equine herpesviruses 4 and 1 located near the C termini elicit type-specific antibody responses in the natural host. J Virol 67: 6332–6338
9. Crabb BS, Studdert MJ (1995) Equine herpesvirus 4 (equine rhinopneumonitis virus) and 1 (equine abortion virus). Adv Virus Res 45: 153–190
10. Crandell RA, Ichimura H, Kit S (1988) Isolation and comparative restriction endonuclease DNA fingerprinting of equine herpesvirus-1 from cattle. Am J Vet Res 49: 1807–1813
11. Csellner H, Walker C, Wellington JE, McLure LE, Love DN, Whalley JM (2000) EHV-1 glycoprotein D (EHV-1 gD) is required for virus entry and cell-cell fusion, and an EHV-1 gD deletion mutant induces a protective immune response in mice. Arch Virol 145: 2371–2385
12. Drummer HE, Studdert MJ, Crabb BS (1998) Equine herpesvirus-4 glycoprotein G is secreted as a disulphide-linked homodimer and is present as two homodimeric species in the virion. J Gen Virol 79: 1205–1213
13. Ficorilli N, Studdert MJ, Crabb BS (1995) The nucleotide sequence of asinine [sic] herpesvirus 3 glycoprotein G indicates that the donkey virus is closely related to equine herpesvirus 1. Arch Virol 140: 1653–1662
14. Fukushi H, Taniguchi A, Matsumura T, Kirisawa R, Masegi T, Hirai K (2000) Comparison of the neuropathogenicity of equine herpesvirus-1 and equine herpesvirus-9 (gazelle herpesvirus-1) in hamsters. In: Equine infectious diseases VIII, Proc, 8th Int Conference of Infectious Diseases. R&W Publications, New Market, pp 513–544
15. Fukushi H, Taniguchi A, Yasuda K, Yanai T, Masegi T, Yamaguchi T, Hirai K (2000) A hamster model of equine herpesvirus 9 induced encephalitis. J Neurovirol 6: 314–319
16. Fukushi H, Tomita T, Taniguchi A, Ochiai Y, Kirisawa R, Matsumura T, Yanai T, Masegi T, Yamaguchi T, Hirai K (1997) Gazelle herpesvirus 1: a new neurotropic herpesvirus immunologically related to equine herpesvirus 1. Virology 226: 34–44
17. Hartley CA, Drummer HE, Studdert MJ (1999) The nucleotide sequence of the glycoprotein G homologue of equine herpesvirus 3 (EHV3) indicates EHV3 is a distinct equid alphaherpesvirus. Arch Virol 144: 2023–2033
18. Ibrahim ESM, Pagmajav O, Yamaguchi T, Matsumura T, Fukushi H (2004) Growth and virulence alterations of equine herpesvirus 1 by insertion of a green fluorescent protein gene in the intergenic region between ORFs 62 and 63. Microbiol Immunol 48: 831–842
19. Jeannmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23: 403–405
20. Kennedy MA, Ramsay E, Diderrich V, Richman L, Allen GP, Potgieter LND (1996) Encephalitis associated with a variant of equine herpesvirus 1 in a Thomson's gazelle (*Gazella thomsoni*). J Zoo Wildlife Med 27: 533–538
21. Kinyili JH, Thorsen J (1979) Antigenic comparisons between herpesviruses isolated from fallow deer in Alberta and the viruses of infectious bovine rhinotracheitis, equine rhinopneumonitis and DN-599, a non-IBR bovine herpesvirus. J Wildlife Dis 15: 339–341
22. Maeda K, Mizukoshi F, Hamano M, Kai K, Iwata H, Kondo T, Matsumura T (2004) Development of an equine herpesvirus type 4-specific enzyme-linked immunosorbent assay using a B-cell epitope as an antigen. J Clin Microbiol 42: 1095–1098

23. Montali RJ, Allen GP, Bryans JT, Phillips LG, Bush M (1985) Equine herpesvirus type 1 abortion in an onager and suspected herpesvirus myelitis in a zebra. J Am Vet Med Ass 187: 1248–1249
24. O'Callaghan DJ, Genty GA, Randall CC (1983) The equine herpesviruses. In: Comprehensive virology, series 2. Plenum Press, New York, pp 215–318
25. Pagamjav O, Sakata T, Matsumura T, Yamaguchi T, Fukushi H (2005) Natural recombination between equine herpesviruses 1 and 4 in the ICP4 gene. Microbiol Immunol 49: 167–179
26. Rebhun WC, Jenkins DH, Riis RC, Dill SG, Dubovi EJ, Torres A (1988) An epizootic of blindness and encephalitis associated with a herpesvirus indistinguishable from equine herpesvirus 1 in a herd of alpacas and llamas. J Am Vet Med Ass 192: 953–956
27. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425
28. Telford EA, Watson MS, McBride K, Davison AJ (1992) The DNA sequence of equine herpesvirus-1. Virology 189: 304–316
29. Weigler BJ (1992) Biology of B virus in macaque and human hosts: a review. Clin Infect Dis 14: 555–567
30. Wolff PL, Meehan TP, Basgall EJ, Allen GP, Sudberg JP (1986) Abortion and perinatal foal mortality associated with equine herpesvirus type 1 in a herd of Grevy's zebra. J Am Vet Med Ass 189: 1185–1186
31. Yanai T, Masegi T, Tomia A, Kudo T, Yamazoe K, Iwasaki T, Kimura N, Katou A, Kotera S, Ueda K (1995) Odontoameloblastoma in a Japanese monkey (*Macaca fuscata*). Vet Pathol 32: 57–59

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