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< Short Communication >

The first virus isolation and partial characterization of equine herpesvirus-4 in a horse, South Korea

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Abstract

An equine herpesvirus-4 (EHV-4) was isolated in nasal swabs collected in a horse showing respiratory clinical signs. Equine dermis cells inoculated with the sample were observed with characteristic viral cytopathic effects after 3 days of postinoculation and the infected cells exhibited bright intracelluar fluorescence by indirect immunofluorescence assay. At the nucleotide level, the partial glycoprotein B gene of the Korean EHV-4 isolate (K001) had 99.9% identity to 1942 strain (GenBank No. M26171). To author's knowledge, the report describes the first isolation and partial characterization of EHV-4 in Korea. The virus can be used for further study of EHV-4.

Key words : Equine herpesvirus-4, Korea, Virus isolation, Immunofluorescence assay, Glycoprotein B

INTRODUCTION

Equine herpesvirus-4 (EHV-4) along with equine herpesvirus-1 (EHV-1) is alpha herpesvirus (a member of the *Alphaherpesvirinae* subfamily) with the clinical symptoms of acute respiratory diseases known as equine rhinopneumonitis (ER) in horse. It is accepted that both EHV-1 and EHV-4 cause respiratory disease, and that EHV-4 rarely causes abortion and is not associated with abortion storms or the neurological syndrome (Telford et al, 1998; Molinkova et al, 2004; Patel and Heldens, 2005). The EHVs have a major economic and welfare impact on all sectors of the horse industry worldwide through their direct clinical effects on the horse, including interference with horse movement for breeding and competition (Sellon and Long, 2014).

In Korea, there have been two cases of EHV-1 reported in an aborted fetus assayed by immunohistochemistry or polymerase chain reaction (PCR) (Bak et al, 1981; Moon et al, 2001). EHV-4 along with EHV-1 was detected in nasal swabs collected from horses suffering from respiratory diseases by PCR methods in Korea, 2008 (Ko et al, 2013). But, the isolation or characterization of EHV-4 in Korea to our knowledge has been not reported so far. Virus isolation is important as this is the only way to secure the isolate for any further analysis. This study was performed to isolate and characterize circulating EHV-4 in Korean equine populations so that in future they will be used as a material for serosurveil1ance and development of vaccine or diagnostics against ER.

Nasal swab was collected from in a 2-year-old foal with the acute phase of the clinical course with respiratory diseases in Korea, 2010. The swab was placed in 1ml of a viral transportation medium, transferred in the laboratory, immediately centrifuged for 5 min at 2,500 rpm. The supernatants were passed through a 0.45 μm membrane filter as previously described (Dynon et al,

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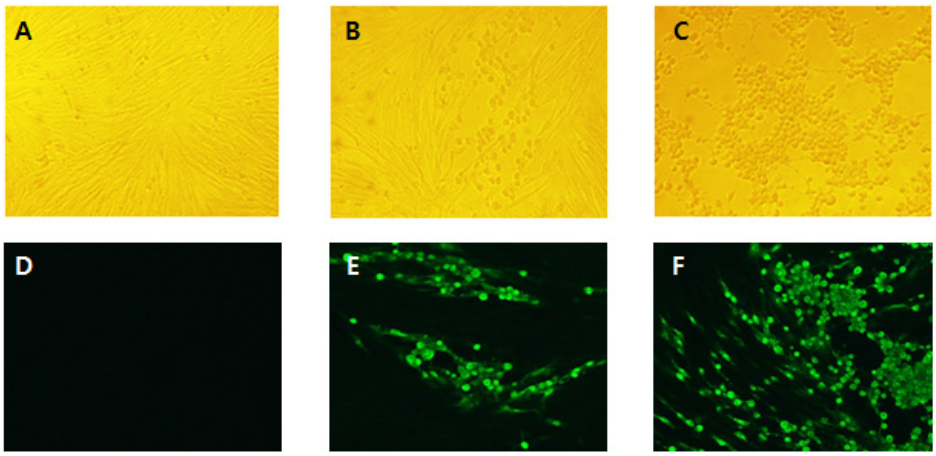

Fig. 1. The cytopathic effects of equine herpesvirus 4 isolate in equine dermis cell. Infected cells were visualized by light (A, B, C) and fluorescence microscopy (D, E, F); magnification x 40. (A & D) 0 days of postinoculation (dpi), (B & E) 3 dpi, (C & F) 4 dpi.

A

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EHV4_M2617   1   ATCCAATCCC ACGTTAATGA GATGCTAAGT AGGATAGCAA CTGCGTGGTG TACACTA
EHV4_K001    1   .......... .......... .......... .......... .......... .......

EHV4_M2617   61  AACAAAGAGC GGACCCTCTG GAATGAGATG GTAAAGGTTA ACCCAAGCGC TATTGTT
EHV4_K001    61  .......... .......... .......... .......... .......... .......

EHV4_M2617   121 GCCACTCTTG ACGAGCGAGT TGCGGCAAGG GTTTTGGGAG ACGTTATAGC CATAACA
EHV4_K001    121 .......... .......... .......... .......... .......... .......

EHV4_M2617   181 TGTGTAAAAA TAGAGGGCAA TGTGTACTTA CAAAACTCTA TGCGCTCCTC GGACAGC
EHV4_K001    181 .......... .......... .......... .......... .......... .......

EHV4_M2617   241 ACGTGCTACT CCCGCCCACC TGTAACGTTT ACCATTACTA AAAATGCAAA CAGCAGA
EHV4_K001    241 .......... .......... .......... .......... .......... .......

EHV4_M2617   301 ACGATAGAGG GCCAGTTGGG AGAAGAAAAC GAGGTTTATA CGGAGCGCAA GCTTATC
EHV4_K001    301 .......... .......... .......... .......... .......... .......

EHV4_M2617   361 CCGTGCGCTA TCAATCAAAA ACGATACTTT AAGTTTGGCA AAGAGTATGT TTACTAT
EHV4_K001    361 ......T... .......... .......... .......... .......... .......

EHV4_M2617   421 AACTACACGT ACGTTCGCAA AGTGCCCCCG ACTGAAATCG AAGTGATCAG CACCTAC
EHV4_K001    421 .......... .......... .......... .......... .......... .......

EHV4_M2617   481 GAACTAAACT TAACTCTTTT GGAAGACCGC GAGTTTCTAC CCCTGGAGGT TTACACG
EHV4_K001    481 .......... .......... .......... .......... .......... .......

EHV4_M2617   541 GCTGAGCTTG AAGACACGGG GCTATTGGAT TACAGCGAGA TACAGCGCCG TAACCAG
EHV4_K001    541 .......... .......... .......... .......... .......... .......

EHV4_M2617   601 CACGCCCTCC GATTCTACGA TATAGACAGC GTTGTCAACG TGGACAACAC TGCTGTC
EHV4_K001    601 .......... .......... .......... .......... .......... .......

EHV4_M2617   661 ATGCAGGGAA TTGCCACCTT TTTTAAAGGC CTTGGTAAGG TGGGAGAGGC AGTTGGG
EHV4_K001    661 .......... .......... .......... .......... .......... .......

EHV4_M2617   721 CTTGTACTTG GAGCGGCTGG CGCGGTTGTT TCTACAGTAT CGGGTATAGC CTCATTT
EHV4_K001    721 .......... .......... .......... .......... .......... .......

EHV4_M2617   781 AACAACCCAT TTGGGGGGCT CGCAATAGGC CTGTTGGTAA TTGCGGGCTT AGTGGCT
EHV4_K001    781 .......... .......... .......... .......... .......... .......

EHV4_M2617   841 TTTTTTGCCT ACCGGTATGT AATGCAACTG CGCAGCAACC CCATGAAAGC TCTATAC
EHV4_K001    841 .......... .......... .......... .......... .......... .......
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B

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EHV4_M2617   1   IQSHVNEMLS RIATAWCTLQ NKERTLWNEM VKVNPSAIVS ATLDERVAAR VLGDVIA
EHV4_K001    1   .......... .......... .......... .......... .......... .......

EHV4_M2617   61  CVKIEGNVYL QNSMRSSDSN TCYSRPPVTF TITKNANSRG TIEGQLGEEN EVYTERK
EHV4_K001    61  .......... .......... .......... .......... .......... .......

EHV4_M2617   121 PCAINQKRYF KFGKEYVYYE NYTYVRKVPP TEIEVISTYV ELNLTLLEDR EFLPLEV
EHV4_K001    121 ..S....... .......... .......... .......... .......... .......

EHV4_M2617   181 AELEDTGLLD YSEIQRRNQL HALRFYDIDS VVNVDNTAVI MQGIATFFKG LGKVGEA
EHV4_K001    181 .......... .......... .......... .......... .......... .......

EHV4_M2617   241 LVLGAAGAVV STVSGIASFI NNPFGGLAIG LLVIAGLVAA FFAYRYVMQL RSNPMKA
EHV4_K001    241 .......... .......... .......... .......... .......... .......
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Fig. 2. Sequence alignments of partial gB region of 1942 strain (EHV4_M26171, GenBank No. M26171) and Korean EHV-4 isolate (EHV4_K001). (A) Nucleotide (897 *nt*) (B) Deduced amino acids (299 *aa*). Dots of the alignment indicate the conserved nucleotide or amino acids sequences.

2007). Equine dermis (E. Derm) cells (ATCC CCL-57) were propagated and used for virus isolation. All cell culture reagents were purchased (Gibco by Life Technologies). The cells were grown in high-glucose DMEM supplemented with 10% fetal calf serum (FCS), 1X Antibiotic-Antimycotic solution, and 2 mM L-glutamine. The prepared samples were inoculated on the monolayers of cells on the coverslips. The detection of EHV-4 antigens in infected cells were done by indirect immunofluorescent assay (IFA) using EHV-4 polyclonal antisera (Biobest Laboratories) as the primary antibody and fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG (Sigma) as the secondary antibody. The coverslips were washed 3 times in PBS and observed specific reactivity using fluorescent microscope. For sequencing of EHV isolate, viral DNA was extracted from nasal swabs using a DNeasy mini kit (QIAGEN, USA) according to the manufacturer's instructions. Extracted DNA was stored at −20°C before use. The first and nested PCR was performed according to the OIE protocol using the Hotstart master PCR kit (QIAGEN). The amplicon was excised from the gel and purified by using a Gel extraction kit (QIAGEN). The purified DNA was sequenced in Macrogen Inc (Seoul, Korea). Sequence analyses were carried out using CLC Main Workbench software (CLC bio, Denmark) and gB sequences of EHV-4 1942 strain previously reported (Riggio et al, 1989).

A Korean EHV-4 strain K001 was isolated in inoculated cells with the supernatant from the nasal swab sample collected from a Thoroughbred. The cells were observed with characteristic viral CPEs after 3 and 4 days of postinoculation by microscopy and exhibited bright intracellular fluorescence (Fig. 1). The sequence of the PCR products amplified from the isolate K001 exhibited 99.8% identity in nucleotide (1 mutation of 897 *nt*) and 99.5% identity in amino acids (1 mutation of 299 *aa*) to the published EHV-4 sequence (GenBank No. M26171) (Fig. 2).

In recent study, the detection rate of EHV-1 and EHV-4 were 5.6% and 7.9%, respectively, in Korea (Ko et al, 2013). The results were examined using PCR. Virus isolation remains the gold standard for laboratory diagnosis of EHV infections and provides clear evidence of the presence of infectious virus in clinical samples (Sellon and Long, 2014). In this study, demonstration of the isolated virus was first done by IFA using EHV-4 antiserum. Bright fluorescence with typical CPE patterns were observed in the infected cells (Fig. 1). We also used PCR methods on according to OIE protocol for differentiation between EHV-1 and EHV-4 because they differ significantly in terms of their genetic aspets (World Organization for Animal Health, 2014). Our isolate exhibited the same size of PCR products for EHV-4, 933 bp of the first product and 581 bp of nested PCR product (data not shown). Gene coding the glycoprotein B (gB) by direct sequencing of PCR products, further supported this discrimination. The partial nucleotide sequence of gB was 897 nucleotides and encoded 299 amino acids (Fig. 2). At the nucleotide and amino acids level, the Korean isolate (K001 strain) exhibited 99.9% and 99.7% homology to 1942 strain, respectively.

The isolation in cell culture of EHV-4 from one horse and confirmation of its presence of EHV-4 by PCR and sequence analysis seems highly significant if only because this is the first time that EHV-4 has been isolated from horses in Korea. This isolate will be valuable to study the serologically epidemiologic understanding of EHV-4 in fields or development of vaccine for respiratory diseases by EHV-4 in horses.

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