Coinfected cases with equine herpesvirus type 1, 4 and *Streptococcus equi* subsp. *zooepidemicus* in thoroughbred horse

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Abstract

The Thoroughbred horse was an approximately 4-years-old castrated male with highly emaciation, nasal epistaxis and subsequently died. Gross necropsy revealed epistaxis and hyperemia on the lung, multiple hemorrhage in muscle, and liver was focally attached to the peritoneum with fibrin. According to polymerase chain reaction (PCR), Equine herpes virus type 1 and 4 (EHV type 1, 4) was detected in the lung and trachea. In bacterial culture from kidney, liver, spleen, muscle and blood, *Streptococcus equi* subsp. *zooepidemicus* was isolated. Based on the gross lesion and PCR, this horse was diagnosed as EHV type 1, 4 and *S. zooepidemicus* coinfection.

Key words : EHV-1 and 4, *Streptococcus equi* subsp. *zooepidemicus*, Thoroughbred horse

INTRODUCTION

Equine herpes virus (EHV) is a common virus occurring in horse populations worldwide. The most common strains are EHV-1 and 4. EHV-1 can cause respiratory diseases, abortions and neurologic disease, while EHV-4 typically causes respiratory disease but can also cause abortions (Azmi and Field, 1993; Ostlund, 1993). EHV-1 has been identified as a cause of abortion outbreaks and, more recently, outbreaks of neurologic disease (Borchers et al, 2006). Respiratory disease caused by EHV is most common in young horses (weanlings and yearlings), while older horses are more likely to transmit the virus without showing symptoms (Reed and Toribio, 2004).

Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*) belongs to the β -hemolytic Group C streptococci, which can cause disease both in animals and humans. *S. zooepidemicus* may be found in the nasopharynx, tonsils, respiratory tract, and on the genital mucous membrane of healthy horses. It is an important cause of respiratory tract infections in foals and young horses, and associated with uterine infections in

mares (Anzai et al, 2002; Korman et al, 2004). Damage from Herpesvirus infection to the protective respiratory mucosal barrier predisposes affected horses to opportunistic bacterial infections. As a result, secondary bacterial rhinopharyngitis (primarily by *S. zooepidemicus*) commonly accompanies EHV infection and contributes to the overall severity of the illness. In the absence of appropriate antibiotic therapy, involvement of the lower airways with the consequent development of bacterial tracheobronchitis, bronchiolitis, or pneumonia can further complicate upper respiratory tract disease initiated by EHV-1 or EHV-4. Secondary invasion by clonally derived *S. zooepidemicus* strains with enhanced virulence can dramatically influence the severity and duration of the disease episode and may be associated with increased levels of morbidity and greater risks for mortality (Timoney, 2004).

CASE AND DISCUSSION

Horse with respiratory distress was died and requested to the Gyeongbuk Veterinary Service Laboratory. Gross

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necropsy revealed epistaxis, hyperemia on the lung, multiple hemorrhage in muscle, and liver was focally attached to the peritoneum with fibrin (Fig. 1).

Molecular identification of EHV and *S. zooepidemicus* isolates was performed by polymerase chain reaction (PCR). The glycoprotein B coding region of EHV and *SodA* gene, *SzP* gene, *CNE* virulence factor, and 16S rRNA gene of *S. zooepidemicus* were then amplified with the oligonucleotide primers described by Younan et al.

(2005) and office international des epizooties Chapter 12. 1. (OIE, 2008), respectively. The sequences of the oligonucleotide primers are given in Table 1.

According to PCR, EHV-1 and 4 were detected in the lung and trachea of Thoroughbred horse. In bacterial culture from heart, kidney, liver, spleen, muscle, and blood, *S. zooepidemicus* was isolated from all cultured samples. The EHV-1 and 4 was confirmed by PCR with the amplicon sizes of 770 bp and 580 bp, respectively.

Fig. 1. Images from gross necropsy. (A) Emaciation; (B) Epistaxis; (C) Hyperemia and hemorrhage on the lung with lobular adhesion; (D) Liver attached to the peritoneum with fibrin.

Table 1. Oligonucleotide primers used in present study

Target antigen	Primer	Sequence (5'→3')	Size of product (bp)
<i>S. zooepidemicus</i>	<i>SodA</i>	CAGCATTCTGCTGACATTCGTCAGG CTGACCAGCCTTATTCACAACCAGC	235
	<i>SeeI</i>	GAAGGTCCGCCATTTTCAGGTACTTTG GCATACTCTCTCTGTCACCATGTCCT	520
	<i>CNE</i>	GCAACTAATCTTAGTGACAACAT AAAGCTGGTATAGCGACTGCCA	906
	<i>ISR*</i>	TTGTACACACCGCCCGTCA GGTACCTTAGATGTTTCAGTT	Size polymorphism
	<i>SzP</i>	ACAAAAGGGAATAAAATGGC TTTACCACTGGGGTATAAGGCT	Size polymorphism
EHV**	EHV-1	CTTTAGCGGTGATGTGGAA AAGTAGCGCTTCTGATTGAG	770
	EHV-4	TGTTTCCGCCACTCTTGAC ACTGCCTCTCCACCTTAC	580

*ISR: 16S rRNA gene, **EHV: Equine herpesvirus.

Fig. 2. PCR amplification for the identification of *S. zooepidemicus* and EHV type 1 and 4. Pannel A: Lane M, molecular size marker (100 bp DNA ladder, Elpis, Korea); Lane 1, *S. equi* ATCC 9528; Lane 2: *S. zooepidemicus* isolates; Lane 3, *SzP* encoding gene; Lane 4, *CNE* encoding gene; Lane 5, 16S rRNA gene (ISR). Pannel B: Lane M, molecular size marker (100 bp DNA ladder, Elpis, Korea); Lane 1, EHV-1; Lane 2, EHV-4.

S. zooepidemicus was identified by *SodA* gene PCR with one amplicon (235 bp), while *S. equi* has two (235 bp and 520 bp). *S. zooepidemicus* was positive for the *SzP* gene with amplicon size of approximately 1,200 bp. Additionally, *CNE*, a 657 amino acid collagen-binding protein encoding gene of *S. zooepidemicus* was also detected. Typical amplicons of the *SzP*, *CNE* and *ISR* genes are shown in Fig. 2.

On the bases of findings at autopsy such as hemorrhage of upper respiratory tract, pneumonia, and epistaxis, we diagnosed this case as equine viral rhinopneumonitis. We conducted exact diagnosis using PCR as well as bacteria test for septicemia, which was suspected to have been caused by secondary bacterial infection, based on the systemic organ hemorrhage and hyperemia. After final diagnosis using PCR, we could confirm that it was due to the coinfection of EHV-1, -4 and *S. zooepidemicus*, important causative agent of upper respiratory tract infection of the horse. Also, *S. zooepidemicus* isolated from the systemic organs including blood, spleen, kidney, liver, lung and muscle, showed positive reaction to both *SzP* and *CNE*, the virulence factor of *S. zooepidemicus*. Therefore, in this case, the coinfection of EHV-1 and -4 caused equine viral rhinopneumonitis, and the immunodepression by viral infection resulted in the pathogenic *S. zooepidemicus* infection and septicemia, which led to the ultimately death.

Generally, relatively light symptoms accompany equine viral rhinopneumonitis caused by primary infection of

EHV-1 or -4, but secondary infection on respiratory system can bring serious symptoms (Ostlund, 1993; Timoney, 2004). However, coinfections of these two rarely leads to death, and there has been no case report in Korea that involves simultaneous infection of EHV-1 and -4, or that shows symptoms due to respiratory infection of *S. zooepidemicus*. Thus, the infectious disease diagnosed in this case seems to be very rare, and the coinfection of EHV-1, -4 and *S. zooepidemicus* is deemed fatal. In addition, we found that the management conditions for the horse were not so satisfactory since there has been no vaccination for critical diseases and proper treatment. In order to prevent the disease caused by those agents, appropriate vaccination and antibiotics treatment, if necessary, are required. Also, when considering that no horse has been newly imported to the farm and it is located in a relatively segregated area, this investigation points out that many strains of EHV and *S. zooepidemicus* could be isolated in horses, which correlates well with the fact that EHV and *S. zooepidemicus* are mucosal commensal organisms in the equine population, and the opportunistic pathogenic characters of these microorganisms (Ostlund, 1993; Timoney, 2004).

REFERENCES

- Anzai T, Timoney JE, Kuwamoto Y, Wada R, Oikawa M, Higuchi T. 2002. Polymerase chain reaction-restriction fragment length polymorphism analysis of the *SzP* gene of *Streptococcus zooepidemicus* isolated from the respiratory tract of horses. *Am J Vet Res* 63(9): 1298-1301.
- Azmi M, Field HJ. 1993. Interactions between equine herpesvirus type 1 and equine herpesvirus type 4: T cell responses in a murine infection model. *J Gen Virol* 74(11): 2339-2345.
- Borchers K, Thein R, Sterner-Kock A. 2006. Pathogenesis of equine herpesvirus-associated neurological disease: a revised explanation. *Equine Vet J* 38(3): 283-287.
- Korman TM, Boers A, Gooding TM, Curtis N, Visvanathan K. 2004. Fatal case of toxic shock-like syndrome due to group C streptococcus associated with superantigen exotoxin. *J Clin Microbiol* 42(6): 2866-2869.
- Office International Des Epizooties (OIE). 2008. Manual of diagnostic tests and vaccines for terrestrial animals, Vol. II. 6th Edition. World Organization for Animal Health, Paris, France. Chapter 12. 1.

- Ostlund EN. 1993. The equine herpesviruses. *Vet Clin North Am Equine Pract* 9(2): 283-294.
- Patel JR, Heldens J. 2005. Equine herpesviruses 1 (EHV-1) and 4 (EHV-4) epidemiology, disease and immunoprophylaxis: a brief review. *Vet J* 170(1): 14-23.
- Reed SM, Toribio RE. 2004. Equine herpesvirus 1 and 4. *Vet Clin North Am Equine Pract* 20(3): 631-642.
- Sutton GA, Viel L, Carman PS, Boag BL. 1998. Pathogenesis and clinical signs of equine herpesvirus-1 in experimentally infected ponies *in vivo*. *Can J Vet Res* 62(1): 49-55.
- Timoney JF. 2004. The pathogenic equine streptococci. *Vet Res* 35(4): 397-409.
- Younan M, Estoepangestie AT, Cengiz M, Alber J, El-Sayed A, Lämmler C. 2005. Identification and molecular characterization of *Streptococcus equi* subsp. *zooepidemicus* isolated from camels (*Camelus dromedarius*) and camel milk in Kenya and Somalia. *J Vet Med B Infect Dis Vet Public Health* 52(3): 142-146.