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First Report on Molecular Detection of Equine Upper Respiratory Infectious Viruses in Republic of Korea

Sungjin Ko DVM, PhD [a], Jun-Gu Kang DVM [a], Jung-Yong Yeh DVM, PhD [b], Jin-San Moon DVM, PhD [b], Gui-Cheol Choi DVM, PhD [c], Sohyun Won BS [a], Joon-Seok Chae DVM, PhD [a]

[a] *Laboratory of Veterinary Internal Medicine and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea*
[b] *Foreign Animal Disease Division, National Veterinary Research and Quarantine Service, Anyang, Republic of Korea*
[c] *Seoul Race Park, Korea Racing Authority, Gwacheon, Republic of Korea*

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ABSTRACT

The prevalence of equine respiratory virus infections among a suspected population of race horses was examined using polymerase chain reaction (PCR). One or more of five equine respiratory viruses were detected in the nasal swabs of 45 of 89 horses (50.6%), and the detection rate of equine herpesvirus type 1 (EHV-1), equine herpesvirus type 4 (EHV-4), equine herpesvirus type 5 (EHV-5), equine rhinitis A virus (ERAV) and equine rhinitis B virus (ERBV) were 5.6%, 7.9%, 39.0%, 2.2%, and 6.7%, respectively. Among the 45 infected horses, 7 were co-infected with EHV and/or equine rhinitisvirus (ERV). Equine influenzavirus and equine arteritisvirus were not detected in any samples. Specific antibodies to EHV-1 and/or EHV-4 were detected in 59 of 73 tested sera (80.8%), using a virus neutralization test. This investigation suggests that equine respiratory viruses are endemic at Seoul Race Park and that the impact of viral infections on race horses' health in Republic of Korea should be evaluated.

1. Introduction

For race horses, infectious respiratory disease is one of the greatest threats affecting racing results [1]. Respiratory disease caused by viruses is responsible for incalculable economic losses within the horse racing industry [1,2]. Respiratory tract illnesses are a very common problem in horses, having a wide range of causes, including bacterial and viral infections, as well as a number of noninfectious factors. The major transmission pathogens are viruses including equine influenzavirus (EIV), equine herpesvirus type 1 (EHV-1), equine herpesvirus type 2 (EHV-2), equine herpesvirus type 4 (EHV-4), equine herpesvirus type 5 (EHV-5), equine arteritisvirus (EAV), equine rhinitis A virus (ERAV) and equine rhinitis B virus (ERBV) [1-3].

EIV is one of the most important respiratory viruses in horses because of its rapid spread among the horse population. EIV infection causes development of typical respiratory signs including acute onset of pyrexia, nasal discharge, coughing, and depression [4,5]. EIV infection often results in cancellation of horseracing events nationally, as well as prohibition of movement of horses internationally [5].

EAV infection in horses has recently been identified in countries such as Australia, New Zealand, and South Africa, which were previously thought to be largely or completely free of the virus [6-9]. This apparent global dissemination and rising incidence of EAV likely reflects the rapid national and international movement of horses for competition and breeding, as well as heightened diagnostic scrutiny as

Corresponding author at: Joon-seok Chae, DVM, PhD, Professor, Veterinary Internal Medicine, College of Veterinary Medicine, Seoul National University, 1, Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea.
E-mail address: jschae@snu.ac.kr (J.-S. Chae).

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a consequence of increasing concern over the potential threat of EAV infection [10].

EHV-1 and EHV-4 are *Alphaherpesviruses* that cause severe lytic infection of host cells and are rapidly cytopathic [11]. Moreover, secondary bacterial infection is well documented and known to be a contributory factor in the development of equine herpetic diseases [12-14]. Of greatest welfare and economic importance of EHV-1 infections are sporadic outbreaks of respiratory, neurological disease, and collective outbreaks of late term abortions that can have hit rates in excess of 50% [1,15-19]. EHV-4 is considered one of the major viral causes of equine acute respiratory disease [20]. In the Republic of Korea (ROK), there has been one case of EHV-1 reported in an aborted fetus assayed by immunohistochemistry [21]. EHV-2 and EHV-5, differentiated from each other in 1992 [22], are *Gammaherpesviruses* which infect host cells but are more slowly cytopathic than *Alphaherpesviruses*. These viruses are widespread throughout the equine population and have been isolated from cases of respiratory disease in horses from different countries [14,23,24]. The pathogenesis of these *Gammaherpesviruses* currently remains unclear; however, various reports suggest their implication in causing respiratory disease [25,26].

ERAV and ERBV are equine respiratory viruses belonging to the family *Picornaviridae*. ERAV is the only non−foot-and-mouth disease virus member of the genus Aphthovirus; and ERBV is the only representative of the genus Erbovirus and consists of three types (ERBV1, 2 and 3) by serological relationship, acid stability, and genomic sequence data [27,28]. These viruses are considered to cause equine respiratory disease and are potentially infectious to humans [29]. Despite the abundance of ERAV and ERBV in horse populations, there have been few reported studies of these viruses, and little is known about their exact prevalence or pathogenesis.

Seoul Race Park (SRP) experienced yearly infectious respiratory disease epizootics, with an estimated incidence of 29.6%, corresponding to 9.3%-13.9% of total veterinary fees from 2001 to 2005 [30]. Treatments are hindered by complex origins and difficulty in determining a causative role for the viral infection. Despite the costs and threats associated with equine respiratory viral infection, to the best of our knowledge, there have been no published estimates of equine respiratory viral infection within the Korean horse population. This study investigated the prevalence and determined the possible involvement of equine viruses in respiratory infectious diseases at SRP (1,574 horses), using molecular detection methods.

2. Materials and Methods

2.1. Reference Strains

Reference strain DNA (killed Equivac EHV-1/-4 equine rhinopneumonitis; Fort Dodge Animal Health, Fort Dodge, IA) and RNA (Duvaxyn IE Plus EIV vaccine, killed virus; Fort Dodge Animal Health; and Artervac equine viral arteritis vaccine, modified live virus; Fort Dodge Animal Health) were kindly provided by Dr. Nicola Pusterla, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis. We screened EHV-5, ERAV, and ERBV without positive reference sequences. Instead, to confirm PCR results, we analyzed nucleotide sequences of all conventional PCR products.

2.2. Samples

Horses were selected from 54 stables (1,574 horses) at SRP from January to December 2008. Trainers were queried regarding the presence of respiratory symptoms in the horses. Horses reported as having an increased frequency of coughing, mucopurulent nasal discharge, and at least one additional clinical symptom (fever, depression, decreased appetite, anorexia, poor exercise performance, or enlarged lymph nodes of the head and neck) were diagnosed as having an infectious respiratory disease. A total of 89 horses suffering from respiratory diseases were included in the study. SRP personnel administered EIV vaccine to race horses, although no vaccines for EHV, EAV, ERAV or ERBV have been administered to race horses in SRP. Individual vaccination histories of 89 horses were confirmed at the Korea Racing Authority homepage (http://studbook.kra.co.kr/studbook.jsp). Nasal swabs were collected from all suspected cases at least 2 weeks from onset of clinical symptoms and were placed in 3 mL of transport medium consisting of Eagle's minimal essential medium (MEM) with nonessential amino acid, 3% fetal bovine serum, 0.08 M NaOH3, 50 μL/mg ampicillin, 100 μL/μg gentamicin, and 5 μL/mg amphotericin B (Invitrogen, US). Specimens were submitted to the laboratory and processed within a few hours. Whole-blood samples were collected from 73 of 89 horses; consent for the remaining horses was refused by the horses' owners. Blood was collected from the jugular vein by using a 10-mL syringe for serological tests. Serum was separated, and samples were stored at −70°C until assayed.

2.3. Polymerase Chain Reaction and Reverse Transcription-PCR

Polymerase chain reaction (PCR) for detection of EHV-1, EHV-4, EHV-5, EIV, EAV, ERAV, and ERBV infections, including details of primers and probes used, are shown in Table 1. Both DNA and RNA viral nucleic acids were extracted from 400 μL of nasal swab samples, using QIAamp mini-kits (Qiagen, Germany) and viral RNA mini-kits (Qiagen, Germany) according to the manufacturer's instructions. For RNA viruses, RT-nested PCR and RT real-time PCR used extracted viral RNA as a template with which to synthesize cDNA, using Sensiscript RT kits (Qiagen, Germany) according to the manufacturer's instructions. The resulting DNA and cDNA were used as PCR templates.

For EHV-1 and EHV-4 real-time PCR, real-time PCR targeting of the glycoprotein B gene was used as described previously [31].

For EHV-5 nested PCR, nested PCR targeting of the glycoprotein B gene was used as described previously [20].

For EIV RT-real time PCR, an EIV RT-real time PCR targeting of matrix gene was used as described previously [32].

For EAV RT-real time PCR, an EAV RT-real time PCR targeting of the ORF7 gene was used as described previously [33].