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# Evaluation of the Usefulness of a PCR Assay Performed at a Clinical Laboratory for the Diagnosis of Respiratory Disease Induced by Equine Herpesvirus Type 1 in the Field

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**J. Equine Sci.**
**Vol. 22, No. 3**
**pp. 53–56, 2011**

*A PCR assay for the diagnosis of respiratory disease induced by equine herpesvirus type 1 (EHV-1) was performed at the clinical laboratory in the Racehorse Clinic of the Ritto Training Center of the Japan Racing Association from December 2007 to March 2008. The assay was performed without the trouble of contamination throughout the study and its turnaround time was approximately 6 hr. The PCR detection rates of EHV-1 among seroconverted horses were 22.2% for nasal swabs and 33.3% for blood samples. However, EHV-1 DNA was also detected in horses without seroconversion at a low rate. These results indicated that the PCR assay should be used as an adjunct method, but would help to make an early diagnosis of EHV-1 infection.*

**Key words:** *EHV-1, PCR assay, clinical laboratory use*

Equine herpesvirus type 1 (EHV-1) is endemic in horse populations throughout the world, and causes acute upper respiratory disease, central nervous disorder, and contagious virus abortion in horses [3, 4, 9, 11]. At the Ritto Training Center (TC) of the Japan Racing Association (JRA), in which approximately 2,100 racehorses were accommodated, many horses suffer from respiratory disease with pyrexia from winter to early spring every year. Our previous virological and serological surveys revealed that EHV-1 was a major cause of this seasonal respiratory disease among the racehorse population [9, 10].

Rapid diagnosis of EHV-1 infection is crucial for effective control of disease spread and treatment of affected horses. However, a conventional virus isolation method takes at least 2–3 days to get the results. On the other hand, several groups have developed sensitive and specific PCR assays that can detect EHV-1 within a 24-hr period. It has been reported that these PCR assays directly detect EHV-1 DNA in nasal swabs or blood samples collected in the field [1, 2, 5, 6, 8]. However, since the PCR assays are carried out mainly in diagnostic laboratories, it takes a few days to get the results due to the transportation of samples from the clinical sites to the laboratories. Therefore, for more rapid diagnosis of EHV-1 infection, it is necessary to perform the PCR assay at the clinical site. In this paper, we report the result of the PCR assay performed at the clinical laboratory in the Racehorse Clinic to detect EHV-1 DNA in clinical samples collected from horses with pyrexia during one epidemic season at the Ritto TC, and evaluate its usefulness for the diagnosis of EHV-1 respiratory disease in the field.

Nasal swabs and blood samples were collected from Thoroughbred racehorses exhibiting clinical pyrexia with body temperature exceeding 38.5°C at the Ritto TC from December 2007 to March 2008. Samples were obtained at the initial clinical examination. Nasal swabs were collected using absorbent cotton with a stick and immersed in 2 m*l* of phosphate buffered saline.

This article was accepted May 12, 2011
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**Table 1.** Comparison of the results of serological assay and PCR asssay

| | | Seroconversion | | |
|---|---|---|---|---|
| | | + | – | total |
| PCR (nasal swabs) | + | 14 | 5 | 19 |
| | – | 49 | 56 | 105 |
| | total | 63 | 61 | 124 |
| PCR (blood samples) | + | 21 | 1 | 22 |
| | – | 42 | 60 | 102 |
| | total | 63 | 61 | 124 |

Blood samples were taken into EDTA tubes; plasma containing buffy-coat cells was removed from the tube and stored in a sterile tube. These samples were kept at –80°C until use.

All procedures for the PCR assay were carried out at the clinical laboratory in the Racehorse Clinic of the Ritto TC. DNA was extracted from nasal swabs and buffy-coat cells using a nucleic acid isolation kit (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche Diagnostic GmbH, Mannheim, Germany) by an automated nucleic extraction system (MagNA Pure LC, Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's instructions. The PCR was carried out using primers previously described for the detection of the glycoprotein C gene of EHV-1 [5]. The thermal cycling program consisted of 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 75 sec, annealing at 60°C for 90 sec, and extension at 72°C for 90 sec, and then a final extension at 72°C for 7 min. PCR products were analyzed by electrophoresis using precast agarose gel cassettes (FlashGel System, Lonza, Rockland, ME, U.S.A.).

Paired sera (3–5-week intervals) were also collected from above mentioned horses at acute and convalescent phases, and were transported to the diagnostic laboratory of JRA (Epizootic Research Center, Equine Research Institute, JRA). For serological confirmation of EHV-1 infection, ELISA using a type-specific region of EHV-1 glycoprotein G as an antigen was carried out as described previously [12, 13]. An equal or more than 4-fold increase of antibody titer between paired sera was considered as seroconversion. The ELISA used in the present study can distinguish antibody to EHV-1 from antibody to EHV-4, which is antigenically related to EHV-1, and only detects antibodies elicited by infection with EHV-1, but not by vaccination with formalin-inactivated EHV-1 vaccine used in Japan [12].

The samples were collected from 124 out of a total of 279 horses that developed pyrexia during the period. PCR positive rates of nasal swabs and blood samples were 15.3% (19/124) and 17.7% (22/124), respectively. EHV-1 DNA was detected in both samples in 9 out of 124 horses (7.3%) tested in this study. On the other hand, the rate of seroconversion to EHV-1 was 50.8% (63/124). The comparison of the results of the PCR assay and the serological assay for EHV-1 infection is shown in Table 1. The PCR detection rates of EHV-1 among seroconverted horses were 22.2% for nasal swabs and 33.3% for blood samples. On the other hands, EHV-1 DNA was also detected in the samples collected from horses without seroconversion (8.2% for nasal swabs and 1.6% for blood samples).

To our knowledge, the present study is the first attempt to evaluate the usefulness of the PCR assay for the diagnosis of EHV-1 respiratory disease in routine equine practice. There is high risk of contamination in performing a PCR assay at the clinical site compared to at the diagnostic laboratory. Therefore, we extracted DNA from the samples using an automated robotic system in a completely closed housing, and the PCR reaction mixtures were prepared on the designated area. Additionally, since complicated sensitive procedures are not suitable for the clinical laboratory, the simple conventional PCR method was used in the present study. The PCR assay was successfully performed without the trouble of contamination throughout the study. The turnaround time of the assay was approximately 6 hr.

The PCR assay used in this study could detect EHV-1 in both nasal swabs and blood samples collected from horses with pyrexia in the field. However, the detection rates in seroconverted horses are relatively low (Table 1). These low detection rates might be due to the less sensitivity of our conventional PCR method. Additionally, if peripheral blood mononuclear cells (PBMCs), which are the primary targets for EHV-1 infection, were used as the sample, the detection rate in

the blood samples could have been higher than those observed in this study. However, the performance of a more sensitive method (e.g., nested PCR or real-time PCR) or purification of PBMCs from the blood samples in the clinical laboratory is not realistic. Furthermore, the respiratory symptoms observed in most seroconverted horses were generally-mild (short lasting fever without other symptoms; the details are not shown), which suggested that the replication of EHV-1 in these animals was limited. Therefore, application of a sensitive method for PCR or PBMCs as the sample may not dramatically improve the detection of EHV-1.

On the other hand, EHV-1 was detected in the samples of the 6 horses without seroconversion (Table 1). Two of them (1 horse positive in nasal swab and 1 horse positive in blood sample) were assumed to be seroconverted, since they showed a 2-fold increase of antibody titer between paired sera. However, no detectable antibody titer (<1:200) was observed in paired sera of the rest of 4 horses, all of which were positive in nasal swab. In these horses, the replication of EHV-1 might be restricted in the mucosa of the upper respiratory tract, resulting in no antibody response. It is unclear whether pyrexias observed in these 4 horses were caused by EHV-1 infection. The PCR positive results observed in the horses without seroconversion was considered not to be due to cross contamination or the false positive reaction. It is because that the positive results randomly appeared with negative results, and all the negative controls (water) gave negative PCR results. Additionally, the PCR used in the present study, which detects EHV-1 gC gene, has been performed for years without the false positive results in the Epizootic Research Center of JRA.

The results obtained in this study indicated that it was difficult to make a certain diagnosis of EHV-1 respiratory disease in the field by the PCR assay performed at the clinical laboratory because of the low detection rates and the possibility to detect the virus that is unrelated to the disease. Therefore, the PCR assay should be used as an adjunct to the diagnosis of EHV-1 infection. Nevertheless, it is considered worthwhile to perform the PCR assay for EHV-1 at the clinical laboratory. In particular, an outbreak of neurological form of EHV-1 infection, the recent increase of which is of major concern to the horse industry worldwide, requires urgent responses including isolation of sick horses and segregation of exposed horses [7]. The PCR assay established in this study can be introduced to routine equine practice, and would help us to make an early diagnosis of EHV-1 infection.

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