



NOTE

Theriogenology

Occurrence of equine coital exanthema (ECE) in stallions in Japan and effectiveness of treatment with valacyclovir for ECE

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ABSTRACT. Equine coital exanthema (ECE) has been reported in many countries, but equine herpesvirus 3 (EHV-3) has been isolated only once in Japan. In 2015, symptoms of ECE were found, and EHV-3 was isolated in two stallions. Valacyclovir, an anti-herpesvirus agent, was administered orally. The stallions rested from mating for more than two weeks, causing enormous financial losses because of their high fees. This is the first study in which valacyclovir was administered for ECE. Though valacyclovir treatment did not shorten the duration of healing, the affected area did not expand after administration of valacyclovir. Valacyclovir therefore seems to be effective for suppression of EHV-3 infection. Further investigation about the administration protocol might be required.

KEY WORDS: acyclovir, ECE, EHV-3, stallion, valacyclovir

Equine coital exanthema (ECE) is caused by equine herpesvirus 3 (EHV-3), which is a member of the *Varicellovirus* genus, subfamily *Alphaherpesvirinae*. The virus is transmitted via both direct contact and indirect contact. It is also transmitted by coitus and artificial insemination [1, 2, 4, 7]. Infection with the virus results in the development of papules, vesicles, pustules and ulcers on the vaginal area in mares and on the penis and prepuce in stallions [1]. These regional symptoms are cured in two to three weeks [1, 5, 7, 11, 16]. Treatments for the disease are very limited. In Japan, firing the affected area with silver nitrate has been used on a regular basis. An antibiotic is used for prevention of complications [7, 16]. Some researchers have investigated the effectiveness of acyclovir, an anti-herpesvirus agent [1, 2, 6, 16]; however, the effectiveness of valacyclovir, a prodrug of acyclovir, has not been reported.

The occurrence of ECE has been reported in many countries [2, 13, 16]. In Japan, symptoms suggesting ECE have been reported, but isolation of EHV-3 was reported in only one case. It was isolated from draft mares in Iwate Prefecture in 2004 [16]. EHV-3 causes latent infection as do other herpesvirus [3–5]. The prevalence of the virus in Japan is unknown.

As reported here, two stallions developed symptoms suggestive of ECE, and EHV-3 was isolated from both stallions. Valacyclovir and acyclovir, anti-herpesvirus agents, were used for treatment, and it was investigated whether valacyclovir is effective for treating ECE.

Case 1: Stallion A was a 14-year-old Thoroughbred. The stallion mated with two or three mares every day from mid February until the occurrence of ECE. On April 10, 2015, some papules were found on the penis, but this stallion mated with three mares on the same day (date of occurrence). The next day, the papules were ruptured, and exudative fluid was observed. The stallion showed signs of pain by palpation of the penis, and mating was impossible. EHV-3 was isolated from a penile swab [12]. On the 2nd day, oral administration of valacyclovir (Valtrex, GlaxoSmithKline K. K., Tokyo, Japan), which is a prodrug of acyclovir and an anti-herpesvirus agent, was started (27 mg/kg/8 hr for two days and 18 mg/kg/12 hr for eight days) [15]. Washing the affected area with hypochlorous acid solution (50 ppm, pH 6.5) and application of 1% nadifloxacin ointment were started on the same day. On the 3rd day, body temperature rose, and appetite was lost. Flunixin meglumin was therefore administered (1.1 mg/kg). The affected area did not expand after starting administration of valacyclovir and gradually dried from the 5th day. Local treatment for the penis was stopped on the 8th day, and oral administration of valacyclovir was stopped on the 11th day. Symptoms of the penile area are shown in Fig. 1. The stallion started mating again on the 18th day, and the stallion had no problems with mating. Antibodies against EHV-3 in sera were detected by a serum neutralizing test using the EHV-3 isolate from the stallion [12] and horse fetal kidney (HFk) cells as described previously [16]. The serum antibody titers are shown in Table 1. One of the three mares the stallion mated with on the date of occurrence developed papules 5 days after mating, and EHV-3 was isolated from a vaginal swab

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Fig. 1. Symptoms of the penile area. (A): Stallion A on the 5th day after onset. (B): Stallion B on the 3rd day after onset

Table 1. Serum EHV-3 neutralizing antibody titers in EHV-3-infected stallions

Stallion	Serum Collection Dates in 2015					
	Apr. 11	Apr. 27	May 27	Jun. 30	Jul.27	Aug. 31
A	<2	4	2	8	8	8
	May 15	Jun. 3	Sep. 16			
B	<2	2	16			

Table 2. Susceptibility of EHV-3 to antiviral compounds

Virus	EC ₅₀ (μg/ml)	
	Valacyclovir	Acyclovir
EHV-3	3.0	2.3

(data not shown).

Case 2: Stallion B was a 13-year-old Thoroughbred. The stallion mated with one or two mares almost every day. On 14 May, 2015, some papules had developed on the stallion's penis, and the number of papules gradually increased (date of occurrence). Some of the papules ruptured, and exudate was observed. EHV-3 was isolated from a penile swab [12]. The stallion was administered valacyclovir orally at the same dose as that for stallion A on the date of occurrence. For local treatment, the penis was washed with hypochlorous acid solution, and 5% acyclovir ointment, an anti-herpesvirus agent, was applied from the 2nd day. The affected area did not expand from the 2nd day, and the ruptured pustules gradually became scarred. Local treatment was stopped on the 7th day, and oral administration of valacyclovir was stopped on the 10th day. Symptoms of the penile area in stallion B are shown Fig. 1. The stallion started mating on the 19th day, and the stallion had no problems with mating. The serum antibody titers are shown in Table 1.

Antiviral activity of valacyclovir (Sigma-Aldrich, St. Louis, MO, U.S.A.) and that of acyclovir (Sigma-Aldrich) against EHV-3 were determined by plaque reduction assays. HFK cells were seeded in 6-well culture plates (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and were cultured in a growth medium (minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS)) at 37°C for 24 hr. Then, the growth medium was removed, and HFK cell monolayers were cultured with various concentrations of each compound, from 1 mg/ml to 250 ng/ml in MEM supplemented with 4% FCS at 37°C for 24 hr. The culture medium was removed again, and HFK cells were infected with 50 to 100 plaque-forming units of EHV-3/well. After 1-hr adsorption, HFK cells were washed three times with MEM and overlaid with MEM containing the same concentration of each compound, 4% FCS and 0.9% Agar Noble (Difco, Detroit, MI, U.S.A.). Plaques were counted two days after infection. The 50% effective concentration (EC₅₀) for reduction in plaque number was calculated from dose-response curves generated from the data.

The EC₅₀ values are shown in Table 2. Both valacyclovir and acyclovir were effective for inhibiting replication of EHV-3.

Symptoms suggestive of ECE have often been observed in Japan, but the exact causes were not investigated in most cases. Isolation of EHV-3 was reported previously in only one case in Japan, and it was isolated from mares [16]. This is the first report of isolation of EHV-3 from stallions in Japan. The two stallions from which EHV-3 was isolated were kept at different studs. The distance between the two studs is more than 70 km, and the stallion handlers did not pass between the two studs in the breeding season. There was no mare that was mated in both of the studs. Thus, it is possible that EHV-3 was prevalent subclinically among Thoroughbreds in Japan.

During the occurrence of ECE, the stallions had to stop mating, because there was pain by palpation of the penis and because

EHV-3 is transmitted by coitus. Both of the stallions stopped mating for more than two weeks. Because their fees for mating were very high, financial losses were enormous and owners who planned to mate their mares with stallion A or B had to change their mating plan. ECE therefore has huge negative effects on the Thoroughbred industry.

Stallions are at high risk for exposure to EHV-3 in the breeding season, because they are in contact with many mares, and popular stallions are very busy and under a lot of stress in the breeding season [2]. According to some reports, stress causes re-activation of the virus latently in infected horses [1, 4, 14].

Approaches to therapy for ECE are very limited. In Japan, firing the affected area with silver nitrate has been used, but this is painful and its effect is unknown. Some researchers have used acyclovir for treatment of ECE. In a previous study, a stallion and some mares with ECE responded well to application of acyclovir, and an inhibitory effect of acyclovir on EHV-3 was shown by plaque reduction assays [6]. Some researchers have used valacyclovir, a prodrug of acyclovir, for treatment of EHV-1 infection, but not for treatment of EHV-3 infection [8–10, 15]. Some studies showed that valacyclovir maintained an effective serum concentration for a longer time than did acyclovir [9, 10, 15]. We therefore expected that valacyclovir would be more effective than acyclovir for treatment of ECE. We used valacyclovir for systemic treatment, and this is the first study in which valacyclovir was used orally for treatment of ECE. However, it took more than two weeks for both stallions to start mating again, and this period is almost the same as that when ECE was treated without an anti-herpesvirus agent [1, 5, 7, 11, 16]. These results suggest that once papules have ruptured, it takes more than two weeks for regeneration of the penile area. Treatment with valacyclovir did not greatly shorten the duration of disease. Garré *et al.* [8] reported that unbound plasma acyclovir concentrations were maintained between 1.7 µg/ml and 3.0 µg/ml after valacyclovir administration (40 mg/kg body, three times daily) in ponies. The isolate used in this study is susceptible to valacyclovir *in vitro* at a concentration of 3.0 µg/ml. Therefore, in this study, the dosage regimen consisting of 27 mg/kg/8 hr for two days and 18 mg/kg/12 hr for eight days might have resulted in a plasma concentration that did not exceed the EC₅₀ value of EHV-3. However, the affected area did not expand after oral administration of valacyclovir, indicating the possibility that valacyclovir suppresses EHV-3 activity clinically.

Stallion A was not a subclinical case and EHV-3 was transmitted from other mares, because the stallion's antibody titer had never increased for eight years before occurrence (data not shown). On the other hand, the antibody titer of stallion B before occurrence was not investigated, and whether the cause of ECE symptoms was re-activation or transmission from mares was unknown. Serum antibody titers did not increase immediately after occurrence in contrast to the immediate increase in antibody titers in previous studies [5, 16], but they increased two months and four months after occurrences in stallion A and stallion B. Oral administration of valacyclovir might suppress the increase in antibody titers.

It has been reported that EHV-3 was transmitted not only from horses with symptoms but also from latently infected horses [1, 2, 4, 7]. Before mating, we checked the vaginal area of all mares, but symptoms of ECE were not found. The vaginal area was washed as in previous studies [1, 2]. In those previous studies, washing the vaginal area reduced the risk of transmission. In our study, the vaginal area was washed with hypochlorous acid solution. Virucidal activity of hypochlorous acid solution against EHV-3 was confirmed in our laboratory (data not shown). In these cases, it was suspected that EHV-3 was inside the vagina and that hypochlorous acid solution did not reach the area of infection.

In 2015, two Thoroughbred stallions developed ECE symptoms, and EHV-3 was isolated by penile swabs. This is the first report of EHV-3 isolation from stallions in Japan and administration of valacyclovir orally for treatment of ECE. Oral treatment with valacyclovir did not greatly shorten the duration of disease. The period for cure of the affected area depended on how long it took for papules and ulcers to heal. The affected area did not expand after administration of valacyclovir, and EHV-3 was susceptible to valacyclovir *in vitro*. Valacyclovir therefore seems to be effective for suppression of EHV-3 replication, but further investigation of the appropriate dosage of valacyclovir for treatment of ECE is required. At present, careful observation and washing of the vaginal area before mating and reducing the stress of stallions are important for prevention of ECE.

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