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Period of excretion of equine herpesvirus 3 (EHV-3) from a stallion before showing clinical signs of equine coital exanthema and the effect of acyclovir treatment on the duration of EHV-3 excretion

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ABSTRACT. In 2017, two Thoroughbred stallions, A and B in Farms A and B, respectively, in Hokkaido in Japan showed clinical signs of equine coital exanthema (ECE). In 2020, stallion C in Farm B showed clinical signs of ECE. Eighteen mares were mated within five days before stallion A developed ECE. Ten mares that mated within 3 days before onset showed clinical signs of ECE on the external genitalia. Equine herpesvirus 3 (EHV-3) was isolated from vaginal swabs from three mares that mated within 2 days before onset. Swabs from 12 mares that mated within 4 days before onset were real-time PCR (rPCR)-positive and nine of those mares had an increased EHV-3 antibody titer. The three stallions were administered valaciclovir orally and topical acyclovir ointment was applied. Treatment started on the next day after onset in stallion A and on the day of onset in stallions B and C. EHV-3 was firstly isolated from penis swabs of stallions A and B before treatment and from penis swabs of stallion C 2 days after treatment. EHV-3 was not isolated after 8, 5 and 8 days from onset in stallions A, B and C, respectively. However, swabs were rPCR-positive for at least 12, 9 and 15 days after onset of stallions A, B and C, respectively. EHV-3 was excreted from the stallions at least within 4 days before the onset of ECE, and acyclovir treatment resulted in the termination of excretion within 8 days after onset.

KEY WORDS: acyclovir, equine coital exanthema, equine herpesvirus 3, excretion, transmission

Equine coital exanthema (ECE) is caused by equine herpesvirus 3 (EHV-3). EHV-3 is a member of the order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Varicellovirus*, and the virus is transmitted by mating [1–3, 12]. In ECE cases, papules, vesicles, pustules and ulcers are observed on the external genitalia [1]. ECE cases have been reported in various countries [2, 15, 17]. Recently, some cases were observed in Thoroughbred stud farms in Hokkaido, Japan [18]. When a Thoroughbred stallion develops ECE clinical signs, the stallion cannot mate for a few weeks because of pain of the lesions and the risk of transmission of EHV-3 to mares [1–3, 11]. This causes a huge economic loss for the Thoroughbred breeding industry. For treatment of ECE, antimicrobials are used to prevent secondary bacterial infection [1, 2]. The effectiveness of topical use of acyclovir ointment has not been investigated in detail [1, 8]. We reported that oral administration of valacyclovir, a prodrug of acyclovir, prevented expansion of the lesions but did not shorten the duration of the resting period to mate [18]. The reason why the resting period to mate was not shortened is unclear, but one possibility is that the acyclovir concentration in plasma did not exceed the theoretical 50% effective concentration of EHV-3 [18]. An affected stallion should be able to start mating as soon as possible; however, it is difficult to decide when the stallion can start to mate.

In our previous studies, we found that EHV-3 infection existed widely without clinical signs in broodmare farms in Hokkaido in Japan and that EHV-3 antibody became positive in a serum neutralization test in affected stallions after they showed clinical signs [14, 18]. It was also reported that EHV-3 was transmitted from horses showing no clinical signs [4, 5]. The results of those previous studies suggested that stallions that developed ECE clinical signs were infected from latently infected mares by mating.

In 2017 and 2020, two Thoroughbred stallions (A and B) and one stallion (C), respectively, in stud farms in Hokkaido in Japan showed clinical signs of ECE. In this study, vaginal swabs were taken from mares that had mated with stallion A before the stallion

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showed clinical signs in order to confirm when the stallion started to excrete EHV-3 and transmit it to mares by mating. The three affected stallions were treated with oral administration of valacyclovir and topical acyclovir ointment. We used about a 1.3-fold higher dose of valacyclovir than that used in our previous study with the expectation of shortening the duration of the disease [18]. Penis swabs were taken from the stallions in order to determine when the excretion of EHV-3 stopped after the onset in the stallions receiving anti-herpesvirus drug treatment. Antibody titers were also investigated in both the mares and stallions in order to determine the immune response to EHV-3 infection.

MATERIALS AND METHODS

Animals

Stallions: Stallion A in Farm A, a 6-year-old Thoroughbred stallion, showed clinical signs of ECE on May 5, 2017 (the first day of occurrence; day 1). One papule was observed on the penis; however, the stallion mated with three mares on day 1. On the next day (day 2), the number of papules had increased and exudative fluid was observed. The stallion felt pain by palpation of the penis and mating was judged to be impossible. Swabs were taken from two sites of the penis (glans and proximal part) on day 2, day 8, day 9, day 11 and day 12. The swab samples were eluted in 1 m*l* of Eagle's minimum essential medium (MEM) supplemented with 50 μg/m*l* of gentamycin as described previously [14] and used for virus isolation and DNA extraction. Sera were taken on day −9, day 20 and day 53. Treatment for ECE was started on day 2. For treatment, the stallion was orally administered 35 mg/kg of valaciclovir (Valtrex, GlaxoSmithKline K. K., Tokyo, Japan), which is a prodrug of acyclovir and an anti-herpesvirus agent, every eight hours for three days and then 25 mg/kg of valaciclovir every 12 hr for nine days [16]. Flunixin meglumin was administered intravenously depending on the pain. Acyclovir ointment was applied on the penis until the lesion was encrusted on day 8. The stallion started mating again on day 17.

Stallion B in Farm B, an 8-year-old Thoroughbred stallion, showed clinical signs of ECE on June 19, 2017. One papule was observed on the penis. Treatment was started on day 1 in the same way as that for stallion A, and mating was stopped. The number of papules had slightly increased on the next day (day 2), but exudative fluid was not observed. Swabs were taken from two sites of the penis (orifice of the urethra and papules) on day 1 to day 9. Sera were taken on day 1, day 9 and day 32. Stallion B did not start mating again until the next breeding season in 2018 because ECE occurred at the end of the breeding season.

The two stallion ECE cases were determined to be unrelated for the following reasons. The distance between the two studs, Farms A and B, was more than 70 km, and the stallion handlers did not pass between the two studs in the breeding season. There were no mares that had been mated by both two stallions.

Stallion C in Farm B, a 14-year-old Thoroughbred stallion, showed clinical signs of ECE on March 27, 2020. Small redden papules were observed on the penis. Treatment was started on day 1 in the same way as that for stallion A, and mating was stopped. The number of papules had slightly increased on the next day (day 2), and exudative fluid was observed. Swabs were taken from two sites of the penis (glans and proximal part) on days 2, 8, 11, 13 and 15. Sera were taken on day 2, day 15 and day 34. Stallion C started mating again on day 13, but the stallion had a scratch on the glans due to mating. The stallion rested for another two weeks.

Mares: Eighteen mares (4–18 years of age) that mated with stallion A from day −5 to day 1 were investigated. Details of the mares are shown in Table 1. Swabs were taken from two sites of the vagina (vaginal vestibule and vulva) in each mare and used for virus isolation and DNA extraction. Sera were also collected on the same day. The external genitalia of each mare were observed carefully and clinical signs that were observed were recorded. The days when swabs and sera were collected are shown in Table 1. Swab samples from mares that mated with stallions B and C were not available, and a follow-up survey as was performed for stallion A was therefore not conducted.

Cell culture and virus

Fetal horse kidney (FHK) cells were cultured in MEM supplemented with 10% fetal bovine serum (FBS) and used for virus isolation and serum neutralization tests. For the maintenance medium, the concentration of FBS was reduced to 4%. The SS-1 strain of EHV-3 was used [14].

Virus isolation

Virus isolation from 100 μ*l* of each supernatant of the swab samples was conducted using FHK cells cultured in a 6-well plate as described previously [14].

Virus titration

Virus titration was performed on an FHK cell monolayer cultured in a 96-well plate by inoculation with serial 10-fold dilutions of samples. After adsorption for 1 hr at 37°C under 5% CO_2, the maintenance medium was added and cultivated at 37°C under 5% CO_2 for 4 days, and viral titers were calculated using Behrens-Kärber's method [13] based on a cytopathic effect (CPE).

DNA extraction

DNA of the SS-1 strain was extracted as total DNA from virus-infected FHK cells as described previously [12]. DNA was extracted from the supernatants of swab samples using a DNeasy Blood and Tissue kit (Qiagen K.K., Tokyo, Japan).

Table 1. The information of the mated mares with stallion A

Days of mating	Mares	Age	Number of foal	Days of collecting samples after mating			
				Swab	Serum-1	Serum-2	Serum-3
Day 1[a)]	A	17	10	5	5	20	78
	B	13	4	4	4	19	76
	C	8	4	4	4	21	80
Day −1	D	14	7	11	11	21	79
	E	5	1	5	5	23	77
	F	6	Maiden	5	5	21	78
Day −2	G	9	2	9	9	21	78
	H	6	2	9	9	21	78
	I	18	10	9	9	22	78
Day −3	J	4	Maiden	10	10	22	79
	K	7	2	10	10	22	79
	L	8	2	10	10	22	79
Day −4	M	6	Maiden	14	14	24	82
	N	11	5	15	15	24	81
	O	10	3	15	15	23	80
Day −5	P	11	4	16	16	26	81
	Q	10	2	16	16	24	82
	R	17	7	16	16	24	81

a) Day 1; the day of occurrence of stallion A.

Polymerase chain reaction (PCR) for detection of EHV-3

PCR assays targeting the gG gene for detection of EHV-3 were carried out as described previously [10, 14]. The size of the PCR product was 518 bp.

Real-time PCR

Real-time PCR (rPCR) for quantification of EHV-3 genomic copies in swab samples was performed using SYBR premix Ex Taq II (Tli RNase H plus) (Takara Bio Inc., Tokyo, Japan) and primers targeting the gG gene on LightCycler 1.5 (Roche Diagnostics GmbH, Mannheim, Germany). The sequences of the forward primer (EHV-3-gG-F2) and reverse primer (EHV-3-gG-R) amplifying a 176-bp fragment were 5′-CATTCTCGTGTCCGGTCCT-3′ and 5′-GGCGTCTCGAAAAGCGAGAG-3′, respectively. A cycling condition was initial denaturation of 1 min at 95°C followed by 45 cycles of 15 sec at 95°C, 30 sec at 60°C and 12 sec at 72°C. DNA standards were prepared as follows. PCR products amplified with the primer pairs (EHV-3-gG-F2 and EHV-3-gG-R) using EHV-3 SS-1 DNA were purified with a High Pure PCR Product Purification kit (Roche Diagnostics GmbH), and the OD value of the purified DNA was measured at 260 nm/280 nm on a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific K. K., Tokyo, Japan). The viral genomic copy number of the DNA standards was calculated by the following formula: DNA copy number (copies/μl)=DNA concentration (g/μl) × 6.02 × 10^{23}/ 660 × DNA length (bp). The DNA standards were used to construct standard curves spanning 10^5 to 10 copies/μl by serial dilutions. Copy numbers were calculated as total number in a swab sample.

Serum neutralization (SN) test

EHV-3 antibody in sera was determined by the SN test using the SS-1 strain as described previously [17].

RESULTS

Stallions, A and B in Farms A and B (in 2017) and stallion C in Farm B (in 2020) were strongly suspected of having ECE because of typical clinical signs. Cytopathic effects (CPEs) characterized by rounded cells and syncytial formation were observed in FHK cells on the day after inoculation of penis swab samples from all the three stallions. By PCR amplification of the partial EHV-3 gG gene, products with the same estimated size, approximately 520 bp, were detected from DNAs extracted from the swabs of those stallions. From these observations, the isolated viruses were identified as EHV-3 [14]. The isolates from stallions A, B and C were designated as S1-17, S2-17 and S1-20, respectively.

Among the 18 mares that mated with stallion A within 5 days before the onset of ECE (day −5) and on the day of onset (day 1), three mares that mated on day 1 (A, B and C), two mares that mated on day −1 (D and E), three mares that mated on day −2 (G, H and I) and two mares that mated on day −3 (K and L) developed clinical signs of ECE (Table 2). Some ruptured papules with exudative fluid and white scar were observed on the vulva. EHV-3 was isolated from two mares that mated on day 1 (A and C) and one mare that mated on day −2 (I). The EHV-3 genome was detected by rPCR in three mares that mated on day 1 (A, B and C), three mares that mated on day −1 (D, E and F), three mares that mated on day −2 (G, H and I), two mares that mated on

Table 2. Detection of equine herpesvirus 3 (EHV-3) genome by rPCR and isolation of EHV-3 from genital swabs taken from the mating mares with stallion A

Days of mating	Mares	Days of collecting samples after mating	Vaginal vestibule		Vulva		Symptoms of ECE	Pregnancy
			Copy numbers in swab	EHV-3 isolation	Copy numbers in swab	EHV-3 isolation		
Day 1[a)]	A	5	1.2×10^7	+	2.0×10^8	+	+	-
	B	4	1.1×10^6	-	5.8×10^5	-	+	+
	C	4	4.8×10^6	-	2.4×10^7	+	+	+
Day −1	D	5			2.4×10^6	-	+	+
	E	5	2.7×10^5	-	1.8×10^7	-	+	+
	F	5			3.2×10^3	-	-	+
Day −2	G	9	5.2×10^4	-	1.6×10^4	-	+	+[b)]
	H	9	1.5×10^5	-	3.4×10^6	-	+	+
	I	9	2.8×10^4	-	3.5×10^6	+	+	-
Day −3	J	10	-	-	-	-	-	+
	K	10	2.6×10^4	-	7.1×10^5	-	+	+
	L	10	1.5×10^5	-	1.5×10^7	-	+	+
Day −4	M	14	-	-	-	-	-	+
	N	15	-	-	-	-	-	-
	O	15	-	-	1.1×10^3	-	-	+
Day −5	P	16	-	-	-	-	-	+
	Q	16	-	-	-	-	-	-
	R	16	-	-	-	-	-	-

a) Day 1; the day of occurrence of equine coital exanthema (ECE) in stallion A. b) The result of pregnancy of mare G was early embryonic death. Other pregnant mare gave birth normally.

Table 3. Equine herpesvirus 3 (EHV-3) antibody in mares

Days of mating	Mare	Serum neutralization titer			EHV-3 genome in genital swab[c)]	Symptoms of ECE
		Serum-1	Serum-2	Serum-3		
Day 1[a)]	A	<2 (5)[b)]	8 (20)	8 (78)	+	+
	B	<2 (4)	<2 (19)	4 (76)	+	+
	C	<2 (4)	<2 (21)	<2 (80)	+	+
Day −1	D	<2 (11)	<2 (21)	4 (79)	+	+
	E	<2 (5)	4 (23)	32 (77)	+	+
	F	<2 (5)	<2 (21)	2 (78)	+	-
Day −2	G	<2 (9)	<2 (21)	<2 (78)	+	+
	H	<2 (9)	8 (21)	<2 (78)	+	+
	I	<2 (9)	8 (22)	<2 (78)	+	+
Day −3	J	<2 (10)	<2 (22)	<2 (79)	-	-
	K	<2 (10)	2 (22)	2 (79)	+	+
	L	<2 (10)	8 (22)	<2 (79)	+	+
Day −4	M	<2 (14)	<2 (24)	<2 (82)	-	-
	N	32 (15)	32 (24)	4 (81)	-	-
	O	<2 (15)	<2 (23)	<2 (80)	+	-
Day −5	P	<2 (16)	<2 (26)	<2 (81)	-	-
	Q	<2 (16)	<2 (24)	<2 (82)	-	-
	R	<2 (16)	<2 (24)	<2 (81)	-	-

a) Day 1; the day of occurrence of equine coital exanthema (ECE) in stallion A. b) Numbers in parenthesis are days of collecting serum samples after mating. c) Data are quoted from Table 2.

day-3 (K and L) and one mare that mated on day −4 (O) (Table 2). Thirteen of the 18 mares, including one mare (G) in which early embryonic death occurred, became pregnant after mating (conception rate of 72.2%).

All of the mares except mare N were EHV-3 antibody-negative in the first serum samples (Table 3). Nine mares showed sero-conversion against EHV-3: one mare that mated on day 1 (A), one mare that mated on day −1 (E), two mares that mated on day −2 (H and I), and two mares that mated on day −3 (K and L) showed sero-conversion in the second serum samples, and one mare that mated on day 1 (B) and two mares that mated on day −1 (D and F) showed sero-conversion in the third serum samples. On the

other hand, three mares, H, I and L, became sero-negative in the third serum samples. EHV-3 antibodies of mare N were observed throughout the observation period without detection of the EHV-3 genome, virus isolation and clinical signs (Tables 2 and 3). Details of EHV-3 antibody titers in each mare are shown in Table 3.

EHV-3 was isolated from the penis swab of stallion A on day 2 (Table 4). EHV-3 was not isolated from the penis swab after day 8. Penis swabs taken until day 12 were rPCR-positive. Copy numbers of the EHV-3 genome were extremely high on day 2 and decreased to more than 3 $\log_{10}$ after day 8. A serum sample was taken on day −9 before this stallion developed clinical signs of ECE. EHV-3 antibody was negative on that day. However, EHV-3 antibody was positive on day 20 and day 53.

EHV-3 was isolated from penis swabs of stallion B collected on days 1, 2, 3 and 4 (Table 5). The virus titers in swab samples decreased drastically after day 2. EHV-3 genomes in swab samples were detected throughout the observation period, but copy numbers were reduced to more than 4 $\log_{10}$ after day 6. In convalescent sera, EHV-3 neutralizing antibody was detected with a low titer.

EHV-3 was isolated from penis swabs of stallion C collected on day 2 (Table 6). EHV-3 genomes in swab samples were detected throughout the observation period, but copy numbers were gradually reduced to almost the detection limit (2.5×10^2 copies/swab) on day 11. EHV-3 neutralizing antibody was detected with a low titer in convalescent sera.

Table 4. Detection of equine herpesvirus 3 (EHV-3) genome by rPCR and EHV-3 isolation from penile swabs and EHV-3 antibody titers in stallion A after onset of equine coital exanthema (ECE)

Days after onset	Copy numbers in swab		EHV-3 isolation		Antibody titer
	Glans	Proximal part	Glans	Proximal part	
−9					<2
2		5.3×10^8		+	
8	2.2×10^5	2.9×10^5	-	-	
9	1.8×10^4	4.0×10^2	-	-	
11	3.3×10^4	1.4×10^5	-	-	
12	5.5×10^3	2.7×10^5	-	-	
20					8
53					2

Table 5. Detection of equine herpesvirus 3 (EHV-3) genome by rPCR and EHV-3 isolation from penile swabs and EHV-3 antibody titers in stallion B after onset of equine coital exanthema (ECE)

Days after onset	Copy numbers in swab		EHV3 isolation ($TCID_{50}$/swab)		Antibody titer
	Orifice of the urethra	Papules	Orifice of the urethra	Papules	
1		9.8×10^9		+ ($10^{6.25}$)	<2
2	3.0×10^7	1.4×10^7	+ ($10^{3.25}$)	+ ($10^{3.25}$)	
3	2.2×10^6	1.8×10^7	-	+ ($10^{1.25}$)	
4	1.9×10^7	8.1×10^6	+ ($10^{1.25}$)	-	
5	3.7×10^5	1.9×10^6	-	-	
6	1.7×10^5	6.7×10^4	-	-	
7	8.3×10^3	2.4×10^5	-	-	
8	-	1.7×10^5	-	-	
9	2.4×10^3	1.4×10^5	-	-	<2
32					2

Table 6. Detection of equine herpesvirus 3 (EHV-3) genome by rPCR and EHV-3 isolation from penile swabs and EHV-3 antibody titers in stallion C after onset of equine coital exanthema (ECE)

Days after onset	Copy numbers in swab		EHV3 isolation ($TCID_{50}$/swab)		Antibody titer
	Glans	Proximal part	Glans	Proximal part	
2	1.8×10^7	1.8×10^7	+ ($10^{4.25}$)	+ ($10^{4.75}$)	<2
8	1.2×10^5	3.1×10^5	-	-	
11	2.6×10^4	8.4×10^2	-	-	
13	4.0×10^2	1.5×10^3	-	-	
15	-	4.7×10^2	-	-	4
34					2

DISCUSSION

Some cases of ECE have recently been observed in Thoroughbred studs in Hokkaido in Japan [18]. It was reported that the lesions healed in about two to three weeks, but it was unclear how many days the affected stallions excreted EHV-3 before developing clinical signs and how many days the stallions treated with an anti-herpetic drug excreted EHV-3.

In this study, it was found that stallion A could transmit EHV-3 to mares within 4 days before the development of clinical signs of ECE. The incubation period of ECE is five to 10 days [1, 21]. Our results suggested that infectious EHV-3 was excreted during the incubation period at least 4 days before the onset of ECE. These observations support the evidence that subclinically infected horses without visibly noticeable lesions can also transmit the virus to their breeding partners [1, 3, 6].

The findings in affected mares in this study suggested that EHV-3 was excreted particularly around the vulva, where more severe clinical signs develop than those on the vaginal vestibule. Mare F and mare O were rPCR-positive, but they did not show clinical signs of ECE. This might be because their clinical signs were mild and were greatly improved when the vulvas of mare F and mare O were observed at five days and 15 days after mating, respectively. Clinical signs of ECE in other mares also seemed to be milder than clinical signs in stallion A, but the reason was unknown. Among the 18 mares, three mares, F, J and M, were maidens and did not show clinical signs of ECE. In general, mucosal immune defenses, including innate immunity, are thought to be depressed during the postpartum period [9, 19]. One possibility is that the three maidens were relatively resistant against microbial infections, including EHV-3 infection, compared with postpartum mares in the breeding season. However, the exact reason is unknown.

Thirteen of the 18 mares were in foal and the conception rate was 72.2%. EHV-3 infection did not have a negative influence on fertility as was previously reported [1].

EHV-3 antibodies were not detected in the first serum samples from any of the mares except mare N collected at 5 to 16 days after mating. Nine mares showing clinical signs of ECE showed sero-conversion against EHV-3 in the second serum samples taken about three weeks after mating or the third serum samples taken about 11 weeks after mating. An increase in EHV-3 antibody was not observed in mares C and G, although these two mares showed clinical signs of ECE and existence of the EHV-3 genomes in swabs taken from the vulva. These results indicate that EHV-3 was transmitted to those mares by mating with stallion A. The reason for our results showing that EHV-3 antibody did not increase drastically and that some mares did not produce EHV-3 antibody might be that EHV-3 infection was limited to the topical area on the external genitalia. It was suggested that peak antibody titers against EHV-3 are relatively low compared to the equine humoral response mounted against systemically infecting herpesviruses of the horse [1, 7].

The three stallions also showed low antibody titers after the onset of ECE. These low reactivities against EHV-3 antibody production might be due to the above suggestion and also due to low viral replication by application of the anti-herpetic drug [18].

The three stallions were orally administered valaciclovir, an anti-herpesvirus medicine, and acyclovir ointment was applied to the lesions after the development of clinical signs. The period of EHV-3 excretion was examined by virus isolation. In stallion A treated after day 2, EHV-3 was isolated on day 2 but not after day 8. In stallion B treated after day 1, EHV-3 was isolated up to day 4. In stallion C treated after day 1, EHV-3 was isolated on day 2 but not after day 8. These results suggested that the anti-herpetic drug treatment might have completely suppressed EHV-1 replication between day 5 and day 8. Experimental infection with EHV-3 in horses revealed that EHV-3 shedding lasted for 12 to 20 days [4, 20]. Therefore, anti-herpetic drug treatment might be effective for reducing virus shedding and viral contamination in farm environments.

In our previous study, anti-herpetic drug treatment did not shorten the healing period of ECE [18]. In that study, the healing period was defined as the period until stallions could start to mate again. In this study, we defined the healing period as the period until the lesions of ECE became encrusted. We increased the amount of the anti-herpetic drugs by about 1.3 times in order to shorten the healing period. Lesions in stallions A and B were encrusted and healed on day 8, and lesions in stallion C were encrusted and healed on day 10. Therefore, the healing period was shortened by using the anti-herpetic drug compared with the healing period of 10 to 14 days without anti-herpetic drug treatment in uncomplicated cases [1]. However, we could not directly compare the efficacy of treatment with an increasing amount of valacyclovir in the present study and the efficacy of valacyclovir treatment in our previous study because the definitions of the healing period were different. These observations in the three treated cases suggested that our treatment protocol is useful for controlling EHV-3 replication and for shortening the healing period, though further experiments are needed to determine the optimal valacyclovir concentration. The time to start mating should be determined on the basis of the conditions of healing lesions. Since it is possible that healing lesions would be damaged during mating, mating of the recovered stallion should be started when the healing lesions have been restored to healthy skin.

In conclusion, it was revealed that stallions could transmit EHV-3 to mares by mating during the incubation period at least within 4 days before showing clinical signs of ECE. Treatment with anti-herpetic drugs, including oral administration of valacyclovir and topical application of acyclovir ointment, can shorten the period of EHV-3 shedding from lesions and the healing period. If a stallion shows clinical signs of ECE, the genitals of mares with which the stallion mated during the incubation period (five days to 10 days) should be carefully observed for several days to prevent EHV-3 shedding to breeding facilities and environments and to prevent transmission of EHV-3 to other horses in the same farm.

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